REGULATION OF PLASMA TRIGLYCERIDE METABOLISM -
SOME NUTRITIONAL AND ENDOCRINE EFFECTS

A thesis submitted for the degree of
Doctor of Medicine
at the University of Cape Town

by

Alan Chait, M.B.Ch.B., M.Sc., M.R.C.P.
Hammersmith Hospital and Royal Postgraduate Medical School
London, 1974

Abbreviations used

VLDL - very low density lipoprotein
LDL - low density lipoprotein
HDL - high density lipoprotein
FFA - free fatty acid
IVFTT - intravenous fat tolerance test
PHLA - post-heparin lipolytic activity
P/S - polyunsaturated/saturated
IHD - ischaemic heart disease

CONTENTS

CHAPTER I

INTRODUCTION

CHAPTER I

During the past two and a half decades, numerous studies on plasma lipids and lipoproteins have appeared in the medical literature. The tremendous interest in this field, no doubt relates largely to the long-known and well established association between elevation of plasma lipids and lipoproteins, and ischaemic heart disease (IHD). Despite many years of study in this field, the nature of this association is still poorly understood. The exact mechanism whereby plasma lipids and lipoproteins influence the pathogenesis of atherosclerosis still remains to be elucidated.

During the past decade, the publication of a number of clinical trials has stimulated further interest in the field. Diets designed to lower plasma lipid levels, resulted in a moderate yet significant reduction in IHD mortality in previously normal individuals (Christakis et al, 1966; Dayton et al, 1969; Miettinen et al, 1972). The implication of these findings in terms of prevention of IHD has obvious attractions.

Another major landmark was the publication by Fredrickson, Levy and Lees (1967) of an improved classification of the clinical hyperlipidaemias which relied on typing patients on the basis of the lipoprotein pattern of their sera rather than on serum lipids alone. Medical interest in the subject was widened and a further spate of publications followed.

Initially, attention was focused largely on serum cholesterol in relation to IHD. This is largely because of the ease with which serum cholesterol values could be determined. With the advent of widely available methods for the determination of serum triglycerides, in recent years considerable interest has been paid to serum triglycerides and the chief triglyceride bearing lipoproteins; their relationship to IHD is now widely accepted and factors controlling and affecting serum triglyceride concentrations have been subject to numerous studies. It is with some of these factors that this thesis is concerned; the mechanisms whereby various dietary factors and pathological states affect serum triglyceride metabolism has been studied in the hope that better understanding of such processes may be of use in the prevention of the development of ischaemic heart disease.

As lipids in general are non-polar molecules with very low solubility in aqueous solutions, their presence in plasma is made possible by the formation of soluble complexes with specific proteins; such water soluble macromolecules are termed lipoproteins. In general, they serve to transport lipids from sites of absorption and synthesis to sites of storage and catabolism.

Lipids and Lipoproteins of Plasma

The four major classes of lipids in plasma are cholesterol and its esters, triglycerides, phospholipids and free fatty acids (FFA). FFA is reversibly bound to albumin. Two moles of FFA are bound per mole of albumin to its major binding sites; a larger number of weaker binding sites are also present (Goodman, 1958).

Four main classes of lipoproteins are detectable in plasma; within each, the relative amounts of cholesterol, triglyceride, phospholipid and protein differ, leading to physicochemical differences which permit of their separation. Considerable heterogeneity exists with each class, this being especially so with the triglyceride rich lipoproteins. Although it is generally considered that the less polar lipids occupy the 'core' of the lipoprotein molecule while the more polar lipids and proteins form the coat, their exact ultrastructure remains the subject of much controversy (Schumaker and Adams, 1969; Forte and Nichols, 1972). The lipid-protein bonding is relatively weak; hydrophobic interactions probably being the main binding force (Chapman, 1969)

The most widely employed methods for the separation of lipoproteins are electrophoresis and ultracentrifugation. The former method relies on the molecular charge and, although useful qualitatively, quantification of lipoproteins by this method is difficult and is not widely practised. Perhaps the most widely used method of quantitating lipoproteins is preparative ultracentrifugation (Havel et al, 1955). After serial runs against background solutions of different densities, lipid determinations are performed on the fractions obtained. Analytical ultracentrifugation (de Lalla and Gofman, 1954), although allowing differential flotation of lipoprotein classes differing by only small density increments, is not generally available due to the high costs of the apparatus. Other methods of separation of lipoprotein which are used, include Cohn fractionation (Cohn et al, 1950), ultrafiltration with nephelometry of the filtrate (Stone and Thorpe, 1966), and various precipitation techniques (Burstein et al, 1970).

Chylomicrons are so called because of their appearance on microscopy of chyle following the ingestion of a fatty meal (Gage and Fish, 1924); the other three lipoprotein classes derive their nomenclature from their electrophoretic and ultracentrifugal behaviour. Their physical characteristics are summarised in Table I (Levy et al, 1971).

Chylomicrons with the lowest density have the highest triglyceride content (80 - 95%) with 0.5 - 2.5% protein. They are normally absent from the plasma during the fasted state, at which time most of the plasma triglyceride is carried on the very low density lipoprotein (VLDL) or prebeta lipoprotein. Fifty-seven to sixty-eight per cent of this lipoprotein class consists of triglyceride, with cholesterol contributing 15 - 18%. Although originally believed to carry endogenous triglyceride only, this lipoprotein class is to-day believed to play a wider role as will be discussed in detail in Chapter VI. Beta or low-density lipoprotein (LDL) is the chief cholesterol bearing lipoprotein of plasma. It has a higher density than VLDL, a cholesterol content of 42 - 46% and a protein content in excess of 20%. The highest density lipoprotein is the alpha lipoprotein, or high density lipoprotein (HDL); this consists roughly of 50% protein, 30% phospholipids, 15% cholesterol and 5% triglyceride.

TABLE I

PHYSICAL CHARACTERISTICS OF MAJOR LIPOPROTEIN FAMILIES

Lipoprotein	S_f[*]	Density gm/ml	Mobility[†]	Molecular Weight	Size Å[‡]
Chylomicrons	> 400	< 0.95	Origin	$10^3 - 10^4 \times 10^6$	750 - 10,000
VLDL	20 - 400	0.95 - 1.006	Pre-β	$5 - 10 \times 10^6$	300 - 800
LDL	0 - 12	1.019 - 1.063	β	$2.1 - 2.6 \times 10^6$	205 - 220
HDL	-	1.063 - 1.21	α	200,000	75 - 100

[*] Lipoprotein flotation rate in Svedberg units (10^{-13} cm/sec/dyne/gm) in a sodium chloride solution of density 1.063 gm/ml(26° C).

[†] Paper electrophoresis.

[‡] As determined by electron microscopy.

A further aspect of lipoprotein composition which is at present being intensively investigated in many laboratories, is that of the protein component of lipoproteins. These so-called apoproteins may be divided into three main groups, including some eight or more individual peptides (Scanu, 1972; Alaupovic et al, 1972). Within each class of lipoprotein as separated by electrophoresis or ultracentrifugation, from one to several polypeptides may be demonstrated.(Scanu, 1972; Alaupovic et al, 1972).

Already, interconversion of some lipoprotein classes have been demonstrated by studying the protein components. For instance, following the demonstration that iodine-labelled peptides of VLDL appears in LDL (Levy et al, 1971; Fidge and Foxman, 1971; Eisenberg et al, 1972), it is becoming generally accepted that the main source of the latter lipoprotein is VLDL.

Serum Lipids as Risk Factors for Ischaemic Heart Disease

Within many Western communities, the incidence of IHD appears to be rapidly increasing (Epstein, 1965; Stamler, 1973). In 1971, statistics showed IHD to be the commonest cause of death in Britain, accounting for 25% of all deaths (Registrar General, 1971). Several risk factors have been identified from prospective studies; among them the hyperlipidaemias have attracted considerable attention.

A risk factor for the development of IHD, is an attribute which increases the probability of an individual subsequently developing the disease. This definition does not include the necessary assumption that the relationship is causal; the characteristic may be predicted by virtue of one of its associations which is directly causally relevant. Strictly speaking, the term 'risk factor' should only apply if proof of a causal relationship has been obtained; the less rigorous definition referred to above is, however, in common usage.

Among all the risk factor studies, serum cholesterol and hypertension appear to have received most attention. A relationship between serum cholesterol and IHD incidence has been demonstrated in most studies (Lande and Spervy, 1936; Morrison et al, 1948; Kannel et al 1961; Stamler et al, 1960, Keys et al, 1963), although the nature of the relationship still needs to be clearly defined. With widespread availability of serum triglyceride determinations for only a decade, attention has recently been focused on its role as a risk factor for IHD. Early work in this field came from Albrink and her co-workers. In 1959, Albrink and Man reported a higher prevalence of elevated serum triglyceride concentrations among survivors of myocardial infarction and subjects with angina, than amongst normal controls. Albrink et al (1961) showed 46% of men with IHD to have elevated cholesterol levels compared with 26% of controls. Eighty-two per cent of the IHD group, however, had triglyceride elevation compared with 46% of the controls. At first sight, this data suggests that serum triglycerides may be a better discriminator of IHD than serum cholesterol. Caution, however, must be exercised in the interpretation of such data because as levels of cholesterol and triglyceride both tend to increase with increasing age in urbanized communities (Schaefer, 1964; Carlson and Lindstedt, 1968), age differences between patient and control groups could be of importance. Also, some samples taken soon after infarction, may show lipid levels quite different to those taken under normal conditions. Furthermore, choosing a cut-off point between normal and hyperlipidaemic raises difficult questions; the choice of an artificially high cut-off point for one of the lipids and an artificially low one for the other, could lead to considerable bias and result in misrepresentation. Nonetheless, a considerable body of evidence has arisen in support of the conclusions of Albrink et al (1961). Foremost in the field has been the group of workers under Carlson, who over the past decade have provided evidence supporting the role of serum triglyceride as an independent risk factor. Carlson (1960), showed that serum triglyceride concentration discriminated better than serum cholesterol concentration between young patients with IHD and normal controls; the reverse trend was, however, noted in the older age group. The latter finding may relate to his finding of a reduced triglyceride level in older subjects, a finding which has been confirmed (Schaefer, 1964; Lewis et al, 1974). It may be that individuals with higher triglyceride levels tend to eliminate themselves from such age distribution studies by dying at an earlier age than their normolipaemic controls. Antonis and Bersohn (1960) also showed raised triglyceride levels in IHD; South African white men over the age of 40, and patients of all ages with IHD, were found to have higher levels of triglyceride than whites under 40, or than

African men of all ages. They suggested that white men in the older age group could be divided into coronary-prone and normal, on the basis of serum triglyceride concentrations. A relationship between IHD prevalence and serum triglyceride levels was also remarked on by Brown et al (1965), although they found triglyceride to be no better an indicator than cholesterol. Kuo (1967) also showed hypertriglyceridaemia to be no more prevalent in individuals with IHD than hypercholesterolaemia, as did Kroman et al (1964). Although Rifkind et al (1968) found the frequency of hypertriglyceridaemia in IHD to exceed that of hypercholesterolaemia by more than a factor of three, combined cholesterol and triglyceride levels were found to best segregate IHD patients from normal. Canellos and Hatch (1962) studied nine young patients, mean age 35 years, following recovery from myocardial infarction. Eight had increased prebeta lipoprotein, although their cut-off point for triglycerides (125 mg/dl) is probably too low by current thinking. Serum cholesterol elevation above 250 mg/dl was only detected in two cases. Elevation of prebeta lipoprotein in IHD had, in fact, been documented considerably earlier (Besterman, 1957), the sera of subjects with IHD having significantly higher levels of prebeta lipoprotein than normal. Serum triglyceride concentrations were, however, not measured in this study. The relationship between IHD and serum lipid concentration has again been studied in two recent investigations. Patterson and Slack (1972) found that a quarter of survivors of myocardial infarction had hyperlipidaemia, defined as a cholesterol or triglyceride level exceeding the mean plus two standard deviations in controls. In a large study involving 500 consecutive survivors of myocardial infarction

Goldstein et al (1973) compared lipid levels with a large group of controls using the 95th percentile as a cut-off point. Hyperlipidaemia was detected in nearly one-third of patients of all ages. Younger subjects had a higher frequency, 60% of male survivors below the age of 40 being affected. Hypertriglyceridaemia with or without associated hypercholesterolaemia was found to occur nearly three times as frequently as hypercholesterolaemia alone. This large retrospective study again raises the possibility that plasma triglyceride may be as important if not more, than cholesterol as a risk factor for the development of IHD and atherosclerosis.

Before this can be regarded as clearly established, the association will need to be studied prospectively in longitudinal epidemiological studies in a manner similar to that for serum cholesterol (e.g. Kannel et al, 1961). To date, only two such prospective studies have been published, namely, the Western Collaborative Group Study (Rosenman et al, 1970) and the Stockholm Prospective Study of Carlson add Böttiger (1972). In the former an elevated serum triglyceride level appeared to confer increased risk of developing IHD. The latter was a nine year follow-up study involving more than three thousand men free from evidence of IHD at the time of recruitment, an elevated serum triglyceride concentration was found to be a risk factor for IHD, the concentration of triglyceride appearing to be at least as important as that of serum cholesterol. Men under the age of 60, whose serum triglyceride levels were in the top quintile of the population studied, had a four times greater chance of developing a new IHD event than did men in the bottom quintile. The study failed to answer the question of whether serum cholesterol or triglyceride is the best predictor of IHD; each, however, appeared to be of predictive value in its own right.

The degree of the problem is perhaps put into perspective when one considers the frequency distribution of serum triglycerides within a population. We recently reported the results of a survey of serum lipid levels in a 'normal' working population in London (Lewis et al, 1974). Cut-off points defining normality were intentionally set high, yet the prevalence of lipid abnormalities was remarkable. Fourteen per cent of men and 3% of women aged 40 - 49 had fasting serum triglyceride concentrations in excess of 2 mMoles per litre (176 mg/dl), cholesterol concentrations exceeding 300 mg/dl in 4.3% of men and 4.8% of women. By these definitions, 17% of men and 8% of women had hyperlipidaemia. Elevated serum triglyceride levels were also shown to occur more frequently than elevated serum cholesterol levels amongst "healthy" Swedes (Micheli et al, 1973). In view of the association between IHD and hyperlipidaemia, the high prevalence found in apparently healthy populations, especially in terms of hypertriglyceridaemia, is of public health importance.

In recent years, a vast volume of research has centred around triglyceride metabolism. Of particular interest has been the factors controlling serum triglyceride concentrations; hopefully by a better understanding of these mechanisms treatment of hypertriglyceridaemia can be attempted on a more rational basis than the largely empirical approaches currently in use. As this thesis focuses on some aspects of control of serum triglyceride concentrate, I propose to briefly outline the physiology of serum triglyceride metabolism.

Triglyceride Metabolism

The two chief triglyceride bearing lipoprotein classes are 'chylomicrons' and 'very low density lipoprotein' (VLDL); considerable heterogeneity exists within these classes in terms of both structure and function, as will be discussed in Chapter VI. The main function of the former lipoprotein class is the transport of dietary fat through plasma, whereas VLDL carry triglyceride largely of endogenous origin. In the fasting state, under normal circumstances, chylomicrons are absent from plasma.

The fasting concentration of plasma lipoprotein triglyceride in a steady state, represents a balance between entry of triglyceride into plasma on the one hand, and removal on the other. A change in the concentration may, therefore, reflect a change in either or both of these variables.

Entry of Triglyceride into Plasma

The two organs known to be capable of secreting the triglyceride-rich lipoproteins into plasma are intestine and liver. The intestinal pathway serves to transport dietary fat into the lymphatic system whence it reaches the blood stream; although chylomicrons have long been known to appear in blood following the ingestion of a fatty meal, considerable evidence is accruing in support of VLDL having a role in dietary fat transport as well (Ockner et al, 1969b; Schlierf and Raetzer, 1972). It has also recently become apparent that a lipoprotein resembling VLDL is continuously delivered into intestinal lymph even in the absence of recent fat ingestion (Baxter, 1966; Ockner et al, 1969a). The intestine thus probably contributes to the transport of endogenous as well as exogenous triglyceride; the fraction of endogenous triglyceride turnover derived from intestinal lymph appears to be less than from liver, but may very well vary in different animal species (Havel, 1970a). The role of the intestine in plasma triglyceride metabolism will be more fully discussed in the section on dietary fat transport (See Chapter VI).

The liver is the other site of triglyceride release into plasma; during the postabsorptive state, it is the chief source of triglyceride entry into plasma in the form of VLDL (Robinson, 1970). The source of the fatty acid components of triglyceride synthesized in the liver varies according to the species and nutritional state. In the postabsorptive state, VLDL triglyceride fatty acids in man derive mainly from free fatty acids (FFA) taken up by the liver from plasma (Freidberg et al, 1961; Havel, 1961). In man, after an overnight fast, FFA are mobilized into plasma by lipolysis of adipose tissue stores (Goodman, 1958); uptake of plasma FFA by the liver appears to depend on plasma FFA concentration in the physiological range and even at most raised levels encountered pathologically (Van Harken et al, 1967; 1969). During fasting, only 30 - 40% reach the liver; most reach muscle where they are oxidised for energy purposes (Robinson, 1973). Of the FFA reaching the liver, a portion is oxidised to carbon dioxide and water, and some partially oxidised to form ketones; a further proportion is esterified to form triglyceride. Other sources of FFA may also be important; during carbohydrate feeding, fatty acids synthesized in the liver, and during fat ingestion, fatty acids from chylomicron triglyceride, may enter the hepatic FFA pool to be used for triglyceride synthesis (Havel, 1970a). In man and the rabbit, the bulk of hepatic FFA is derived from plasma; plasma FFA concentrations may, therefore, be a major determinant of hepatic triglyceride secretion in these species (Freidberg et al, 1961; Havel, 1961; Havel et al, 1962; Havel, 1970a). In the rat, only a small proportion of hepatic FFA is derived from the plasma FFA pool. During carbohydrate feeding in rats fatty acids synthesized in the livers have been shown to be of importance (Windmueller and Spaeth, 1967).

Much of our knowledge of the hepatic synthesis and release of lipoprotein derives from studies with the isolated perfused liver. In the hepatocyte, the enzymes necessary for incorporation of fatty acids into triglycerides are contained mainly in microsomal fraction (Stein and Shapiro, 1957; Tzur and Shapiro, 1964) which consists of smooth and rough endoplasmic reticulum and the Golgi apparatus. Although the exact site of esterification is not known with certainty, the smooth endoplasic reticulum is the most likely, as toxic agents which damage the rough endoplasmic reticulum tend not to interfere with this process (Jones and Armstrong, 1965; Stein and Stein, 1965). Fatty acyl CoA reacts with glycerol -3- phosphate to form diglyceride, which reacts with a further molecule of fatty acyl CoA yielding triglyceride. Glycerol -3- phosphate is derived from glycerol the reaction being catalyzed by glycerokinase, or from glycolysis by the action of glycerol -3- phosphate dehydrogenase on dihydroxyacetone phosphate (Marinetti, 1970). The newly formed triglyceride may either be deposited in the liver cell as a lipid droplet, or transported from it in the form of VLDL. The key determinant of whether the latter occurs, appears to be the availability of apoprotein which is essential for lipoprotein secretion (Jones et al, 1967). After synthesis in the rough endoplasmic reticulum, the protein passes to the smooth endoplasmic reticulum where combination with lipid occurs. Inhibition of protein synthesis by puromycin prevents lipid transport out of the liver without inhibiting the formation of triglyceride (Robinson and Seaknis, 1962; Jones et al, 1967). It is of interest then that raising the FFA concentration in the perfusing medium results in an increased availability of protein released by the liver, and incorporation of aminoacids into the apoproteins of VLDL (Ruderman et al, 1968). Finally, release of the newly synthesized lipoprotein molecule appears to occur via the microtubular system (Neutra and Leblond, 1966).

Removal of Triglyceride from Plasma

It is only in recent years that a better understanding of the removal of lipoprotein triglyceride from plasma has emerged. During the last few years, emphasis has shifted from the liver as a major site of lipoprotein removal to adipose tissue and muscle. Current opinion is that at these latter sites, the enzyme lipoprotein lipase is of major importance in the removal of triglyceride from plasma (Robinson, 1970).

The function of the liver in triglyceride removal appears to be negligible. It was originally thought to play a role following the observation that a large proportion of endogenously labelled lipoprotein triglyceride could rapidly be recovered from livers following reinjection (Bragdon and Gordon, 1958; Stein and Shapiro, 1960), as much as 35% being recovered in the liver soon after injection (Borgström and Jordan, 1959; Olivecrona, 1962). The triglyceride appears to be taken up intact, little evidence existing for hydrolysis (Borgström and Jordan, 1959; Stein and Shapiro, 1960; Reiser et al, 1960). Histological studies were in agreement that at least some of the lipid seen in hepatic sinusoids and in the perisinusoidal space were seen as intact particles (Ashworth et al, 1960).

It was believed that chylomicrons passed through large fenestrations up to 5 μ in width in the capillary endothelium, as seen under the electron microscope (Bennett et al, 1959; Majno, 1965); entry into the subendothelial species of Disse would be feasible, after which they could come into direct contact with liver parenchymal cells.

Robinson (1963), however, suggested that the triglyceride fatty acids may be outside the liver cells in spaces in communication with the hepatic sinusoids, and Havel and Goldfein (1961) showed that exclusion of the liver from the circulation had little effect on the removal of triglyceride fatty acids from plasma in dogs. These findings suggested the possibility that after pulse injection, the liver was reversibly taking up chylomicrons by trapping them in the space of Disse. More recent studies (Wisse, 1970) suggests that the gaps seen in the capillary endothelium are, in fact, artefacts due to fixation. When tissues were fixed by perfusion of the liver with gluteraldehyde, multiple fenestrated sieves $<$ 0.1 μ diameter were seen; entry of chylomicra through these into the space of Disse is thus unlikely.

The metabolism of chylomicron triglyceride has also been investigated in the isolated perfused liver system. Uptake of triglyceride has been shown to be limited unless heparin is present in the system (Felts, 1965; Mayes and Felts, 1966). Heparin is known to activate lipoprotein lipase which has been shown to exist in liver (Le Quire et al, 1963; Mayes and Felts, 1966; Engelberg, 1966). Hydrolysis of triglyceride under these unphysiological conditions may thus have accounted for the uptake in the presence of heparin. The liver is, however, the major site of removal of chylomicron cholesterol (Nestel et al, 1963), almost all radioactivity being recovered in the liver after infusion of chylomicrons containing labelled cholesterol. Excluding the liver from the circulation also resulted in a marked reduction in the rate of removal of chylomicron cholesterol. Havel (1970a) has suggested that chylomicron uptake by the liver may occur following prior modification of the complex by extra-hepatic lipoprotein-lipase. Such a view would suggest the liver to play a minor role in uptake of lipoprotein triglyceride, yet to be of major importance in the uptake of lipoprotein cholesterol. Consistent with this viewpoint are the findings of Redgrave (1970); ten minutes after injecting doubly labelled chylomicrons into rats, 80% of the cholesteryl ester label was found in liver compared with only 25% of the chylomicron triglyceride. In rats in which the liver was excluded from the circulation cholesteryl esters accumulated in plasma whereas triglyceride was cleared by peripheral tissues. The residual labelled cholesterol in plasma was shown to be present in remnant particles which when injected into other rats, appeared in the liver more rapidly than did chylomicrons. This suggests the first step in chylomicron metabolism to be extrahepatic triglyceride removal, probably by lipoprotein lipase, followed by removal of the cholesteryl ester and remaining triglyceride in the remnant particle, by the liver.

Lipoprotein Lipase

Electron microscopic studies of capillaries have shown chylomicrons to be confined within the vascular lumen (Williamson, 1964; Schoefl and French, 1968). When the endothelial cells are damaged by vascular enzymes (French, 1963), or when separated as in newborn animals (Suter and Majno, 1965), chylomicra still do not penetrate to extravascular tissue, but are held up by the basement membrane of the endothelial cells. This suggests that before lipoprotein triglyceride fatty acids are removed from blood, some change in their structure is required. There is now abundant evidence that the means whereby such a change occurs is by hydrolysis of lipoprotein triglyceride by lipoprotein lipase (Robinson and French, 1960; Robinson, 1963). The main evidence comes from studies involving triglyceride labelled in glycerol and fatty acid moeities with different isotopes. Following the injection of chylomicrons containing such triglycerides, a change in the ratio of the labels found in the major sites of uptake, adipose tissue and muscle, occurs in such a manner so as strongly to suggest prior hydrolysis of the triglyceride by a lipase (Olivecrona, 1962; Olivecrona and Belfrage, 1965; Jones and Havel, 1967). The enzyme lipoprotein lipase is present in these and other tissues which take up triglyceride fatty acids from plasma. It is not present in brain, an organ not capable of removing triglyceride fatty acids from plasma. In the fed state, a high proportion of the total lipoprotein lipase activity in the body is found in adipose tissue and muscle, consistent with a role of the enzyme in these organs for removing lipoprotein triglyceride from plasma (Robinson, 1970).

This process involves hydrolysis of the triglyceride moeity of VLDL or chylomicrons; the liberated FFA enters the adipocyte where it is re-esterified to form triglyceride. The glycerol moeity released by hydrolysis, does not enter the cell (Havel, 1965; Olivecrona and Belfrage, 1965; Jones and Havel, 1967).

Lipoprotein lipase is absent from the blood other than after its release from tissue sites by heparin. In adipose tissue and muscle, the enzyme is present in the capillary endothelial cell (Robinson, 1963; Hamosh and Evans, 1972). Not all the enzyme in a given tissue is localized to this site; in adipose tissue, for example, some enzyme is detectable in the adipocyte (Rodbell, 1964). This fraction appears to be non-functional in respect of uptake of lipoprotein triglyceride (Cunningham and Robinson, 1969) and may be a precursor of functional enzyme at the endothelial cell surface to where it must be transported (Cunningham and Robinson, 1969; Robinson and Wing, 1970).

Chylomicrons and artificial triglyceride emulsions have been shown by electron microscopy to be attached to the luminal surface of the capillary endothelium and sometimes to be partially enclosed by capillary endothelium; no particles have, however, been visualized in the fat cell or extracellular space (Suter and Majno, 1965; Blanchette-Mackie and Scow, 1971), suggesting that these complexes cannot cross the capillary endothelium as intact particles. Using electron microscopic cytochemical techniques, it has been possible to show that hydrolysis of lipoprotein triglyceride in adipose tissue occurs extravascularly within capillary endothelial cells and in the subendothelial space, but not in the capillary lumen or near the fat cells (Scow et al, 1972). This sequestration of chylomicrons and VLDL at the capillary endothelial surface permits an association to take place between lipoprotein lipase and its substrate; it, however, appears to depend on the presence of certain peptides in the apoprotein moeity of chylomicrons and VLDL. These peptides which are held in common with high density lipoprotein (HDL) function as activators (Havel et al, 1970; La Rosa et al, 1970; Krauss et al, 1973). They are not attached to chylomicrons when secreted by the intestinal cells and appear to be added when the particle comes into contact with plasma (Lossow et al, 1967; Robinson, 1970). Based on the biochemical and electron microscopic cytochemical studies of perfused adipose tissue, Scow et al (1972) have proposed a scheme of how lipoprotein triglyceride fatty acid may cross the capillary wall into endothelium. When chylomicrons or VLDL becomes attached and partially enveloped by the endothelial cell, triglyceride within the lipoprotein is hydrolyzed to FFA and diglyceride. The FFA is released into the blood stream and diglyceride taken into a vacuole or microvesicle. Hydrolysis to FFA and monoglyceride occurs during transport across the endothelial cell, the product being released into endothelial space. Further hydrolysis of the monoglyceride occurs, and the FFA released from this fraction and from the hydrolysis of diglyceride, enters the fat cell where they are re-esterified; glycerol and the now triglyceride-poor remnant (Redgrave, 1970) are released back to the blood stream. This hypothesis is consistent with the findings that about two-thirds of labelled chylomicron triglyceride fatty acid enters the fat cell, one-third being released into the circulation (Scow et al, 1972).

FFA Uptake

The FFA released by hydrolysis of lipoprotein triglyceride crosses the cell membrane and enters the tissues. Whereas tissue uptake of plasma FFA released by lipolysis of adipose tissue triglyceride stores is dependent on plasma FFA concentration and blood flow (Van Harken et al, 1967; 1969), the uptake of FFA released by hydrolysis by lipoprotein lipase depends on the activity of the enzyme in the particular tissue (Robinson, 1973). Control in the removal of triglyceride fatty acid from blood may thus be effected by variation in the activity to the needs of the organism. In the fed state, for instance, plasma triglyceride fatty acids in excess of immediate energy needs, may be taken up by adipose tissue, re-esterified, and stored as depot fat. Conversely during fasting, VLDL triglyceride fatty acids are taken up by muscle where they may be utilized as a source of energy (Robinson, 1970). Indeed, during periods of caloric excess, adipose tissue lipoprotein lipase activity rises, whereas during periods of calorie deficit, smooth and cardiac muscle enzyme activity rises while that in adipose tissue falls Borensztajn et al, 1970; Borensztajn and Robinson, 1970). A further example of how the enzyme activity may result in localization of triglyceride uptake in response to a need, is during lactation. Mammary gland lipoprotein lipase and triglyceride uptake increase markedly following parturition, at which time there is a vastly increased requirement of lipid for milk formation (Barry et al, 1963; McBride and Korn, 1964).

The correlation of lipoprotein lipase activity and tissue triglyceride uptake thus permits of a system with the potential of determining the site of removal of triglycerides by varying the activity of the enzyme in a particular tissue according to a need. The factors controlling these tissue specific changes in lipoprotein lipase activity are, however, poorly understood. Hormonal factors are probably involved in that insulin increases lipoprotein lipase activity, whereas the catecholamines, ACTH (Wing et al, 1966; Wing and Robinson, 1968), glucagon and thyroid stimulating hormone (Nestel and Austin, 1969), inhibit the enzyme. The effect of these hormones on lipoprotein lipase is thus opposite to their effect on the 'hormone-sensitive lipase' responsible for the hydrolysis of adipose tissue triglyceride. Catecholamines, ACTH and glucagon thus tend to mobilize fat from adipose tissue stores, whereas lipoprotein lipase promotes uptake and storage of triglyceride. Insulin appears to be of prime importance in the latter process; the reason why the lipoprotein lipase activity is stimulated in one tissue while simultaneously inhibited in another, in response to a hormone, is however, not clear.

A further factor of importance in tissue uptake of FFA liberated by the action of hydrolysis, is the FFA concentration gradient between the capillary endothelium and the cell. This aspect has received relatively little attention. Once across the cell membrane, FFA is re-esterified by combining with glycerol -3- phosphate to reform triglyceride. As adipose tissue has very low glycerol kinase activity (Steinberg, 1962; Margolis and Vaughan, 1962; Marinetti et al, 1964), the glycerol -3- phosphate must derive from glycolysis. The entry of glucose into the adipocyte and factors controlling glycolysis, may thus also affect triglyceride removal from plasma, as impaired re-esterification would tend to diminish the FFA concentration gradient.

The means by which triglyceride enters and leaves plasma has been outlined in brief; under conditions where the endogenous plasma triglyceride level is constant, input and output must be equal. An increase in plasma triglyceride pool size can either result from enhanced triglyceride input, diminished output, or both these factors. Considerable research over the past few years has attempted to show which of these factors is operative in various pathological states. Because of numerous assumptions on which many of the methods of estimating plasma triglyceride turnover are based, controversy still exists as to the pathogenesis of the lipid elevation in both primary and secondary hyperlipidaemic states (Havel, 1970; Nikkilä and Kekki, 1971; Havel, 1972). Thus, some authorities have regarded primary endogenous hypertriglyceridaemia to be due to defective triglyceride removal (Ryan and Schwartz, 1965; Sailer et al, 1966; Porte and Bierman, 1969; Havel et al, 1970; Boberg, 1971), whereas others have thought the primary defect to be one of enhanced input of triglyceride into plasma (Farquhar, 1965; Olefsky et al, 1974).

It is well known that hyperlipoproteinaemia may be due to underlying disease (Fredrickson et al, 1967). In the absence of any detectable cause, the hyperlipoproteinaemia is referred to as primary; each category of primary hyperlipoproteinaemia has been shown to have a population with a common genetic basis, the remainder probably being the resultant of various constitutional and environmental factors. Environment may also effect the expression of a genetic defect, predisposing an individual to the development of hyperlipoproteinaemia. Factors of importance in respect of this are diet, in particular the effect of over-nutrition and the type and amount of fat and carbohydrate ingested. Alcohol and drugs may also modify lipid and lipoprotein levels and metabolism to a considerable degree.

Abnormal lipoprotein patterns arising as a result of alcohol and drug ingestion have been classified as primary hyperlipoproteinaemia (Beaumont et al, 1970). Such an approach may not be entirely justified; in the absence of alcohol intake or oral contraception medication, an entirely normal lipoprotein pattern may prevail. Other authorities prefer to regard the hyperlipidaemias arising as a result of these factors as secondary (Steinberg, 1972). In view of environmental influences it is perhaps not surprising that under different circumstances, a particular genotype might manifest itself in a variety of patterns of serum lipoprotein. Different 'types' of disorders occurring in members of the same family may to some extent be explained on this basis. There would thus seem to be advantage in always thinking of serum lipid and lipoprotein concentration and pattern in an individual, as the resultant of the interaction of various environmental factors with other factors in the genetic constitution. In addition, underlying causes known to be associated with elevations of serum lipids and lipoprotein may exert a further influence.

In this thesis, I have attempted to study some of the factors affecting serum triglyceride metabolism. In Chapter III the effect of diabetes mellitus is investigated. Diabetes has frequently been regarded primarily as a disorder of carbohydrate metabolism; disturbance of plasma lipids and lipoproteins, however, also occur remarkably frequently, and may be an important factor predisposing to the premature vascular disease seen commonly amongst diabetics. The pathogenesis of the lipid disturbances are thus of interest with a view to their therapy and possible benefit in the prevention of atherosclerosis.

Two nutritional factors which I have studied are alcohol and dietary fat. A well-known hazard of excessive alcohol intake is the development of fatty liver; alcohol may also result in hypertriglyceridaemia, although this probably only occurs in a minority of drinkers. Nonetheless, alcohol-induced elevation of serum lipid levels may be a factor predisposing the drinker to yet another ill-effect of alcohol, that of the development of atherosclerosis. Identification of individuals particularly susceptible to the effect of alcohol on serum triglycerides might, therefore, be of value in the prevention of atherosclerosis and the associated IHD and peripheral vascular disease (PVD). The effect of alcohol on serum triglyceride is discussed in Chapter IV.

Many Western societies tend to ingest excess calories as judged by the exceedingly high frequency of obesity in such communities (Montegriffo, 1968). High intake of fat especially of the saturated variety tend to prevail in more affluent societies; both the total fat intake and that of saturated fat has been shown to correlate significantly with the incidence of IHD within populations (Masironi, 1970; Stamler et al, 1970). As the type and amount of dietary fat influences serum lipids, dietary fat intake may be linked to atherosclerosis via an effect on plasma lipid metabolism. Much work, especially epidemiological has for the past 15 years or so, centred around the effect of dietary fat on serum cholesterol concentration. However, since the advent of precise easy methods of determining serum triglyceride, and the realisation that triglyceride may also be important as a risk factor for IHD, considerable attention has recently been focussed on this lipid. This thesis looks at some effects of dietary fat on serum triglyceride metabolism. Chapter V describes studies on polyunsaturated dietary fat. This was studied in view of its use in the therapy of certain hyperlipoproteinaemic disorders, and also because of its potential recommendation for widespread use in order to try and lower the alarming incidence of IHD in Western societies. The plasma transport of dietary fat has also been investigated in Chapter VI; the triglyceride bearing lipoproteins formed during ingestion of fat have been studied and discussed in relation to classification of disorders of lipoprotein metabolism based on lipoprotein profiles in plasma.

In many of the experiments, quantitative measurements of lipoprotein lipid was performed using preparative ultracentrifugation. Removal of triglyceride from plasma was assessed by a relatively new technique, the intravenous fat tolerance test. The theoretical consideration relating to this test is discussed in the Methods section on page 58 . This test was chosen because of its simplicity, and because of its good correlation with more difficult and more invasive methods of investigation of triglyceride turnover.

Alaupovic, P., Lee, D.M. and McConathy, W.J. (1972) Biochim. Biophys Acta. 236, 29.

Albrink, M.J., Meigs, J.W. and Man, E.B. (1961) Amer. J. Med. 31, 4.

Albrink, M.J. and Man, E.B. (1959) Arch. intern. Med. 103, 4.

Antonis, A. and Bersohn, I. (1960) Lancet I, 998.

Astworth, C.T., Sternbridge, V.A. and Sanders, E. (1960) Amer. J. Physiol. 198, 1326.

Barry, J.M., Bartley, W. Linzell, J.L. and Robinson, D.S. (1963) Biochem. J. 89, 6.

Baxter, J.H. (1966) J. Lipid Res. 7, 158.

Beaumont, J.L., Carlson, L.A., Cooper, G.R. Fejfar, Z., Fredrickson, D.S. and Strasser, T. (1970) Bull. Wld Hlth. Org. 43, 891.

Bennet, H.S., Luft, J.N. and Hampton, J.C. (1959) Amer. J. Physiol. 196, 381.

Besterman, E.M.M. (1957) Brit. Heart J. 19, 503.

Blanchette-Mackie, E.J. and Scow, R.O. (1971) J. Cell Biol. 51, 1.

Boberg, J. (1971) M.D. Thesis. Acta Univ. Upsal. 105.

Borensztajn, J. and Robinson, D.S. (1970) J. Lipid Res. 11, 111.

Borensztajn, J., Otway, S. and Robinson,D.S. (1970) J. Lipid Res. 11, 102.

Borgström, B. and Jordan, P. (1959) Acta Soc. Med. Upsal. 64, 185.

Bragdon, J.H. and Gordon, R.S. (1958) J. clin. Invest. 37, 574.

Brown, D.F., Kinch, S.H. and Doyle, J.T. (1965) New Engl. J. Med. 273, 947.

Burstein, M., Scholnick, H.R. and Morfin, R. (1970) J. Lipid Res. 11, 583.

Canellos, G.P. and Hatch, F.T. (1962) Circulation 26, 648.

Carlson, L.A. (1960) Acta med. scand. 167, 399.

Carlson, L.A. and Lindstedt, S. (1968) Acta med. scand.suppl. 493.

Carlson, L.A. and Böttiger, L.E. (1972) Lancet I, 865.

Christakis, G. Rinzler, S.H., Archer, M., Winslow, G., Jampel, S., Stephenson, I., Friedman, G., Fein, H., Kraus, A. and James, G. (1966) Am. J. Publ. Hlth 56, 299.

Chapman, D. (1969) In Structural and functional aspects of lipoproteins in living systems (eds. E. Tria and A.M. Scanu) Academic Press, London, New York, p.3.

Cohn, E.J., Gurd, F.R.N., Surgenor, D.M., Barnes, B.A., Brown, R.K., Derouaux, G., Gillespie, J.M., Kahnt, F.W., Lever, W.F., Liu, C.H., Mittleman, D., Mouton, R.F., Schmid, K. and Urovna, E. (1950) J. Amer. Chem. Soc. 72, 465.

Cunningham, V.J. and Robinson, D.S. (1969) Biochem. J. 112, 203.

Dayton, S., Pearce, M.L., Hashimoto, S., Dixon, W.T. and Tomiyasu, U. (1969) Circulation 40, Suppl.2.

de Lalla, O. and Gofman, J. (1954) Methods of Biochem. Anal. 1, 459.

Engelberg, H. (1966) Circulation 34, III 10a.

Eisenberg, S., Bilheimer, D.W. and Levy, R.I. (1972) Biochim. Biophys. Acta 280, 94.

Epstein, F.H. (1965) J. chron. Dis. 18, 735.

Farquhar, J.W., Gross, R.C., Wagner, R.W. and Reaven, G.M. (1965) J. Lipid Res. 6, 119.

Felts, J.M. (1965) Ann. N.Y. Acad. Sci. 131, 24.

Fidge, G. and Foxman, C.J. (1971) Aust. J. Exp. Biol. Med. Sci. 49, 581.

Forte, T. and Nichols, A.V. (1972) Adv. Lipid Res. 10, 1.

Fredrickson, D.S., Levy, R.I. and Lees, R.S. (1967) New Engl. J. Med. 276, 34, 94, 148, 215, 273.

Friedberg, S.J., Klein, R.F., Trout, D.L., Bogdonoff, M.E. and Ester, E.H.,Jr. (1961) J. clin. Invest. 40, 1846.

French, J.E. (1963) In Biochemical Properties of Lipids (ed. A.C. Frazer), Elsevier, Amsterdam. p.296.

Gage, S.H. and Fish, P.A. (1924) Amer. J. Anat. 34, 1.

Goldstein, J.L., Hazzard, W.R., Schrott, H.G., Bierman, E.L. and Motulsky, A.G. (1973) J. clin. Invest. 52, 1533.

Goodman, D.J. (1958) J. Amer. Chem. Soc. 80, 3892.

Hamosh, M. and Evans, A.J. (1972) Fed. Proc. 31, 297.

Havel, R.J., Eder, H.A. and Bragdon, J.H. (1955) J. clin. Invest. 34, 1345.

Havel, R.J. (1961) Metabolism 10, 1031.

Havel, R.J. and Goldfein, A. (1961) J. Lipid Res. 2, 389.

Havel, R.J., Felts, J.M., van Duyne, C.M. (1962) J. Lipid Res. 3, 297.

Havel, R.J. (1965) In Handbook of Physiology V: Adipose Tissue (eds. A.E. Renold and G.F. Cahill) Amer. Physiol. Soc. Washington D.C. p.499.

Havel, R.J. (1970) In Atherosclerosis: Proceedings of 2nd International Symposium (ed. R.J. Jones) Springer-Verlag p.210.

Havel, R.J., Shore, V.G., Shore B. and Bier, D.M. (1970) Circulat. Res. 27, 595.

Havel, R.J., Kane, J.P., Balasse, E.O., Segel, N. and Basso, L.V. (1970) J. clin. Invest. 49, 2017.

Havel, R.J. (1972) Adv. exp. med. Biol. 26, 57.

Jones, A.L. and Armstrong, D.T. (1965) Proc. Soc. exp. Biol. (N.Y.) 119, 1136.

Jones, A.L., Ruderman, N.B. and Hemera, M.G. (1967) J. Lipid Res. 8, 129.

Jones, N.L. and Havel, R.J. (1967) Amer. J. Physiol. 213, 824.

Kannel, W.B., Dawber, T.R., Kagan, A., Revotskie, N. and Stokes, J.III. (1961) Ann. intern. Med. 55, 33.

Keys, A., Taylor, H.L., Blackburn, H., Brozek, T., Anderson, J.T. and Simonson, E. (1963) Circulation 28, 381.

Krauss, R.M., Herbert, P.H., Levy, R.I. and Fredrickson, D.S. (1973) Circ. Res. 33, 403.

Kroman, H., Nodine, J., Bender, S. and Brest, A. (1964) Am. J. Med. Sci. 248, 571.

Kuo, P.T. (1967) J. Amer. med. Assoc. 201, 87.

Lande, K.E. and Spervy, W.M. (1936) Arch. Pathol. 22, 301.

La Rosa, J.C., Levy, R.I., Herbert, P., Lux, S.E. and Fredrickson, D.S. (1970) Biochem. Biophys. Res. Comm. 41, 57.

Le Quire, V.S., Hamilton, R.L., Adams, R. and Merrill, J.M. (1963) Proc. Soc. exp. Biol. (N.Y.) 114, 104.

Levy, R.I., Bilheimer, D.W. and Eisenberg, S. (1971) In plasma lipoproteins (ed. R.S. Smellie), Academic Press, London, New York. p.3.

Lewis, B., Chait, A., Wootton, I.D.P., Oakley, C.M. Krikler, D.M., Sigurdsson, G., February, A., Maurer, B. and Birkhead, J. (1974) Lancet I, 141.

Lossow, W.J., Lindgren, F.T. and Jensen, L.C. (1967) Biochim. Biophys. Acta. 144, 670.

Majno, G. (1965) In Handbook of Physiology II Circulation Vol.3 (eds. W.F. Hamilton and P. Dow) Amer. Physiol. Soc. Washington D.C.

Margolis, S. and Vaughan, M. (1962) J. biol. Chem. 237, 44.

Marinetti, G.V., Erbland, J.F. and Brossard, M. (1964) In Metabolism and Physiological Significances of Lipids (eds. R.M.C. Dawson and D.N. Rhodes), Wiley, New York. p.71.

Marinetti, G.V. (1970) In Comprehensive Biochemistry : Lipid Metabolism (eds. M. Florkin and E.H. Stortz), Elsevier, Amsterdam. p.117.

Mayes, P.A. and Felts, J.M. (1966) Biochem. J. 99, 43p.

Micheli, H., Weiswange, A., Pometta, D., Lewis, B., Chait, A., Oakley, C., Krikler, D., Mancini, M., Orienti, P., Paggi, E., Carlson, L.A., Eriksson, M. and Boberg, J. (1973) Europ. J. clin. Invest. 3, 255.

Miettinen, M., Turpeinen, O., Karvonen, M.J., Elosuo, K. and Paavilainen, E. (1972) Lancet II, 835.

Morrison, L.M., Hall, L. and Chaney, A.L. (1948) Amer. J. med. Sci. 216, 32.

McBride, O.W. and Korn, E.D. (1964) J. Lipid Res. 5, 459.

Nestel, P.J., Havel, R.J. and Bezman, G. (1963) J. clin. Invest. 42, 1313.

Nestel, P.J. and Austin, W. (1969) Life Sci. 8, 157.

Neutra, M. and Leblond, C.P. (1966) J. Cell Biol. 30, 137.

Nikkilä, E.A. and Kekki, M. (1971) Scand. J. clin. Lab. Invest. 27, 97.

Ockner, R.K., Hughes, F.B. and Isselbacher, K.J. (1969a) J. clin. Invest. 48, 2079.

Ockner, R.K., Hughes, F.B. and Isselbacher, K.J. (1969b) J. clin. Invest. 48, 2367.

Olefsky, J., Farquhar, J.W. and Reaven, G.M. (1974) Europ. J. clin. Invest. 4, 121.

Olivecrona, T. (1962) J. Lipid Res. 3, 439.

Olivecrona, T. and Belfrage, P. (1965) Biochim. Biophys. Acta 98, 81.

Patterson, D. and Slack, J. (1972) Lancet I, 393.

Porte, D., Jr. and Bierman, E.L. (1969) J. Lab. clin. Med. 73, 631.

Redgrave, T.G. (1970) J. clin. Invest. 49, 465.

Registrar General (1971) Statistical Review of England and Wales. Stationery Office.

Reiser, R., Williams, M.C., Sorrels, M.F. (1960) J. Lipid Res. 1, 241.

Rifkind, B.M., Lawson, D. and Gale, M. (1968) J. Atheroscler. Res. 8, 167.

Robinson, D.S. and French, J.E. (1960) Pharmacol. Rev. 12, 241.

Robinson, D.S. and Seaknis, A. (1962) Biochim. Biophys. Acta 62, 123.

Robinson, D.S. (1963) Adv. Lipid Res. 1, 133.

Robinson, D.S. (1970) In Comprehensive Biochemistry: Lipid Metabolism (eds. M. Florkin and E.H. Stortz), Elsevier, Amsterdam, p.51.

Robinson, D.S. and Wing, D.R. (1970) In Adipose Tissue: Regulation and Metabolic Functions (eds. B. Jeanrenaud and D. Hepp) Thieme, Stuttgart, p.41.

Robinson, D.S. (1973) J. clin. Path. 26, Suppl. 5, 5.

Rodbell, M. (1964) J. biol. Chem. 239, 753.

Rosenman, R.A., Friedman, M., Straus, R., Jenkins, C.D., Zyzanski, S.J. and Wurm, M. (1970) J. chron. Dis. 23, 173.

Ruderman, N.B., Richard, K.C. Valles de Bourges, V. and Jones, A.L. (1968) J. Lipid Res. 9, 613.

Ryan, W.G. and Schwartz, T.B. (1965) Metabolism, 14, 1243.

Sailer, S., Sandhofer, F., and Braunsteiner, H. (1966) Klin. Wschr. 44, 1032.

Scanu, A.M. (1972) Biochim. Biophys. Acta 265, 471.

Schaefer, L.E. (1964) Amer. J. Med. 36, 262.

Schlierf, G. and Raetzer, H. (1972) Nutrit. Metab. 14, 113.

Schoefl, G.I. and French, J.C. (1968) Proc. Roy. Soc. (London) B169, 153.

Schumaker, V.H. and Adams, G.H. (1969) Ann. Rev. Biochem. 38, 113.

Scow, R.O., Hamosh, M., Blanchette-Mackie, E.J. and Evans, A.J. (1972) Lipids 7, 497.

Stamler, J., Lindburg, H.A., Berkson, D.M., Schaffer, A., Muller, W. and Poindexter, A. (1960) J. chron. Dis. 11, 405.

Stamler, J. (1973) Med. Clin. Nth. Amer. 57, 5.

Stein, Y. and Shapiro, B. (1957) Biochim. Biophys. Acta. 24, 197.

Stein, Y. and Shapiro, B. (1960) J. Lipid Res. 1, 326.

Stein, O. and Stein, Y. (1965) Israel J. med. Sci. 1, 378.

Steinberg, D. (1962) In Fat as a Tissue (eds. K. Rodahl and B. Issekutz) McGraw-Hill, New York. p.127.

Steinberg, D. (1972) Circulation 45, 247.

Stone, M.C. and Thorpe, J.M. (1966) Clin. chim. Acta 14, 812.

Suter, E.R. and Majno, G. (1963) J. Cell. Biol. 27, 163.

Tzur, R. and Shapiro, B. (1964) J. Lipid Res. 5, 542.

Van Harken, D.R., Brown, T.O. and Heimberg, M. (1967) Lipids 2, 231.

Van Harken, D.R., Dixon, C.W. and Heimberg, M. (1969) J. biol. Chem. 244, 2278.

Williamson, J.R. (1964) J. Cell Biol. 20, 57.

Windmueller, H.G. and Spaeth, A.E. (1967) Arch. Biochem. 122, 362.

Wing, D.S., Salaman, M.R. and Robinson, D.S. (1966) Biochem. J. 99, 648.

Wing, D.R. and Robinson, D.S. (1968) Biochem. J. 106, 667.

Wisse, E.J. (1970) Ultrastructure Res. 31, 125.

CHAPTER II

METHODS

CHAPTER II

1. BLOOD SAMPLING

All venous blood samples for fasting lipids and lipoproteins were collected after a 12 - 14 hour overnight fast. Venous stasis was avoided as this is known to lead to errors due to haemoconcentration (Koerselman et al, 1961).

In studies in which repeated blood samples were required, subjects were cannulated with polyethylene catheters (A-caths 1968 or 1962 Baxter, England) fitted with a three-way stopcock. In those studies in which sampling was frequent, no special precautions were taken to prevent clotting in the cannula. When a longer period was required between samples, saline was injected to keep the cannula free of blood. In all cases, 1 ml. of blood was withdrawn and discarded before the actual sample was taken.

After allowing time for clotting and retraction, serum was separated by centrifugation at 1,500 rpm at 4°C. Samples for lipid estimations were stored at -20°C in cases in which estimations were not performed immediately.

Samples for ultracentrifugation were mixed with ethylene diamine tetra-acetic acid (EDTA) to a final concentration of 0.05% and kept at 4°C until spun. Ultracentrifugation was always commenced within 48 hours of sampling. Fractions obtained by ultracentrifugation were stored at -20°C before analysis.

Serum for lipoprotein electrophoresis was stored at 4°C and run within 72 hours of sampling.

2. DETERMINATION OF SERUM TRIGLYCERIDE BY A SEMI-AUTOMATED METHOD

The method used was essentially that of Cramp and Robertson (1968), with triolein standards replacing trilaurin and trimyristin. It has also been found satisfactory for use with lipoprotein fractions separated by ultracentrifugation. The method is based on extraction of lipid from the serum sample, followed by the liberation of the glycerol moeity of triglyceride by alkaline hydrolysis. The released glycerol is oxidized to formaldehyde which is measured fluorimetrically after condensation with acetyl acetone in the presence of ammonia. It has been shown to agree with the Van Handel and Zilversmit (1957) manual reference method (Cramp and Robertson, 1968).

Procedure

A 1:20 dilution of serum in isopropanol (BDH Biochemical Reagent, England) prepared with a Hooke and Tucker autodilator (0.3 ml. to 5.7 ml.), is allowed to stand overnight in the presence of Zeolite containing copper sulphate (Technicon, U.S.A.). The latter reagents are necessary for the removal of phospholipid and glucose, both of which may cause false readings. In the flow system of the Technicon autoanalyzer, the triglyceride is hydrolyzed by IM KOH, the released glycerol oxidised to formaldehyde, and finally condensed with acetylacetone in the presence of excess ammonium ions to form diacetyldihydrolutidine in a 58°C constant temperature water bath. After activation at 405 nm., the product emits fluorescence at 510 nm., the increase in fluorescence being measured on a Mark V Locarte fluorimeter (Locarte Co., London) coupled to a 10 mv. recorder.

The standard used was triolein (Sigma Chemical Co. Ltd., U.S.A. 99% pure). Purity was checked by thin layer chromatography; only one spot was detected. Each group of 40 estimations had a set of six standards on either side of it. Four pooled samples and four drift checks are included in the 40 samples. As the same isopropanol extracts of serum were also used for cholesterol determinations, the triglyceride stock standard also contained cholesterol (BDH Biochemical Reagents, England).

A typical standard curve is shown in Figure 1.

Reproducibility experiment

A serum sample was divided into 10 aliquots, and its triglyceride content determined on 10 separate occasions.

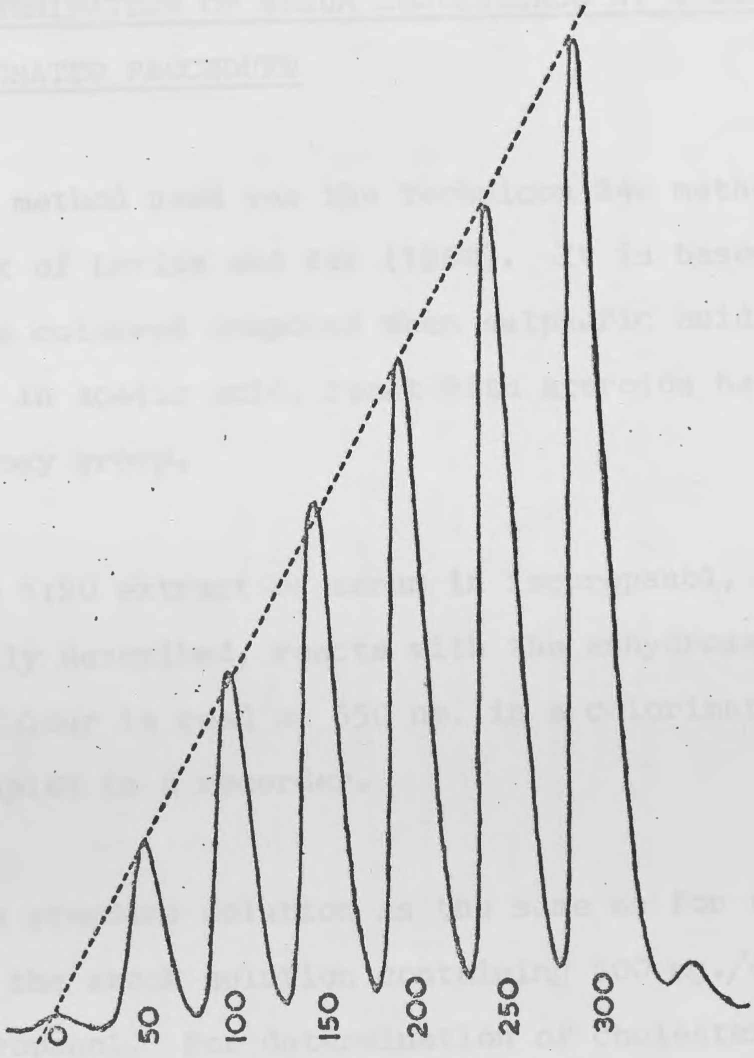

Fig.1. Triglyceride standard curve

Triglyceride concentration = 160 mg./dl. ± 7 (SD)

Coefficient of variation = 4.6%

3. DETERMINATION OF SERUM CHOLESTEROL BY A SEMI-AUTOMATED PROCEDURE

The method used was the Technicon 24a method, modified from that of Levine and Zak (1964). It is based on the formation of a coloured compound when sulphuric acid and ferric chloride in acetic acid, react with steroids having the Δ 5,1 hydroxy group.

The 1:20 extract of serum in isopropanol, prepared as previously described, reacts with the anhydrous reagent at $95^{o}C$. Colour is read at 550 nm. in a colorimeter with a flow cell coupled to a recorder.

The standard solution is the same as for the triglyceride method, the stock solution containing 500 mg./dl. of cholesterol in isopropanol. For determination of cholesterol in lipoprotein fractions which contain bromide, the latter must be removed as it interferes with colour development. This is achieved by the addition of Dowex 2 ion exchange resin (Dow Chemical Co., U.S.A.) in the chloride form, to the isopropanol extract.

A typical standard curve is shown in Figure 2.

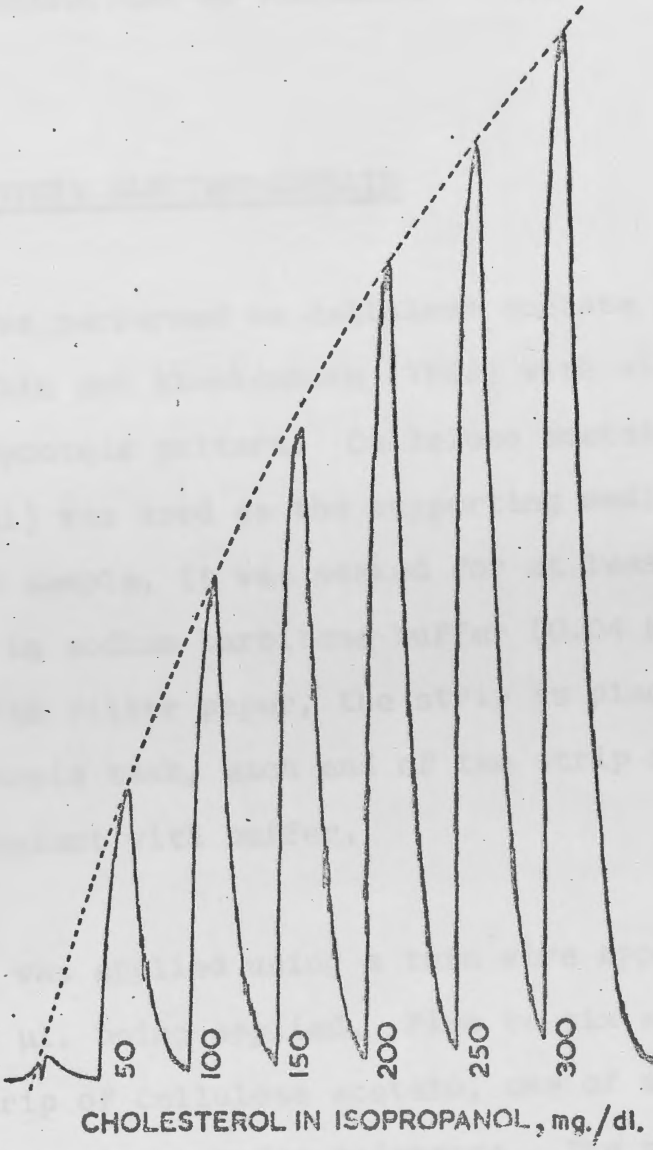

Fig. 2 Cholesterol standard curve

Reproducibility experiment

The cholesterol concentration of a sample of serum was determined on 10 separate occasions.

Cholesterol concentration = 554 mg./dl. ± 10 (SD)

Coefficient of variation = 1.7%

4. LIPOPROTEIN ELECTROPHORESIS

This was performed on cellulose acetate according to the method of Chin and Blankenhorn (1968) with visual inspection of the lipoprotein pattern. Cellulose acetate (Cellogel, Reeves Angel) was used as the supporting medium. Before application of the sample, it was soaked for at least 10 minutes in 1% albumin in sodium barbitone buffer (0.04 M, pH 8.6). After blotting with filter paper, the strip is placed on a Shandon electrophotesis tank, each end of the strip resting on filter paper in contact with buffer.

Serum was applied using a twin wire applicator (Chemetron, Italy) 1.5 µl. being applied. Five to six samples could be run per strip of cellulose acetate, one of which was always a normal control serum for reference. The samples were electrophoresed at a constant current of ½ mA/cm. for 50 minutes.

Staining of the lipid was performed using Oil red O staining solution, prepared by refluxing 40 mg./dl. Oil red O (Allied Chemical, U.S.A.) with 70% methanol:water. The stain can be kept for one week at 37°C. After overnight staining at 37°C, the strips were rinsed free of excess stain in tap water and briefly immersed in a solution of hypochlorous acid, freshly prepared by adding 5 ml. of sodium hypochloride solution (BDH 10 - 14% available chlorine) to 100 ml. of 5% $^{v}/v$ acetic acid. This treatment causes considerable bleaching of the background. Using this technique with cellogel, resulted in chylomicrons remaining at the point of application.

5. LIPOPROTEIN ULTRACENTRIFUGATION

Preparative ultracentrifugation of lipoproteins was performed according to the method of Carlson (1973), based upon that of Havel et al (1955).

Apparatus

M.S.E. Superspeed 50 preparative ultracentrifuge (Measuring and Scientific Instruments Equipment, Sussex, England).

Anglehead Rotor 18 x 6.5 ml. (M.S.E. no. SP 909. Identical in radius, capacity and 26° angle to the Beckman 40.3 rotor used in the method of Havel et al (1955) and of Hatch and Lees, 1968).

Tube-slicer (Beckman - Spinco, U.S.A.).

Cellulose nitrate centrifuge tubes (0.5" x 2.5" - Beckman - Spinco, U.S.A.).

VLDL separation

This was achieved as follows: 4 ml. of serum were transferred to 6.5 ml. Beckman cellulose nitrate centrifuge tubes. 2 ml. saline, density 1.006, prepared by making 11.4 g.NaCl, 0.1 g. EDTA and 1 ml. 1M NaOH up to 1 litre with water and adding an additional 3 ml. was carefully layered on top of the serum. The samples were centrifuged at 105,000 g for 16 hours at 15°C leading to an opalescent upper layer containing the VLDL, a clear saline layer, and the residual contents of the serum as an infranatant. The VLDL was removed by tube slicing and both the tube cap and the tube slicer were carefully rinsed with saline, density 1.006.

LDL separation

4 ml. of the infranatant after tube slicing, was transferred to another cellulose nitrate tube and 2 ml. of a NaCl - NaBr solution were added to increase the density to 1.182. This solution was prepared by adding 24.98 g.NaBr to 100 ml. of the d = 1.006 solution. After capping, mixing was achieved by inversion, following which the samples were centrifuged for 20 hours at 105,000 g. The supernatant containing the LDL was separated by tube slicing. The infranatant contains HDL and other serum proteins.

Chylomicron separation

In samples in which a creamy layer was observed to float upwards following overnight storage at 4°C, or in studies on alimentary lipaemia, separation of chylomicrons (corresponding to particles with $S_f > 400$) was performed prior to VLDL separation. This was achieved by spinning 4 ml. of serum under a 2 ml. layer of saline density 1.006 for 30 minutes at 17,500 rpm. The opalescent upper layer was removed by tube slicing and rinsing of the tube cap and slicer, the bottom layer being made up to 5 ml., of which 4 ml. were used for VLDL separation.

The fractions so obtained were extracted and triglyceride and cholesterol contents determined as described above. In addition, total serum cholesterol and triglyceride were measured and recoveries calculated. Recoveries of between 90 - 110% were regarded as acceptable.

Recovery experiment

The total triglyceride and cholesterol concentration in six samples of serum were compared with the sums of the fractions separated by ultracentrifugation.

Triglyceride Recovery = 101% ± SD 5
Cholesterol Recovery = 103% ± SD 3

Preparation of three fractions of VLDL from serum

Gustafson et al (1966) reported a method for separation of up to five sub-fractions of the VLDL fraction (including chylomicrons). In some experiments, three such fractions were collected as follows:

4 ml. of serum was layered under 2.5 ml. of d = 1.006 solution in a cellulose nitrate ultracentrifuge tube. After capping, it was centrifuged for 30 minutes at 17,500 rpm (0.66×10^6 g-minutes). The lipoprotein fraction equivalent to the $S_f > 400$ fraction floats to the top.

The top fraction (of about 1.5 ml. volume) was separated as before and made up to 2 ml. volume.

The infranatant is quantitatively washed into 5 ml. volumetric flask, from which 4 ml. are pipetted into a fresh centrifuge tube and layed under 2.5 ml. of d = 1.006 saline. Samples are then spun at 35,000 rpm for 60 minutes (5.4×10^6 g-minutes) to yield a fraction equivalent to S_f 100 - 400.

The fraction is collected as above, and the procedure repeated with a further spin of 16 hours at 40,000 rpm to separate the S_f 20 - 100 sub-fraction.

0.3 ml. of the fraction collected are taken for determination of cholesterol and triglyceride content as described on pages 41 and 44 .

6. PLASMA FREE FATTY ACID CONCENTRATION

This was measured by the method of Goss and Lein (1967) which is a one-phase modification of the Dole (1956) two-phase method. Venous blood was drawn after a 12 - 14 hour overnight fast from the recumbent subject who had been resting for 20 - 30 minutes prior to venesection. Smoking was not permitted on the morning of the study. The blood was taken into heparinized tubes, immediately placed in ice, and centrifuged at 1,500 rpm at 4°C for 10 minutes. One ml. aliquots of the plasma so obtained were added to 5 ml. Dole extraction mixture, consisting of isopropanol; n-heptane; 0.5 M sulphuric acid, 20:5:1, and mixed for four minutes on a vortex mixer. A further 3 ml. heptane, 2 ml. distilled water (which had been recently boiled to remove the dissolved CO_2) were added and the mixture mixed for a further four minutes. On standing upright overnight, two phases separated by a sharp interface are obtained; 3 ml. of the upper heptane phase were removed for titration. All solvents used were either BDH 'Analar' grade or redistilled. Standards consisted of a range of concentrations of palmitic acid (Sigma Chemical Co., U.S.A., 99% pure) in heptane after having been shown to be chromatographically pure using thin layer chromatography. For the standards, 2 ml. of heptane and 3 ml. of water were added after the Dole solution, to adjust for solvent differences. Blanks, containing water instead of plasma, were also used.

Titration of the heptane phase against ethanolic sodium hydroxide (0.016 M), using 0.5 ml. ethanolic thymol phthalein (0.05%) as indicator, resulted in a one-phase system because of the miscibility of the heptane and ethanol. Titration was carried out using an Algar glass microsyringe coupled to a micrometer screw gauge (Burroughs Wellcome and Co., England), mixing being achieved by a continuous stream of nitrogen which had been rendered CO_2 free by passage through a KOH solution.

The colour change for the standard is from colourless to blue, while that for plasma samples is from pale yellow to pale green.

After substraction of blanks from readings for the samples and standards, a standard curve was drawn on each occasion; the sample value for FFA were determined from this. A typical standard curve is shown in Figure 3.

7. PLASMA FFA TURNOVER

The method is based on that of Lewis et al (1966) in which FFA flux through plasma is determined by measuring the equilibrium specific activity of FFA in plasma during a constant infusion of ^{14}C albumin-bound palmitate.

Preparation of the isotope for injection

Palmitic acid of specific activity $55.2\,\mu ci/M$ (Radiochemical Centre Amersham, Bucks.), after blowing off the benzene carrier, was dissolved in 3 ml. of ethanol, adjusted to pH 8 with potassium hydroxide, diluted to 10 ml. with 0.15 M saline. It was then autoclaved at 15 psi for 15 minutes. Radiochemical purity in the product was checked by thin layer chromatography and sterility of the product confirmed by culture. The preparation was stored at $-20^{o}C$ until the morning of use.

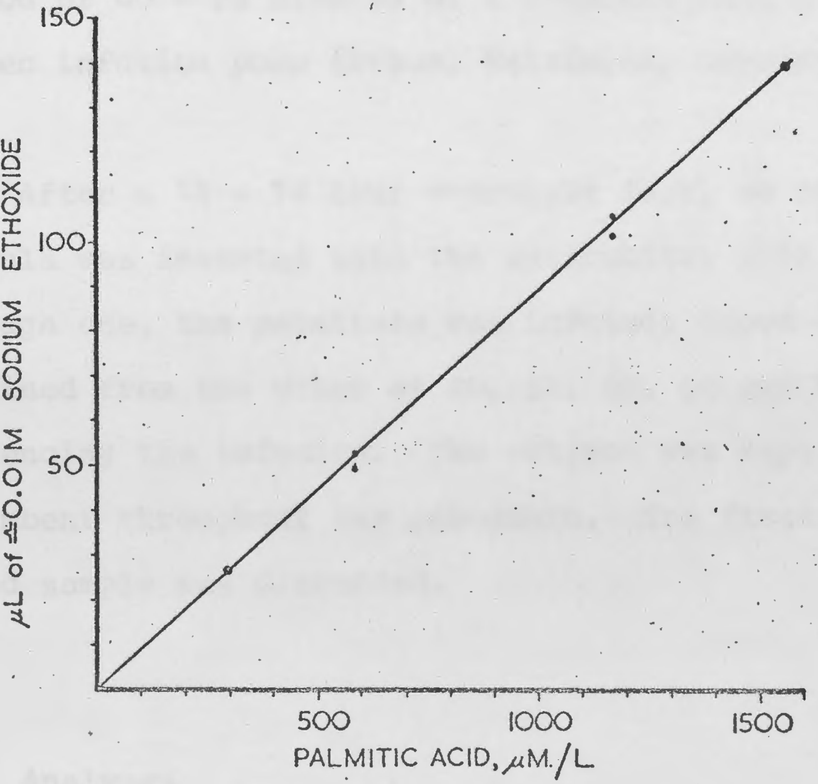

Fig. 3. Free fatty acid standard curve

Procedure

On the morning of the study, approximately 15 µci of the palmitate was added slowly, with swirling, to a solution containing 0.25 g. of fatty acid poor albumin (Lister Institute, Elstree, Herts.). Electrophoresis showed all the radioactivity to travel with the albumin band. The complex was diluted with 0.15 M saline and about 10 µci infused intravenously over a period of 60 - 70 minutes at a constant rate using an electric driven infusion pump (Braun, Melsingen, Germany).

After a 12 - 14 hour overnight fast, an indwelling plastic cannula was inserted into the anticubital vein of each arm. Through one, the palmitate was infused; blood samples were obtained from the other at 20, 30, 40, 50 and 60 minutes after commencing the infusion. The subject was kept quiet and recumbent throughout the procedure. The first 1 ml. of each blood sample was discarded.

Analyses

Part of each sample was used for determination of FFA levels as previously described. A further aliquot was used to determine radioactivity in the FFA fraction after separation on silica-potassium columns by the method of McCarthy and Duthie (1962). This separation of FFA is necessary because of the appearance of endogenously labelled triglyceride from about 20 minutes (see Chapter V, page 286). No significant labelling of cholesterol esters or phospholipid was shown to occur during the first hour of infusion.

The absorbant for the columns was prepared as follows: 45 g. of Silicic acid (Malinkrodt 100 mesh, Malinkrodt Chemical Co., U.S.A.) was suspended in methanol and the finer particles decanted off. Ten per cent potassium hydroxide in methanol was then added and the mixture evaporated to dryness under reduced pressure using a rotary evaporator; the dry powder which contains approximately 20% $^{W}/v$ KOH in silicic acid, was stored in an airtight container. Columns consisting of 13 x 1 cm. glass columns fixed at one end to 100 ml. round-bottomed flasks, and at the other end to glass sintered discs, to allow a flow rate of 30 ml./hour with diethyl ether, and 2 g. of silica KOH, were used.

Into each column was slurried 2 g. of the silica KOH powder in diethyl ether (BDH 'Analar'). The Dole extract was evaporated to dryness, dissolved in 2 ml. of ether and applied to the column. Fifty ml. of ether eluted the neutral lipids. FFA were eluted by 50 ml. of 4% formic acid in ether followed by 100 ml. of ether. The FFA elvates were dried in a rotary evaporator then transferred quantitatively to counting vials with ether. After evaporation to dryness, counting in a liquid scintillation spectrometer with quench correction by an internal standard of ^{14}C-n hexadecane (Radiochemical Centre, Amersham, Bucks.) was performed using 10 ml. of a scintillation solvent with the following composition: Toluene 2½ litres (BDH A.R.); 2,5 - diphenyloxazole (Packard) 10 g.; 1-4-bis/2-(4-methyl-5-Phenyloxazolyl)7 Benzene (Packard) 1.25 g. Counting efficiency was never less than 70%.

Specific activity of FFA in plasma was then calculated for the 20, 30, 40, 50 and 60 minute samples and shown in all cases to have achieved equilibrium by 40 minutes. In many cases, equilibrium had been reached by 20 - 30 minutes. (A typical result is shown in Figure 4). The equilibrium specific activity was derived by taking the mean of the 40, 50 and 60 minute samples.

Recovery experiment

(a) Free fatty acid

^{14}C palmitic acid was diluted in a Dole extract of plasma (to provide carrier FFA). Aliquots were taken for either counting or were applied to the column and counted after elution as described above.

Result = 94% ± SD 4 (n = 6)

(b) Triglyceride

A similar experiment was performed using ^{3}H triolein (Amersham Radiochemical Centre, Amersham, Bucks.). Aliquots were counted before and after recovery of the neutral lipid fraction.

Result = 96% ± SD 3 (n = 5)

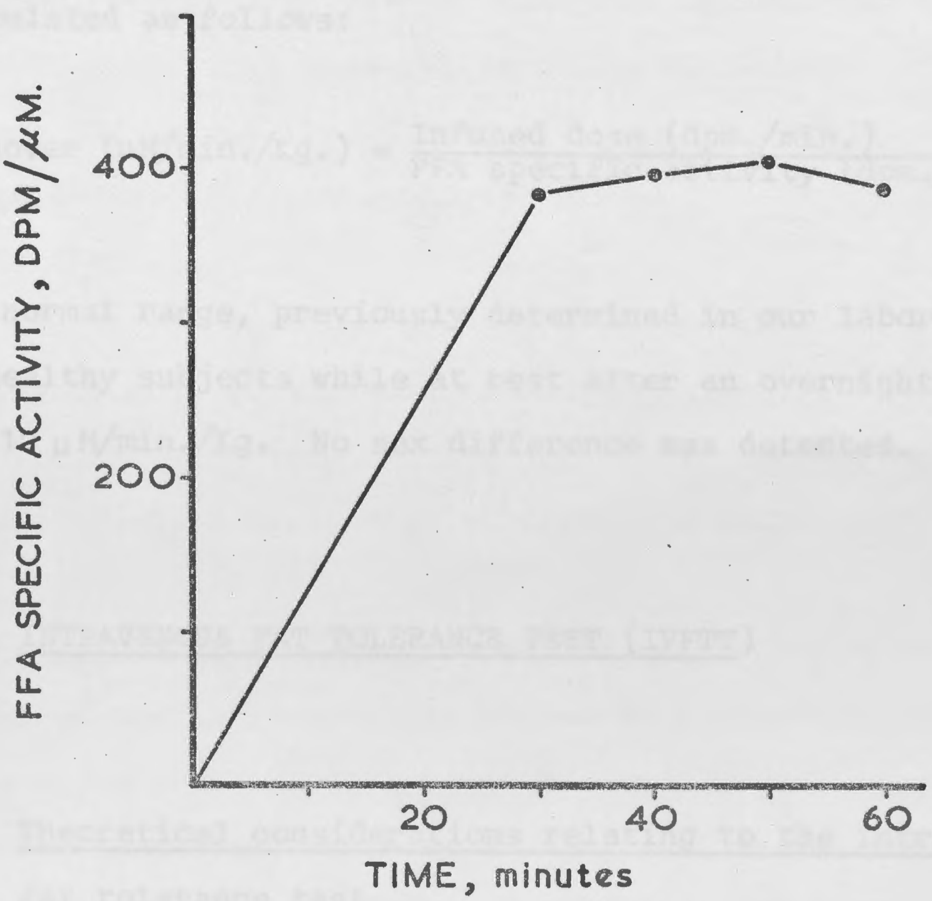

Fig. 4. Plasma FFA specific activity during infusion of ^{14}C palmitate

Calculation of FFA turnover

To calculate the infused dose (dpm./ml.), 50 µl. of the infusate was evaporated in a counting vial, and counted in a similar manner to the plasma samples. The rate of infusion was obtained by measuring the volume of a precisely timed sample of the infusion. FFA turnover through plasma was then calculated as follows:

$$\text{Turnover } (\mu\text{M/min./Kg.}) = \frac{\text{Infused dose (dpm./min.)}}{\text{FFA specific activity (dpm./}\mu\text{M x weight (Kg.)}}$$

The normal range, previously determined in our laboratory in 12 healthy subjects while at rest after an overnight fast, was 6 - 12 µM/min./Kg. No sex difference was detected.

8. INTRAVENOUS FAT TOLERANCE TEST (IVFTT)

Theoretical considerations relating to the intravenous fat tolerance test

Tolerance tests have the advantage of being able to detect latent and mild metabolic abnormalities. Thus, glucose intolerance can be detected with the oral glucose tolerance test or its modifications. Similarly, oral fat tolerance tests have been used to attempt to detect mild or latent abnormalities of lipid metabolism. For example, Angervall (1964) showed alimentary lipaemia to be of a greater degree and more prolonged in subjects with atherosclerosis than in normal controls. The need for an intravenous fat tolerance test arose because of difficulties in the interpretation of oral fat tolerance tests; this is largely due to the many processes influencing alimentary lipaemia, such as gastric emptying, and intestinal absorption. Furthermore, the nature of the lipoprotein carrier depends to some extent on the fat load ingested (see Chapter VI); as VLDL and chylomicrons have been shown to be removed from the circulation at different rates (French and Morris, 1957; Nestel, 1964), the amount of fat ingested could influence removal rates.

The IVFTT developed by Boberg et al (1969) is a loading test, analogous with the intravenous glucose tolerance test. It involves a non-steady state and depends on the removal of an exogenously administered triglyceride emulsion from plasma.

Early studies on the removal of triglyceride from plasma relied on the intravenous injection of labelled chylomicrons (French and Morris, 1957; Borgström and Jordan, 1959; Havel and Goldfein, 1961). Due to the instability and difficulty of standardization of chylomicrons, which are also difficult to collect in any quantity, especially so in man, the removal of synthetic triglyceride emulsions has been studied. The emulsion found to be most suitable for the purpose, and which has been used by Boberg et al (1969) in their intravenous fat tolerance test, is commercially available Intralipid (Vitrum, Stockholm). It has been shown to closely resemble chylomicrons in respect of both particle size and kinetic behaviour. Hallberg and Wersall (1964) have shown Intralipid particles to resemble chylomicrons in particle size under the electron microscope. In dogs, their rates of elimination are the same (Carlson and Hallberg, 1963); similar observations have been made in man (Hallberg, 1965a and b). When compared as substrates for post-heparin lipoprotein lipase activity, the kinetics of Intralipid and chylomicrons, are remarkably similar (Boberg and Carlson, 1964).

The injected triglyceride was originally separated from endogenous triglyceride on a polyvinylpyrrolidone (PVP) gradient. This method, initially described by Gordis (1962), is capable of separating chylomicron containing plasma into exogenous and endogenous fractions; it depends on aggregation of lipoprotein molecules and subsequent flotation, the latter process being size dependent. Hallberg (1964) using such columns with plasma obtained following the intravenous injection of Intralipid, showed that the triglyceride emulsion could be made to separate into a top phase, whereas endogenous triglycerides were contained in a middle phase. The advantage of the PVP gradient method over total plasma triglyceride determination is that endogenous triglyceride, probably including recirculated secondary particles, is separated from exogenous by the former method. Separation on PVP columns is, however, very time consuming and Lewis et al (1972) described a modification of the intravenous fat tolerance test in which the disappearance of the injected Intralipid was measured nephelometrically. This was shown to correlate well with values obtained using the PVP gradient method, and has the distinct advantage of being much quicker and easier to perform. The nephelometric method was used in all the investigations described in this thesis.

Whereas intravenous glucose tolerance is defined by only one rate which characterizes a first order reaction, two rate constants have been shown to be measurable with the intravenous fat tolerance test (Boberg et al, 1969). Following administration of a dose of 0.1 g. Intralipid per kilogram body weight to man, disappearance of the triglyceride follows an exponential slope, thus exhibiting first order kinetics. Serum triglyceride concentrations following the injection of this dose usually range between 100 and 150 mg./dl. When the intravenous fat tolerance test is carried out at the higher dose of 0.25 - 0.3 g. per kilogram body weight, disappearance is initially linear, followed by exponential removal. Saturation of the removal mechanism is implied, because a constant amount of triglyceride is removed per unit time. The slope of the zero order part of the removal curve then gives a measure of maximal removal capacity (K_1), in contradistinction to the fractional removal rate (K_2), measured by the later part of the curve.

Nikkilä and Kekki (1971a) have criticized the intravenous fat tolerance test on the grounds that removal of a fat emulsion is not a valid indicator of the removal of endogenous triglyceride. Although, as discussed above, Intralipid appears to be a good kinetic model for chylomicrons, the latter triglyceride bearing lipoprotein is removed from plasma at a much more rapid rate than endogenous triglycerides. Both chylomicrons and VLDL are, however, believed to be removed from plasma by a common mechanism (Havel, 1965). Further evidence of chylomicrons and VLDL sharing a common saturable removal mechanism has more recently been presented by Brunzell et al

(1973). It has, therefore, been suggested that the IVFTT may be used as a method to study the removal of endogenous plasma triglyceride (Boberg et al, 1969; Carlson and Rössner, 1972; Rössner et al, 1974).

The validity of the test is suggested by several points. Boberg et al (1969) have shown that in a population of normolipaemic and hypertriglyceridaemic subjects, K_2 values and serum triglyceride concentrations were inversely related, showing a hyperbolic relationship. A similar finding in a population of normolipaemic subjects is shown in Figure 9 on page Values obtained for fractional triglyceride turnover by chemically measuring arteriovenous differences across the splanchnic bed showed a similar relation to serum triglyceride concentration as was observed for K_2 (Boberg, 1971). This non-isotopic method has, however, been criticised on the basis of analytical error (Nikkilä and Kekki, 1971a).

Nevertheless, a significant positive correlation was shown to exist by Rössner et al (1974) when fractional turnover rate constants determined by the IVFTT were compared with those determined by the chemical plasma triglyceride secretion method ($r = +0.6$; $p < 0.01$) and by the plasma triglyceride clearance method ($r = +0.7$; $p < 0.001$) in the same subjects. Values obtained with the IVFTT were, however, about 30% higher. Unfortunately, in some cases there was a long time interval between the studies, and triglyceride concentrations were not always equal.

The low turnover of triglyceride in the presence of high triglyceride levels showed in these studies, may be interpreted as suggesting defective triglyceride removal from plasma to be the mechanism of the hypertriglyceridaemia.

Alternatively, it could be argued that K_2 values reflect plasma triglyceride pool size, high triglyceride concentrations resulting in low K_2 values, and vice versa. The experiments of Nestel (1964), on removal of chylomicrons from plasma, are frequently quoted in support of this latter concept, and Nikkilä and Kekki (1971a) have further criticised the intravenous fat tolerance test on this basis. It has been suggested that any change in serum triglyceride concentration leads to a reciprocal change in K_2, even if the change in triglyceride is due to enhanced secretion. The highly significant correlation with fractional triglyceride turnover measured chemically by arteriovenous difference across the splanchnic bed, however, mitigates strongly against this argument (Rössner et al, 1974). K_2 has also been shown to be independent of the amount of exogenous (Hallberg, 1965b), or endogenous triglyceride (Hallberg, 1965a; Boberg and Hallberg, 1968) during constant infusion of Intralipid at different rates.

Further evidence against K_2, as measured by the IVFTT, being a reflection of plasma triglyceride pool size, is the lack of change in K_2 value when serum triglyceride concentration rises during oral contraceptive therapy (Rössner et al, 1971), and during high carbohydrate feeding (Nestel and Barter, 1973). Conversely, Gustafson and Sannersfedt (1973) showed an increasing K_2 during drug treatment of hyperlipoproteinaemia to be unassociated with a change in serum triglycerides. Two further examples are presented in this thesis; two situations are described in which there is a change in plasma triglyceride pool size without a corresponding change in K_2. The marked reduction in serum triglyceride concentration achieved by withdrawing alcohol from patients with alcoholic hypertriglyceridaemia was not accompanied by a change in triglyceride fractional removal rates (see page 206), nor was the reduction in serum triglyceride concentration in response to substituting saturated by polyunsaturated dietary fat (see page 283). The substantial change in serum triglyceride concentrations (and hence presumably triglyceride pool size) in these two situations, without reciprocal change in K_2, further strengthens the argument against triglyceride pool size determining the value obtained with the IVFTT.

The significance of these values has been further tested in this thesis, by performing the IVFTT at the higher dose in some diabetics. In studies described on page 144, maximal removal rate (K_1) varied in a similar fashion to fractional removal rate (K_2) when the diabetes was controlled. If a constant plasma volume is assumed, K_1 is independent of pool size consideration, thus further strengthening the validity of the IVFTT.

In view of these considerations, the IVFTT does appear to be a useful and valid test, despite the actual half-life of the injected emulsion being more rapid than that of endogenous triglycerides. Perhaps its main advantage is the ease and rapidity with which it can be performed, with relatively little discomfort on the part of the subject undergoing the investigation. It provides a rapid and reproducible (Carlson and Rössner, 1972) semi-quantitative measure of fractional turnover, which correlates with, but is not equal to, the fractional turnover of VLDL triglyceride.

Method

The method used was that of Boberg et al (1969) adapted to nephelometric measurement (Lewis et al, 1972). In most cases, Intralipid was injected at a dose of 0.1 g./Kg. body weight; some subjects in addition, had the test repeated using 0.25 - 0.3 g./Kg. body weight.

Procedure

A pulse injection of ten per cent Intralipid (Vitrum, Stockholm) batch no. 199523 was administered intravenously via an indwelling polyethylene cannula, to subjects who had been fasted for 12 - 14 hours.

The cannula was then flushed by withdrawing blood to clear it of Intralipid. Blood samples were withdrawn at five minute intervals for a period of 40 minutes, in each case, 1 ml. first being withdrawn and discarded, to clear the cannula and tap off old blood. When the large dose of Intralipid was used, the test was carried on for 60 minutes.

The blood samples were taken into heparinized tubes, kept on ice and centrifuged for 10 minutes at 600 rpm to permit separation of the plasma, without permitting flotation of the triglyceride emulsion. 0.2 ml. of the plasma was diluted with 9.8 ml. 0.15 M saline using an autodilutor (Hook and Tucker, England). The test tubes used were carefully cleaned to eliminate dust particles which may contribute to light scattering. The light scattering index was then measured on a nephelometer (Scientific Furnishing Ltd., Poynton, Cheshire). Plasma taken before the injection of the Intralipid was used as a blank and subtracted from the sample readings.

Calculations of fractional removal rate (K_2)

As standard curves plotting the concentration of triglycerides against light scattering intensity, were shown to be straight lines passing through the origin (see Figure 5), the log of the light scattering indices minus the blanks were plotted directly against time. At the low dose of Intralipid, the removal was exponential in all cases measured. From the graph, the half life (T/2) was calculated and the fractional removal rate (K_2) derived as follows:

$$K_2 = \frac{0.693}{T/2}$$

A typical example is shown in Figure 6.

The only cases which failed to give an adequate curve were those subjects who were intensely lipaemic, having both chylomicrons

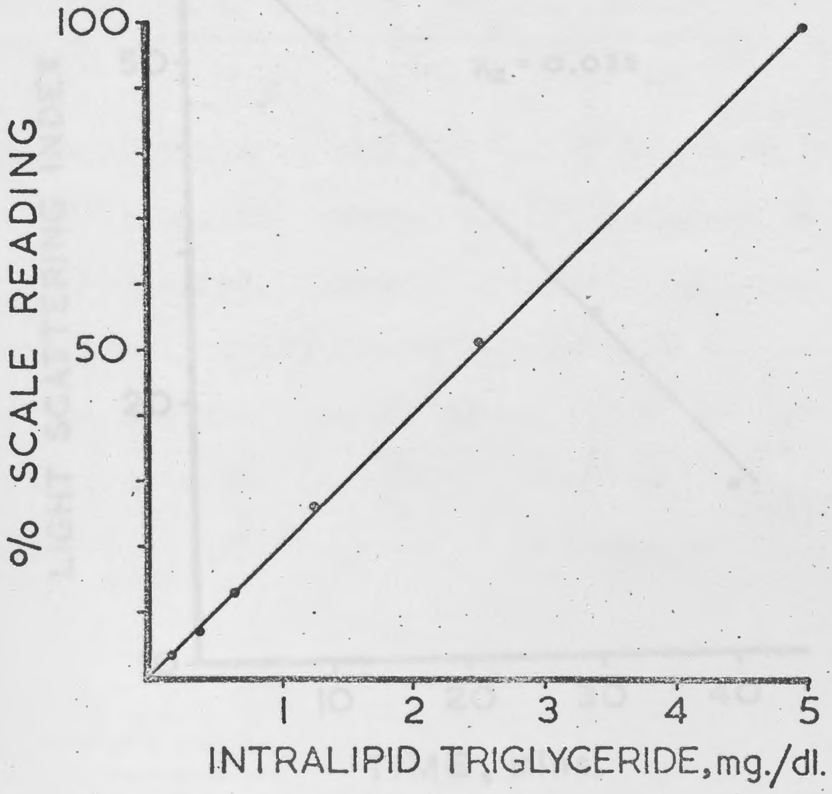

Fig. 5. Intralipid standard curve

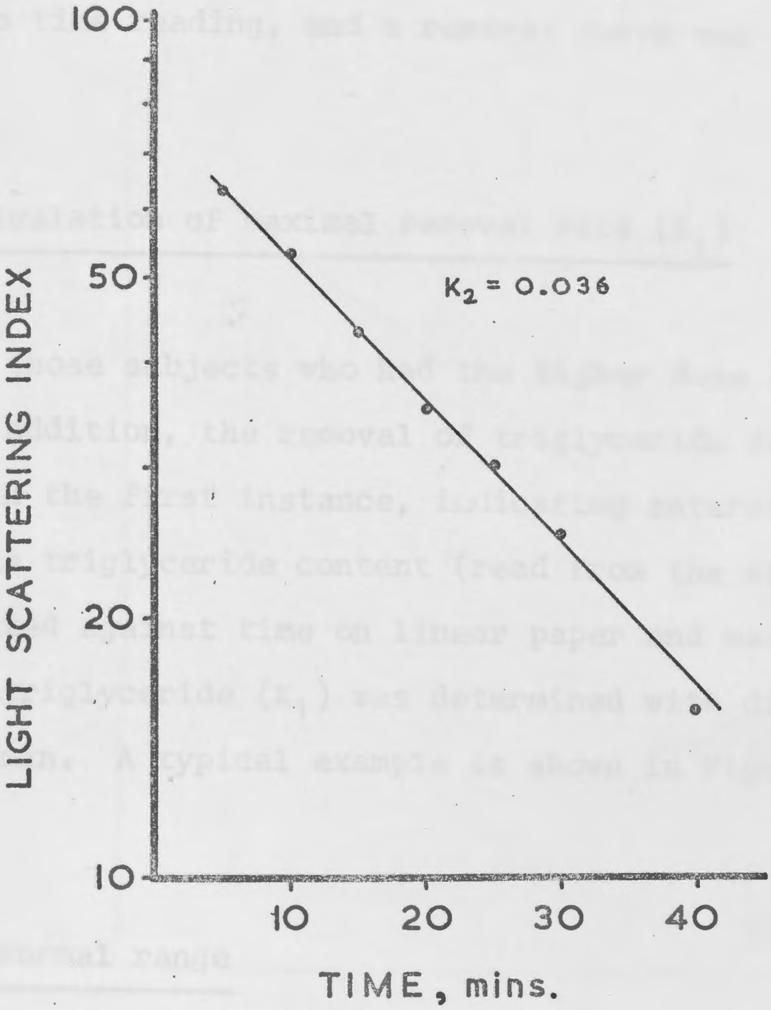

Fig. 6. Typical K_2

and VLDL in excess in the fasting state. In such cases, injection of the triglyceride emulsion which raised the triglyceride level by about 100 - 150 mg./dl., made little difference to the zero time reading, and a removal curve was unobtainable.

Calculation of maximal removal rate (K_1)

In those subjects who had the higher dose (0.25 - 0.3 g./Kg.) in addition, the removal of triglyceride from plasma was linear in the first instance, indicating saturation. The exogenous triglyceride content (read from the standard curve) was plotted against time on linear paper and maximal removal rate of triglyceride (K_1) was determined with dimensions of mg./dl./min. A typical example is shown in Figure 7.

K_2 Normal range

In order to establish a normal range for K_2, the IVFTT was performed in a sample of normal volunteers. The subjects included healthy members of hospital and laboratory staff (52 males, 9 females). The test was also performed on a sample of healthy, working volunteers at a factory; they were part of a larger survey performed in order to determine the frequency of hyperlipidaemia in a healthy British population (Lewis et al, 1974). To supplement the 60 - 69 year age group, retired members of the factory staff were examined. Of 276 volunteers for this study, the IVFTT was performed on 38 males and 26

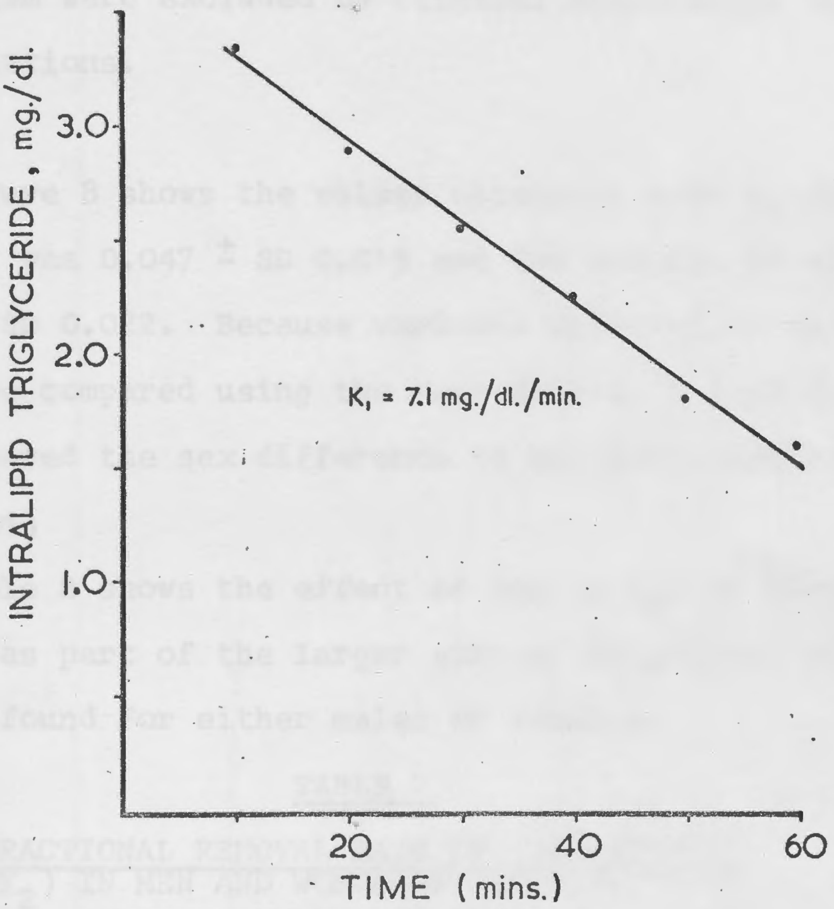

Fig. 7. Typical K_1

females selected randomly. All were habitually consuming 'typical' British diets; adherence to therapeutic diets such as reducing, diabetic or modified fat diets were grounds for exclusion. In these subjects, who were part of the larger survey, IHD, endocrine and other diseases known to affect lipid metabolism were excluded by clinical examination and appropriate investigations.

Figure 8 shows the values obtained; mean K_2 for men of all ages was 0.047 ± SD 0.015 and for females of all ages 0.074 ± SD 0.022. Because variance differed in the two sexes, they were compared using the Mann-Whitney U test (Siegel, 1956) which showed the sex difference to be statistically significant. ($p < 0.01$)

Table 2 shows the effect of age on K_2, in those subjects studied as part of the larger survey; no obvious change with age was found for either males or females.

TABLE 2

FRACTIONAL REMOVAL RATE OF TRIGLYCERIDE (K_2) IN MEN AND WOMEN OF DIFFERENT AGES

Age (yrs.)	Mean (min.$^{-1}$)	SD	SEM	n
Males				
20 - 29	0.054	0.018	0.007	6
30 - 39	0.046	0.014	0.005	8
40 - 49	0.050	0.024	0.009	7
50 - 59	0.042	0.009	0.003	9
60 - 69	0.047	0.010	0.004	6
Females				
20 - 29	0.113	0.073	0.033	5
30 - 39	0.076	0.022	0.008	7
40 - 49	0.072	0.022	0.011	4
50 - 59	0.071	0.033	0.012	7
60 - 69	0.076	0.096	0.005	3

The two standard deviation normal range for men is thus 0.017 - 0.077 min.$^{-1}$ and for women 0.030 - 0.118.

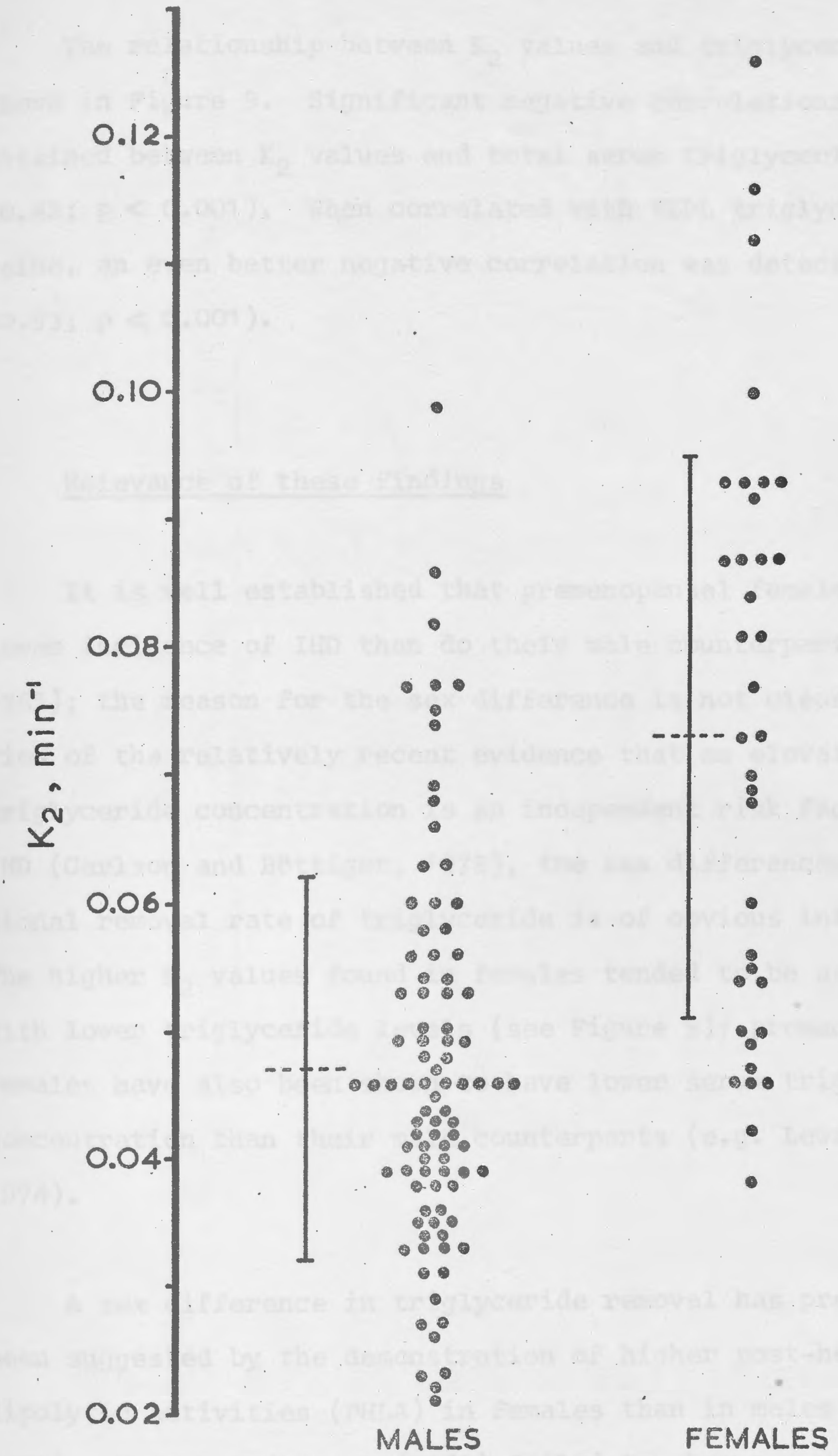

Fig. 8. K_2 in normal males and females (brackets indicate mean ± SD)

The relationship between K_2 values and triglycerides is shown in Figure 9. Significant negative correlations were obtained between K_2 values and total serum triglyceride ($r = -0.42$; $p < 0.001$). When correlated with VLDL triglyceride value, an even better negative correlation was detected ($r = -0.53$; $p < 0.001$).

Relevance of these findings

It is well established that premenopausal females have a lower incidence of IHD than do their male counterparts (Epstein, 1965); the reason for the sex difference is not clear. In view of the relatively recent evidence that an elevated serum triglyceride concentration is an independent risk factor for IHD (Carlson and Böttiger, 1972), the sex differences in fractional removal rate of triglyceride is of obvious interest. The higher K_2 values found in females tended to be associated with lower triglyceride levels (see Figure 9); premenopausal females have also been shown to have lower serum triglyceride concentration than their male counterparts (e.g. Lewis et al, 1974).

A sex difference in triglyceride removal has previously been suggested by the demonstration of higher post-heparin lipolytic activities (PHLA) in females than in males (Boberg, 1971). Nestel and Havel (1962) failed to show a sex difference in adipose tissue lipoprotein lipase, although Persson (1972) showed levels to be higher in women than in men, lending support to the present findings. Nikkilä and Kekki (1971b) who

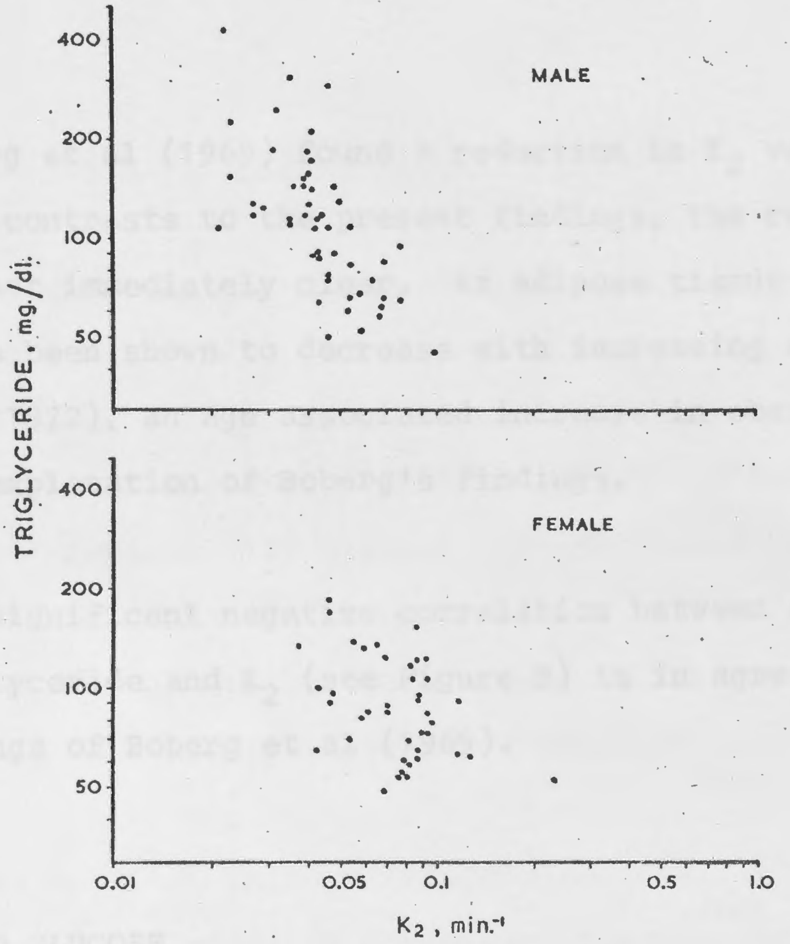

Fig. 9. Correlation between serum triglyceride and K_2

studied triglyceride turnover using infusions of labelled glycerol, showed females to have a more efficient plasma triglyceride removal system than males; their findings of equal production rates in the two sexes would then explain the lower plasma triglyceride concentration found in females. These findings have more recently been confirmed by Olefsky et al, (1974).

Boberg et al (1969) found a reduction in K_2 values with age; this contrasts to the present findings, the reason for which is not immediately clear. As adipose tissue lipoprotein lipase has been shown to decrease with increasing obesity (Persson, 1972), an age associated increase in obesity is one possible explanation of Boberg's findings.

The significant negative correlation between serum and VLDL triglyceride and K_2 (see Figure 9) is in agreement with the findings of Boberg et al (1969).

9. BLOOD GLUCOSE

A modified alkaline potassium ferricyanide method was used on the Technicon Autoanalyzer. Unlike the glucose oxidase method, this is not specific for glucose and can be affected by other reducing substances; results can be up to 10% higher than with the specific enzyme method. Glucose determinations were kindly performed by the routine chemical pathology laboratory.

10. SERUM INSULIN

Insulin was determined by radioimmunoassay. ^{125}I insulin competes with the unlabelled hormone for sites on a specific antibody. The assay was kindly performed by the Hammersmith Hospital Immunoassay service.

11. POST-HEPARIN LIPOLYTIC ACTIVITY (PHLA)

PHLA was determined by a modification of the method of Fredrickson et al (1963), but using Intralipid (Vitrium, Stockholm) as substrate. This method relies on the release of lipoprotein lipase into plasma following the intravenous injection of heparin. It assumes that the amount of enzyme released into circulation is proportional to that available for triglyceride uptake by the tissues. Difficulties in interpretation of PHLA are discussed more fully on page

In the original assay of Fredrickson et al (1963), the substrate used was Ediol, a commercial coconut emulsion; the advantage over other substrates available at that time was its stability. A disadvantage, however, is its high content of partial glycerides especially monoglyceride, as monoglyceride hydroloses are known to occur both in adipose tissue (Vaughan et al, 1964; Kupiecki, 1966) and in post-heparin plasma (Shore and Shore, 1961; Greten et al, 1969; Biale and Shafrir, 1969). PHLA is also more completely inhibited by IM Nacl (Boberg and Carlson, 1964; Boberg, 1969) and PHLA values are lower (Elkeles

1973), when Intralipid rather than Ediol is used as substrate. Intralipid has subsequently been used as a substrate for lipoprotein lipase assays by many investigators, and has been used in the present investigation.

Procedure

Heparin (Weddel Pharmaceuticals, London) 10 units/Kg. body weight was injected intravenously after a 12 - 14 hour overnight fast. Just prior to injection, a 10 ml. venous blood sample was taken and placed in an iced tube containing 0.5 ml. of 0.1 M sodium citrate. After centrifugation for 10 minutes at 1,500 rpm at 4°C, the plasma was separated and immediately frozen at -20°C until the assay was performed. In order to negate the effects of possible variations between assays, samples from individual subjects were stored until the end of each experiment, in order that they could be assayed simultaneously.

Preparation of the substrate

Twenty per cent fraction V Bovine Albumin (Armour Pharmaceuticals Co. Ltd., England), 0.7 ml. Tris (previously corrected to pH 8.1), and Heparin (Weddel Pharmaceuticals, London), 20 units/ml. were mixed in ratio of 4:2:1. To this was added two parts of 10% Intralipid which had previously been pre-incubated with fasted human plasma for 30 minutes at 37°C in a ratio of 1:1.

2.5 ml. post-heparin plasma was added to 4.5 ml. substrate, mixed by inversion and incubated for 120 minutes in a Gallencamp-shaking water bath (100 cycles/min. at 37°C). At 0, 30, 60, 90 and 120 minutes, 1 ml. samples of the incubation mixture were removed and added to 5 ml. Dole solution for estimation of free fatty acid concentration by the titration method previously described. An approximately linear release of FFA occurs throughout the period of observation (see Figure 10). The slope of the line provides a measure of the amount of the FFA released per unit time; i.e. the lipolytic activity of the post-heparin plasma, expressed as μM FFA released per litre of plasma per minute. The FFA standard curve was as described in the section dealing with FFA determination (see page 50.

12. DETERMINATION OF THE RELATIVE INCORPORATION OF PALMITIC AND LINOLEIC ACIDS INTO SERUM VERY LOW DENSITY LIPOPROTEIN-TRIGLYCERIDES (VLDL-TG)

This was assessed by simultaneously infusing albumin-bound ^{3}H-palmitate and ^{14}C-linoleate intravenously into normal men, and measuring the appearance of the labels in serum VLDL-TG.

The absolute rate of incorporation cannot be reliably calculated from incorporation curves, mainly because of simultaneous removal of triglyceride from plasma, and because of the possibility of recycling (Eaton et al, 1969). These problems are further discussed on page 303 . However, in the experiment described in Chapter V, no difference in the rates

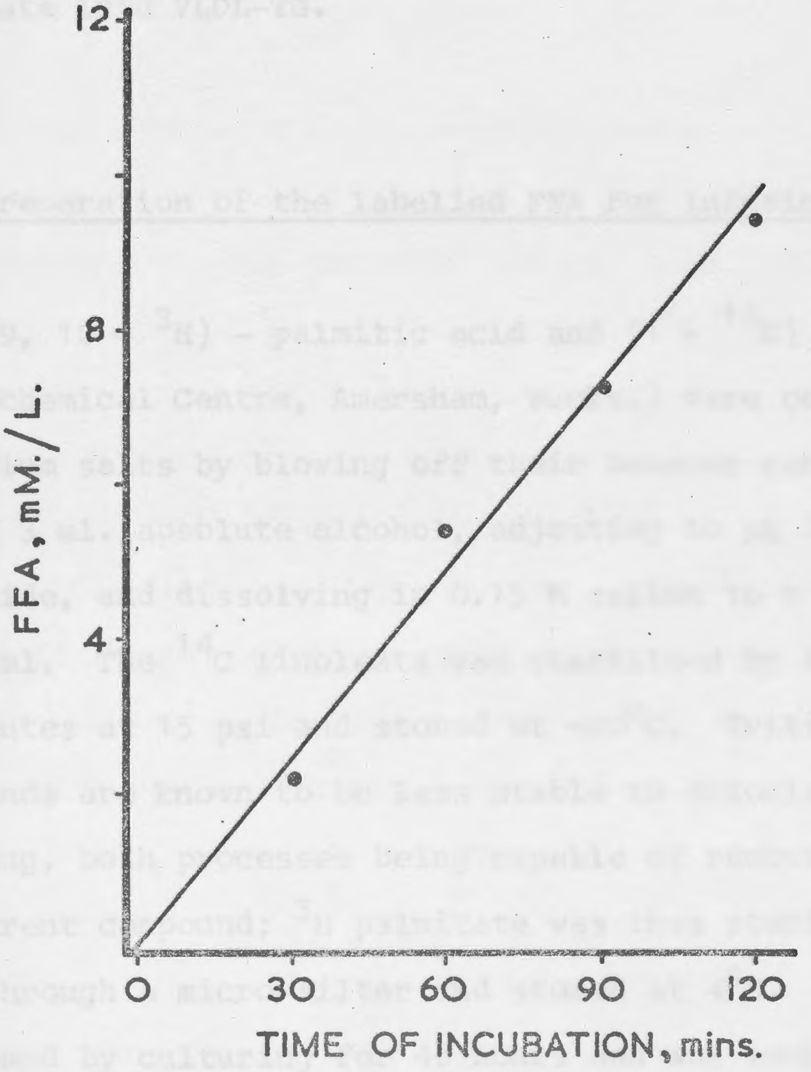

Fig. 10. FFA released during incubation with post-heparin plasma

of disappearance of ^{14}C and ^{3}H from the endogenously labelled triglyceride was discernible. In view of this, the linear increase in radioactivity was taken to be a measure of incorporation of free fatty acid into triglyceride for the purpose of calculating the ratio of incorporation of palmitate and linoleate into VLDL-TG.

Preparation of the labelled FFA for infusion

(9, 10 - ^{3}H) - palmitic acid and (1 - ^{14}C) - linoleic acid (Radiochemical Centre, Amersham, Bucks.) were converted to their potassium salts by blowing off their benzene carrier, dissolving in 3 ml. absolute alcohol, adjusting to pH 8 with potassium hydroxide, and dissolving in 0.15 M saline to a final volume of 10 ml. The ^{14}C linoleate was sterilized by autoclaving for 15 minutes at 15 psi and stored at -20°C. Tritium labelled compounds are known to be less stable to autoclaving and deep freezing, both processes being capable of removing the ^{3}H from the parent compound; ^{3}H palmitate was thus sterilized by passing through a micro-filter and stored at 4°C. Sterility was confirmed by culturing for 48 hours and was rechecked at intervals. Radiochemical purity in the product was confirmed by the presence of a single spot on thin layer chromatography (TLC).

Immediately before the experiment, approximately 10 μci ^{14}C linoleate and 25 μci ^{3}H palmitate were added slowly with mixing to a solution of fatty acid poor human albumin (Lister

Institute, London) containing 0.5 g. human serum albumin in 2 ml. The infusate was made up to its final volume by dilution with 0.15 M sterile saline.

Procedure

After a 12 - 14 hour overnight fast, polyethylene cannulae were inserted into each antecubital vein and stoppered with a three-way tap; the cannulae and tap were kept patent by sterile saline. A 30-minute interval was allowed to elapse before commencing the infusion in order to allow the subjects to assume a basal state. A base-line sample for determination of lipid concentrations was obtained prior to infusion; the fatty acid albumin complex was then infused at a constant rate over a four-hour period by an electric driven infusion pump (Braun, Melsingen, Germany). During this interval, the subjects lay quietly, being disturbed only for blood sampling. 20 ml. samples were obtained at hourly intervals during the infusion, for measurement of the incorporation of the ^{3}H and ^{14}C labels into VLDL-TG. An aliquot for determination of FFA concentration and composition was put into a heparinized tube, the plasma immediately separated by centrifuging for 10 minutes at 1,500 rpm at 4°C, and stored at -20°C until analysed. FFA concentration was measured, as described on page 50 . FFA composition was determined by gas-liquid chromatography (GLC) after prior separation of FFA from other plasma lipids by TLC (see page 82). The remainder of the blood sample was allowed to clot; after clot retraction, the serum was separated by centrifugation for 10 minutes at 1,500 rpm. EDTA was then added to a final concentration of 0.05%. A 4 ml. aliquot was ultracentrifuged for 20 hours at 105,000 g mins. under a 2 ml. layer of d = 1.006 saline to separate the VLDL fraction. This fraction was collected quantitatively by tube slicing and washing of the tube slicer and cap as described on page 48 . The radioactivity in the lipids of VLDL and serum were then determined as described below.

After the infusion, blood was sampled hourly for a further 4 hours to determine the isotope ratio in VLDL-TG during the disappearance phase.

Separation of lipid by thin-layer chromatography (TLC)

The method used to determine the radioactivity in labelled lipids of plasma VLDL was essentially that of Boberg (1966).

Thin layer glass plates (20 x 20 cm.) were coated with silicic acid (Silica Gel G, Merck A G, Darmstadt, Germany) to a thickness of 0.5 mm. by the pouring technique, using silicic acid (15 g.) slurried in 35 ml. of a solution containing 2% toluene scintillation solution in ethanol. This procedure allows rapid drying of the plates, and visualization of lipid spots under ultraviolet light, without the necessity for other indicators, many of which are unsuitable for radioactive work because of their quenching properties. After drying at room temperature, plates were activated for 30 minutes at 110°C and stored over phosphorus pentoxide.

Lipids were extracted in duplicate as follows (Boberg, 1966): 1 ml. plasma, or VLDL was mixed with 5 ml. methanol followed by 10 ml. chloroform. Aqueous and organic phases were separated by the addition of 15 ml. 0.2 M sodium hydrogen phosphate, the upper aqueous phase being separated by suction after overnight storage followed by low speed centrifugation. The chloroform phase was evaporated to dryness under a nitrogen stream. 0.3 ml. hexane was added, and after mixing on a vortex mixer, 0.2 ml. was carefully spotted on to a thin layer plate with a Hamilton syringe (Hamilton Micromesure, Netherlands) using a syringe holder especially designed for TLC.

Duplicate spots of each sample were made. One was used for determination of radioactivity in the various lipid fractions; the FFA spot of the other was used for the determination of fatty acid composition by GLC.

Separation was achieved at room temperature in unlined jars using a solvent mixture of petroleum ether; diethyl ether; acetic acid (82; 17.5; 0.5) in an ascending system. After a period of about 45 minutes, or when the solvent front had reached two-thirds to three-quarters of the way up the plate, the plates were removed and the solvent allowed to evaporate at room temperature. After about 30 minutes, the lipid fractions were visualized in ultraviolet light and compared with the spots from a standard solution containing palmitic acid, triolein, cholesterol palmitate and lecithin (Sigma Chemical Co., U.S.A.). Marks were made on the silicic acid outlining each spot. Using a razor blade, the silicic acid containing the lipid spot was quantitatively scraped into a glass counting vial. 10 ml. toluence scintillation fluid (see page 55 for preparation) containing 5% methanol allows intravial elution of the lipid (Boberg, 1966). Phospholipid elution is incomplete by this method, hence when determining radioactivity in this fraction, the spot was scraped into a column and the lipid eluted with 10 ml. ethanol. The ethanol was then evaporated under a nitrogen stream and 10 ml. scintillation fluid added. Counting was performed on a Beckman liquid scintillation spectrometer, each sample being counted in duplicate. Quenching was corrected for by external standardization, values for ^{14}C and ^{3}H dpm being read off quench curves.

Recovery experiment

FFA

An appropriate amount of ^{14}C palmitate was added to a chloroform-methanol extract of plasma. The phases were split, the chloroform phase evaporated to dryness, and hexane added as previously described. Duplicate aliquots were applied to thin layer plates; one was immediately scraped into a vial for counting, the other separated as described. FFA and triglyceride spots were scraped into vials and counted.

Result

FFA counts recovered in FFA spot: 98.3% ± SD 3.0 (n = 6)

FFA counts recovered in triglyceride spot: 0.5% ± SD 0.4 (n = 6)

Triglyceride

The above experiment was repeated using appropriate amounts of ^3H triolein.

Result

Triglyceride counts recovered in triglyceride spot:

94.8% ± SD 3.5 (n = 5)

Triglyceride counts recovered in FFA spot:

1.4% ± SD 1.0 (n = 5)

Determination of the specific activities of plasma free palmitic and linoleic acids, using gas liquid chromatography (GLC)

In order to determine the specific activity of free palmitic and free linoleic acid in plasma, duplicate extracts of plasma were fractionated by TLC as described on page The FFA spot from one specimen was counted as previously described; that from the other was methylated and its composition analysed by GLC (Gurr and James, 1971).

Methylation was performed in stoppered tubes at 75°C for 90 minutes following the addition of 6 ml. 5% sulphuric acid in dry methanol. The methylated fatty acids were then dried under a nitrogen stream, re-extracted into re-distilled diethyl ether, and analysed in a Pye series 104 (Pye Co., Cambridge, England) gas liquid chromatograph. The samples were applied to a glass column (5' x ¼") packed with 10% FFAP (Phase Separations Ltd., Flintshire, England) at an isothermal oven temperature of 205°C. Detection was by flame ionization, the detector output being attached to a recorder. The emergent peaks were identified by comparing their retention times with authentic standards (kindly supplied by Unilever Ltd., Bedford). The area under the peaks was calculated by multiplying the peak height by its width at half its height. The percentage of linoleate and palmitate components was then calculated:

$$\% = \frac{\text{Area of linoleate or palmitate peak} \times 100}{\text{Total area of all peaks}}$$

The concentration of free linoleic and palmitate acid in plasma was then calculated after the total plasma FFA concentration was measured as previously described (see page 50).

The plasma specific activity of free palmitic and free linoleic acid was then calculated as follows:

$$\frac{\text{Radioactivity (dpm/ml.)}}{\text{Concentration (mg./ml.)}}$$

By the time of the first sample, the plasma FFA specific activity had reached a plateau; the mean value for the 1, 2, 3 and 4 hour samples was used.

Calculation

Incorporation of each fatty acid into VLDL-triglyceride was calculated as follows:

Incorporation ratio =

$$\frac{\text{Rate of increase of } ^{3}\text{H in VLDL-TG}}{\text{Specific activity of free palmitate}} \div$$

$$\frac{\text{Rate of increase of } ^{14}\text{C in VLDL-TG}}{\text{Specific activity of free linoleate}}$$

Interpretation of results obtained using this method, and the assumptions on which it is based are discussed in Chapter V (see page

Calculation of the plasma turnover of the individual fatty acids was calculated as described on page 58 , e.g.

$$\text{Palmitate turnover } (\mu\text{M/min.}) = \frac{\text{Infused dose of palmitate (dpm/min.)}}{\text{Free palmitate specific activity (dpm/}\mu\text{M.)}}$$

13. STATISTICAL METHODS

In most studies described in this thesis, variables are compared under different conditions in the same subject, using the paired t-test (Snedecor, 1956).

The Mann-Whitney U test was used for comparison of two populations with unequal distribution (Siegel, 1956).

Linear regressions were calculated according to the method of least squares, and standard procedures were used to calculate Pearson's correlation coefficients (r). Where the data appeared not to be distributed with bivariate normality, Spearman's rank correlation test was used (Siegel, 1956).

Probabilities (p) have been expressed using two tailed tests.

14. CONSENT

Fully informed consent was obtained and witnessed in all cases studied.

CHAPTER II - REFERENCES

Angervall, G. (1964) Acta med. scand. 176. suppl.424.

Biale, G. and Shafrir, E. (1969) Clin. chim. Acta 23, 413.

Boberg, J. and Carlson, L.A. (1964) Clin. chim. Acta. 10, 420.

Boberg, J. (1966) Clin. chim.Acta 14, 325.

Boberg, J. and Hallberg, D. (1968) J. Atheroscler. Res. 8, 351.

Boberg, J., Carlson, L.A. and Hallberg, D. (1969) J. Atheroscler. Res. 9, 159.

Boberg, J. (1969) Lipids 5, 452.

Boberg, J. (1971) M.D. Thesis Acta Univ, Upsal.

Borgström, B. and Jordan, P. (1959) Acta Soc. med. Upsalien. 64, 185.

Brunzell, J.D., Hazzard, W.R., Porte, D.,Jr. and Biermen, E.L. (1973) J. clin. Invest. 52, 1578.

Carlson, K. (1973) J. clin. Path. 26, suppl. 5, 32.

Carlson, L.A. and Hallberg, D. (1963) Acta physiol. scand. 59, 52.

Carlson, L.A. and Böttiger, L.E. (1972) Lancet I, 865.

Carlson, L.A. and Rössner, S. (1972) Scand. J. clin. Lab. Invest. 29, 271.

Chin, H.P. and Blankenhorn, D.H. (1968) Clin. chim. Acta 20, 305.

Cramp, D.G. and Robertson, G. (1968) Analyt.Biochem. 25, 246.

Dole, V.P. (1956) J. clin. Invest. 35, 150.

Eaton, R.P., Berman, M. and Steinberg, D. (1969) J. clin. Invest. 48, 1560.

Epstein, F. (1965) J. chron. Dis. 18, 735.

Elkeles, R.S. (1973) Clin. Sci. 44, 23p.

Fredrickson, D.S., Ono, K. and Davis, L.L. (1963) J. Lipid Res. 4, 24.

French, J.E. and Morris, B. (1957) J. Physiol. 138, 326.

Gordis, E. (1962) Proc. Soc. exp. Biol. (N.Y.) 110, 657.

Goss, J.E. and Lein, A. (1967) Clin. Chem. 13, 36.

Greten, H.R., Levy, R.I. and Fredrickson, D.S. (1969) J. Lipid Res. 10, 326.

Gurr, M.I. and James A.T. (1971) In Lipid Biochemistry. An Introduction. Chapman & Hall, London p.13.

Gustafson, A., Alaupovic, P. and Furman, R.H. (1966) Biochemistry 5, 632.

Gustafson, A. and Sannerfedt, R. (1973) Europ. J. Pharmacol. 5, 259.

Hallberg, D. (1964) Acta physiol. scand. 62, 407.

Hallberg, D. and Wersall, J. (1964) Acta chir. scand. suppl.325, 23.

Hallberg, D. (1965a) Acta physiol. scand. (1965) 64, 299.

Hallberg, D. (1965b) Acta physiol. scand. 65, 279.

Haton, F.T. and Lees, R.S. (1968) Advan. Lipid Res. 6, 1.

Havel, R.J., Eder, H.A. and Bragdon, J.H. (1955) J. clin. Invest. 34, 1345.

Havel, R.J. and Goldfein, (1961) J. Lipid Res. 2, 389.

Havel, R.J. (1965) In Handbook of Physiology (Section V): Adipose Tissue (eds. A.E. Renold and G.F. Cahill) Amer. Physiol. Soc. Washington D.C. p.499.

Koerselman, H.B., Lewis, B. and Pilkington, T.R.E. (1961) J. Atheroscler. Res. 1, 85.

Kupiecki, F.P. (1966) J. Lipid Res. 7, 230.

Levine, J.B. and Zak, B. (1964) Clin. chim. acta 10, 38.

Lewis, B., Wittman, W., Krut, L.H., Hansen, J.D.L. and Brock, J.F. (1966) Clin. Sci. 30, 371.

Lewis, B., Boberg, J., Mancini, M. and Carlson, L.A. (1972) Atherosclerosis 15, 83.

Lewis, B., Chait, A., Wootton, I.D.P., Oakley, C.M., Krikler, D.M., Sigurdsson, G., February, A., Maurer, B. and Birkhead, J. (1974) Lancet I, 141.

McCarthy, R.D. and Duthie, A.H. (1962) J. Lipid Res. 3, 117.

Nestel, P.J. and Havel, R.J. (1962) Proc. Soc. exp. Biol. (N.Y.) 109, 985.

Nestel, P.J. (1964) J. clin. Invest. 43, 943.

Nestel, P.J. and Barter, P. (1973) Amer. J. clin. Nutrit. 26, 241.

Nikkilä, E.A. and Kekki, M. (1971a) Scand. J. clin. Lab. Invest. 27, 97.

Nikkilä, E.A. and Kekki, M. (1971b) Acta med. scand. 190, 49.

Olefsky, J., Farquhar, J.W. and Reaven, G.M. (1974) Europ. J. clin. Invest. 4, 121.

Persson, B. (1972) M.D. Thesis. Univ. Göteberg.

Rössner, S., Larsson-Cohn, U., Carlson, L.A. and Boberg, J. (1971) Acta med. scand. 190, 301.

Rössner, S., Boberg, J., Carlson, L.A., Freychuss, U. and Lassers, B.W. (1974) Europ. J. clin. Invest. 4, 109.

Shore, B. and Shore, V. (1961) Amer. J. Physiol. 201, 915.

Siegel, S. (1956) Nonparametric statistics for the behavioural sciences. McGraw-Hill, New York.

Snedecor, G.W. (1956) Statistical Methods, Ames, Iowa.

Van Handel, E. and Zilversmit, D.B. (1957) J. Lab. clin. Med. 50, 152.

Vaughan, M., Berger, J.E. and Steinberg, D. (1964) J. biol. Chem. 239, 401.

CHAPTER III

DIABETES MELLITUS

CHAPTER III, SECTION A

Diabetes Mellitus has until recently, been viewed as a disorder primarily of carbohydrate metabolism. The metabolism of carbohydrates and lipids are, however, closely related, most tissues of the body being able to utilize either fat or carbohydrate as metabolic fuels: the amount of each used tends to vary reciprocally with different energy demands and under different nutritional conditions. It is, therefore, not surprising that disturbed metabolism of one tends often to be associated with changes in the metabolism of the other. It is becoming increasingly apparent that in diabetes, lipid metabolism in addition to carbohydrate metabolism, is frequently deranged. The lipid abnormalities may be of at least equal importance. This is especially so as hypertriglyceridaemia has been found to frequentl accompany diabetes. The role of the latter as a risk factor for the development of atherosclerosis has been discussed previously (see page 10). Serum triglyceride concentration provided better discrimination that cholesterol concentration between diabetic patients with or without atherosclerosis (Albrink et al, 1963; Schrade et al, 1963; Santen et al, 1972).

The association that exists between diabetes mellitus and hypertriglyceridaemia is complex. At one end of the spectrum, hypertriglyceridaemia tends to occur in patients with classical diabetes; on the other hand, patients with primary endogenous hypertriglyceridaemia have mild abnormalities of glucose toleranc (Albrink and Davidson, 1966; Fredrickson et al, 1967; Glueck et al, 1969). Considerable overlap between glucose intolerance and hypertriglyceridaemia thus occurs, the relationship being made more complex by the lack of significant correlation between the magnitude of hypertriglyceridaemia and the degree of glucose intolerance (Reaven et al, 1963; Carlson and Wahlberg, 1966; Albrink and Davidson, 1966; Reaven et al, 1967; Glueck et al, 1969). A further difficulty is the lack of any obvious causal relationship between the two variables. Glucose intolerance and mild hypertriglyceridaemia are thus probably mediated by a common close association with environmental factors of which obesity appears to be the most important (Bierman et al, 1968). This interrelationship, as well as the effect of insulin, is discussed in greater detail on page 105.

The metabolism of glucose and lipids is closely interrelated. The extent to which each of the two major metabolic fuels, glucose and FFA is utilized for energy purposes depends largely on the nutritional status of the individual. A reciprocal relationship tends to exist. In the postprandial period, carbohydrate is the chief source of energy, whereas during the postabsorptive state, most of the energy needs of the organism are supplied by FFA mobilized from tissue stores (Havel, 1972). Control of this complex interrelationship is largely effected by the action of insulin.

THE FED STATE

During the absorption of food, both carbohydrate and triglyceride enter the circulation; the former mainly as monosaccharides via the portal vein, the latter as lipoprotein particles via the thoracic duct. Insulin released in response to feeding promotes the uptake of glucose by various tissues where they are metabolized for energy purposes. Glucose in excess of immediate needs, tends to be stored in the adipocyte as triglyceride. Insulin tends to promote the synthesis of lipoprotein lipase (Wing et al, 1966), an enzyme which results in uptake of triglyceride fatty acids from chylomicrons and VLDL; it also increases the formation of glycerol -3- phosphate from glucose, thereby permitting re-esterification of these fatty acids to form triglyceride stores (Vaughan, 1961). Lipogenesis may also take place in the adipocyte from acetyl CoA or glucose. In human adipose tissue, incubated with ^{14}C glucose in vitro, most of the label is found in glyceride-glycerol (Galton, 1971); fatty acid synthesis from acetyl CoA has also been demonstrated (Bray, 1972; Goldrick and Galton, 1974). A further way in which glucose promotes the storage of excess energy in the form of fat is by insulin induced inhibition of hormone-sensitive lipase, thereby diminishing the mobilization of FFA from adipose tissue stores.

THE FASTING STATE

During this period, the organism depends on energy stored during periods of caloric excess. FFA concentrations increase in plasma, providing the major metabolic fuel at this time. This is largely attributable to increased activity of hormone-sensitive lipase, the activity of which increases with reduced availability of insulin (Owen and Reichard, 1971). In man, FFA is utilized by most tissue, including brain (Cahill and Owen, 1968). During the post-absorptive state, about one-third of the FFA transported through liver is esterified with glycerol -3- phosphate to form triglyceride; of this a proportion is secreted as lipoprotein, the so-called endogenous triglyceride (Havel, 1972).

THE "GLUCOSE FATTY ACID CYCLE"

Randle et al (1963) have shown that certain tissues, such as muscle, normally metabolize FFA in preference to glucose. Fatty acid oxidation is believed to exert an inhibitory effect on glycolysis and oxidation of glucose in muscle, during periods of carbohydrate deprivation. Acetyl CoA resulting from the oxidation of FFA, leads to an increased formation of citrate, which inhibits phosphofructokinase (Randle et al, 1963). This rate limiting enzyme in glycolysis is also inhibited by high adenosine triphosphate levels (Mansour et al, 1962; Passonneau and Lowry, 1962) such as may result from the oxidation of FFA. After carbohydrate ingestion, the release of insulin results in a suppression of FFA mobilization and oxidation, thereby facilitating the uptake and oxidation of glucose. Glucose then tends to enter the adipocyte where glycolysis occurs. The glycerol -3- phosphate which is formed by the action of glycerophosphate dehydrogenase esterifies the FFA resulting in triglyceride formation. The cycle is thus completed.

Randle et al (1965) have suggested that the metabolic effects of diabetes could all be secondary to an increased production of FFA, which results in impaired muscle utilization of glucose. According to this theory, a fall in FFA availability should result in a stimulation in glucose utilization. However, when fatty acid availability was reduced by nicotinic acid, both Miettinen et al (1969) and Balasse and Ooms (1973) showed a reduction in glucose tolerance. These findings are thus inconsistent with this hypothesis. Csorba et al (1966) also failed to show a correlation between the fluxes of FFA and glucose, and Butterfield et al (1965) showed the insulin resistance of obesity to be unrelated to elevated FFA concentrations in plasma. Lasserset al (1971), however, showed a significant negative correlation between plasma FFA concentration and uptake of carbohydrate substrates by the human heart.

FFA elevation is thus unlikely to be the primary event in diabetes, the hallmark of which is believed to be insulin deficiency, absolute or relative, (Renold et al, 1972). As insulin exerts a regulatory effect at many sites in the pathways of both carbohydrate and lipid metabolism, it is perhaps not surprising that lipid abnormalities occur frequently among diabetics.

INSULIN - ITS PHYSIOLOGICAL ROLE IN RELATION TO TRIGLYCERIDE METABOLISM

As the metabolic defects in diabetes mellitus are considered to result from an absolute or relative deficiency of insulin (Renold et al, 1972), I will begin by briefly outlining the physiological effects of this hormone, particularly in relation to metabolic processes affecting plasma triglyceride metabolism.

FREE FATTY ACID MOBILIZATION

Triglyceride fatty acids of depot fat reach the liver after mobilization of free fatty acids from storage fat, and transport through plasma as a free fatty acid albumin complex (Goodman, 1958). Lipolysis of adipose tissue triglyceride is mediated by a triglyceride lipase, the activity of which is controlled by hormones; it is, therefore, frequently referred to as hormone sensitive lipase. Vaughan and Steinberg (1963) showed lipolysis to be stimulated by adrenalin, adrenocorticotrophic hormone, glucagon and growth hormone. It is, however, inhibited by insulin (Jungas and Ball, 1963). Mahler et al (1964) suggested that basal lipolysis was unaffected by insulin, only hormonal activation being suppressed. One means whereby insulin and other hormones may affect triglyceride metabolism is by their effect on the supply of precursor fatty acids to the liver, where they are precursors of triglyceride formation.

One of the more exciting advances in biochemistry in recent years, has been the discovery of cyclic 3'5' adenosine monophosphate (cyclic AMP), and the elucidation of its role as a second messenger in hormone action (Sutherland et al, 1965; Butcher, 1968; Robinson et al, 1968). Its role as a mediator of hormone action was initially discovered in relation to liver phosphorylase, but it has subsequently been shown to be operative in lipolysis as well (Butcher and Sutherland, 1967).

Cyclic AMP is formed from adenosine triphosphate by the action of a membrane bound enzyme, adenyl cyclase; this occurs in response to a hormone becoming attached to a receptor site at the target cell membrane and activating adenyl cyclase (Kuo and de Renzo, 1969). The cyclic AMP produced, activates a protein kinase, which catalyzes the transfer of phosphate from ATP to the inactive form of an enzyme, resulting in activation of the latter enzyme. Destruction of cyclic AMP is catalyzed by the ubiquitous enzyme phosphodiesterase (Cheung, 1967). A hormone may thus affect a cyclic AMP mediated reaction by stimulation of adenyl cyclase or by inhibition of phosphodiesterase. Inhibition of a cyclic AMP mediated reaction may be due to a reduction in adenyl cyclase activity or stimulation of phosphodiesterase.

In fat cells, hormone sensitive lipase has been demonstrated to act via the cyclic AMP system (Butcher and Sutherland, 1967). Hormones such as catecholamines, ACTH and glucagon, stimulate adenyl cyclase and hence lipolysis (Birnbaumer et al, 1969). Insulin, on the other hand, inhibits mobilization of fatty acids from adipose tissue (Perry and Bowen, 1962; Jungas and Ball, 1962) The anti-lipolytic effect of insulin has been shown to probably be due to stimulation of phosphodiesterase (Das and Chain, 1972), although Blecher et al (1968) showed insulin to be without effect on the activity of this enzyme in adipose tissue. Evidence, however, also exists for an inhibitory effect on adenyl cyclase (Jungas, 1966). As a result of these effects, insulin, when administered in vivo, results in a marked and almost immediate reduction of plasma FFA (Dole, 1956) and glycerol concentrations (Hagen, 1963; Mueller and Evans, 1963). The reduced plasma FFA levels and turnover observed after insulin administration are thought to be due entirely to its peripheral effect on free fatty acid and mobilization (Havel and Carlson, 1963; West and Passey, 1967). Free fatty acid uptake by the liver appears to depend on plasma levels and to occur independently of insulin effect. Heimberg et al (1966) and Van Harken et al (1967; 1969), using the isolated perfused rat liver, have shown the uptake of free fatty acids to depend on its concentration in the perfusate, the fractional extraction rate remaining unchanged. Pre-treatment of rats with insulin did not increase the rate of uptake of palmitate by the perfused liver. Penhos et al (1968), however, showed an increased uptake of FFA when insulin was added to the perfusate, as had previously been shown when insulin was administered to the animal in vivo (Heimberg et al, 1965). The effect of insulin in inhibiting FFA mobilization from adipose tissue is to reduce the supply of FFA to the liver where it is a precursor for triglyceride formation. A potential for limiting triglyceride synthesis and secretion thus exists.

HEPATIC LIPOGENESIS

If the fall in plasma FFA levels and hence hepatic uptake, are compensated for by an increased hepatic lipogenesis, the overall input of FFA into the hepatic triglyceride pool could remain unchanged. Whereas the supply of circulating FFA has been shown to be related to the production of plasma triglyceride by liver (Nestel and Steinberg, 1963; Heimberg et al, 1969), it has also been claimed that de novo fatty acid synthesis in rats may be an important determinant of lipid release by the liver (Windmuellier and Spaeth, 1967). The effect of insulin on hepatic lipogenesis, however, shows marked species differences; most studies have been performed in rats and results obtained should be extrapolated to man with caution.

Synthesis of free fatty acids occur mainly extramitachondrially, although an intramitachondrial system also exists (Masoro, 1968). Acetyl CoA, generated within the mitachondria, mainly from carbohydrate utilization, is the main building block for fatty acid synthesis. Malonyl CoA is generated from acetyl CoA, the reaction being catalyzed by acetyl CoA carboxylase following which the 'fatty acid synthetase' complex catalyzes the formation of long chain fatty acids (Wakil, 1961). FFA synthesis is markedly depressed by fasting and stimulated by feeding (Goldman and Cahill, 1964). Insulin could affect hepatic lipogenesis by altering the supply of precursors such as acetyl CoA, by stimulating hepatic glucose utilization, or it could have a more direct effect on the enzymes of fatty acid synthesis and of glycolysis; an inducing effect on the latter enzymes is known to occur (Weber and Hird Convery, 1966). In vitro experiments suggest that insulin increases hepatic lipogenesis; early experiments (Medes et al, 1952) showed insulin to increase acetate lipogenesis in liver slices from fed animals. Fasted animals were also shown to demonstrate this effect if glucose was added to the medium (Masri et al, 1952), insulin alone having no effect. Although glucose alone augmented acetate lipogenesis, the combination of glucose and insulin caused stimulation which exceeded that of glucose alone. Liver perfusion studies have confirmed these results (Altman et al, 1951; Haft, 1967; 1968). Haft (1967) has shown insulin to increase both acetate and glucose lipogenesis in livers of fed and fasted rats, in the presence of physiological concentrations of glucose in the medium. The increased glucose oxidation in this study was insufficient to account for increased lipogenesis. Conflicting results have, however, been obtained in other studies, for example Boden and Willms (1966) failed to demonstrate an effect of insulin on lipid metabolism of livers of normal rats. Glucose lipogenesis in alloxan diabetic rats was, however, restored within three hours of insulin administration.

A further possibility is that FFA concentration could affect lipogenesis; in vivo effects of insulin in reducing FFA mobilization could thus indirectly affect lipogenesis. Nikkilä (1969) comments that the increased incorporation of labelled glucose into esterified fatty acids in fed rats after insulin administration (Goldman and Cahill, 1964) could be due either to genuine enhanced lipogenesis or less dilution of radioactive fatty acid due to reduced FFA mobilization. He has shown in experiments of similar design to Goldman and Cahill (1964), that when FFA mobilization in fed rats is inhibited by nicotinic acid rather than insulin, a reduced incorporation by both labelled acetate and glucose into hepatic and plasma triglyceride resulted; it, therefore, seems unlikely that the acute effect of insulin on hepatic lipogenesis is mediated by insulin induced changes in FFA availability.

HEPATIC TRIGLYCERIDE FORMATION AND SECRETION

It is apparent from the foregoing that insulin on the one hand lowers hepatic uptake of free fatty acids by inhibiting lipolysis, while in the other direction, the evidence suggests increased de novo fatty acid synthesis. The resultant effect of insulin on input of FFA into the hepatic fatty acid pool in man remains uncertain, but is clearly of importance in determining hepatic triglyceride formation.

Using ^{14}C palmitate, Rubenstein and Rubenstein (1966) were unable to demonstrate an increased triglyceride formation after the addition of insulin to rat liver slices, nor were Heimberg et al (1966) able to show increased esterification of palmitate in response to insulin in the isolated perfused rat liver. Triglyceride production by the perfused liver did, however, fall after administration of anti-insulin serum (Woodside and Heimberg, 1972). Nikkilä and Ojala (1966) obtained similar results in intact rats, although Penhos et al (1968) reported inhibition of triglyceride output after addition of insulin to the perfusate of livers from fed, but not from fasted rats. Studies in humans have shown essentially similar findings. A single dose of insulin has been shown not to influence the rate of appearance of ^{14}C palmitate in plasma triglyceride (Csorba et al, 1966), similar results being obtained during constant infusion of insulin (Nestel, 1967). Although the bulk of evidence points against insulin having an effect on hepatic triglyceride output, Topping and Mayes (1972) have reported a direct stimulation of VLDL secretion by insulin in perfused rat livers. Analogous results with liver slices have been obtained by Letarte and Fraser (1969).

The net effect of insulin on triglyceride output by the liver in the intact animal is thus probably related to reduced plasma FFA, although this may be compensated for by enhanced lipogenesis.

TRIGLYCERIDE REMOVAL

As early as the 1930's, it was known that insulin diminished alimentary lipaemia in dogs (Rony and Ching, 1930). The rate of removal of an artificial triglyceride emulsion from the blood of rats has also been shown to be increased by insulin pretreatment (Heimberg et al, 1966).

As described previously, removal of triglyceride from plasma is mediated largely through tissue lipoprotein lipase. Insulin has been shown to induce lipoprotein lipase in incubated adipose tissue (Hollenberg, 1959; Salaman and Robinson, 1966) possibly through induction of the enzyme (Nikkilä and Pykälistö, 1968). Post-heparin lipoprotein lipase activity in plasma, however, does not seem to increase after insulin administration to normal subjects (Jones et al, 1966). A further possible site of insulin action in the uptake of triglyceride by adipose tissue is on re-esterification of the fatty acid liberated by lipoprotein lipase. As there appears to be little glycerokinase in human adipose tissue (Steinberg, 1962; Margolis and Vaughan, 1962), re-esterification depends on glycolysis and glycerophosphate dehydrogenase for the glycerol -3- phosphate necessary for re-esterification. The entry of glucose into fat tissue and its subsequent metabolism favours this process (Vaughan, 1961).

EFFECT OF INSULIN ON SERUM TRIGLYCERIDES

The various sites of insulin action as related to lipid metabolism have been outlined above. The net effect of acute insulin administration in man is a reduction of serum triglycerid concentration in both normal subjects (Jones and Arkey, 1965) and in subjects with hypertriglyceridaemia (Schlierf and Kinsell, 1965). The chronic effect of hyperinsulinism is less clear. In normal subjects, fasting serum triglyceride levels and insulin response to an oral glucose load, show a strikingly positive correlation (Abrams et al, 1969). Hyperinsulinism has also been observed in hypertriglyceridaemia (Reaven et al, 1967). Nikkilä (1969) has suggested that insulin may be one of several factors promoting hepatic triglyceride secretion in subjects with endogenous hypertriglyceridaemia. Elkeles et al (1971) also found hypertriglyceridaemia to be more frequent in maturity onset diabetics with a moderate insulin response than in those with a poor insulin response. This relationship does not appear to occur universally; Glueck et al (1969), for instance, found high, normal, or low insulin responses in roughly equal proportions in patients with hypertriglyceridaemia, as did Nikkilä and Taskinen (1970). In the latter study, infusion of an artificial triglyceride emulsion resulted in a prompt increase in plasma insulin and insulin secretion rate; this observation may partly explain the hyper-responsiveness of the pancreatic cell in hypertriglyceridaemia and obesity.

EFFECT OF OBESITY

The picture is further complicated when one considers the interaction of obesity. Obesity has frequently been shown to be associated with hypertriglyceridaemia; correlations between serum triglyceride concentrations and obesity exist whether the latter is assessed by relative weight, ponderal index, skin fold thickness or measurements of per cent body fat (Albrink and Meigs, 1964; Hollister et al, 1967; Evans and Ostrander, 1967; Ford et al, 1968; Rifkind et al, 1968). Obesity, however, appears to result in higher triglyceride levels in diabetics than in controls without diabetes (Braunsteiner et al, 1966; Sailer et al, 1966). Braunsteiner et al (1966) showed serum triglyceride concentrations to increase by a mean of 8 mg./dl. for every 10 Kg. increase in body weight in non-diabetic subjects, and by 28 mg./dl. and 39 mg./dl. in diabetics on tolbutamide and insulin respectively.

The co-existence of obesity and maturity onset diabetes has already been referred to; diabetics of this type frequently being obese (Joslin et al, 1935). Furthermore, diabetes occurs more commonly amongst obese than non-obese individuals (Bierman et al, 1968), and obese subjects have been shown to have reduced forearm uptake of glucose which correlates well with indices of obesity (Butterfield and Wichelow, 1968; Wichelow and Butterfield, 1971). This raises the question of whether obesity can cause diabetes or conversely, whether diabetes can lead to obesity. The nature of the association between these two is not clear; an abnormality of insulin secretion could, however, provide the missing link.

In obese subjects without carbohydrate intolerance, an elevation of basal insulin and of insulin response has been demonstrated (Karam et al, 1965; Perley and Kipnis, 1966; Bagdade et al, 1968a; Chiles and Tzagournis, 1970); this response appears disproportionately large for any blood glucose level (Cerazi and Luft, 1967). When, however, insulin response is expressed as a percentage increment over basal levels, Bierman et al (1968) have shown that the correlation between obesity and insulin response can be eliminated. When this approach was adopted in diabetics, a negative correlation was observed; carbohydrate intolerance was associated with a progressive impairment in insulin response. Despite the lack of a bimodal distribution these authors suggest that impairment of insulin response to glucose characterizes the individual with carbohydrate intolerance. Although insulin responses in obese diabetics may exceed those in thin diabetics, in neither instance is this response adequate to prevent carbohydrate intolerance.

Reduced insulin response thus characterizes diabetes; increased insulin response occurs commonly in obesity, yet obesity and diabetes frequently co-exist. This may be explained by the larger adipocytes found in obese subjects (Salans et al, 1968; Hirsch and Knittle, 1970) which have been shown to be insulin resistant in vivo (Wichellow and Butterfield, 1971) and in vitro (Salans and Dougherty, 1971). Obesity may thus act as a further stress on persons genetically predisposed to diabetes, facilitating expression of the diabetic tendency. For obese individuals to preserve normal glucose tolerance in the presence of peripheral insulin resistance, an enhanced insulin response is required. This is supported by the observation of increased pancreatic islet cell tissue in obese subjects (Ogilvie, 1933), presumably due to increased stress on insulin secreting cells imposed by the enhanced response.

Although raised plasma FFA levels have been reported in obesity (Dole, 1956; Gordon, 1960; Opie and Walfish, 1963), their role in the production of insulin resistance is not clear. Randle et al (1965) claimed an abnormal elevation of plasma FFA to have a primary role in the pathogenesis of carbohydrate intolerance by preventing muscle utilization of glucose, which would lead to insulin resistance and hyperinsulinaemia. These findings are, however, in contrast with those of Miettinen et al (1969) and Balasse and Ooms (1973) who failed to show an improved removal of glucose from blood following the reduction of plasma FFA with nicotinic acid. Bagdade et al (1968a) have shown that accelerated lipolysis occurs only when obesity and carbohydrate intolerance co-exist; they suggest that obesity alone, with its associated hyperinsulinism, limits fat mobilization, and that thin subjects with mild carbohydrate intolerance have sufficient insulin secretion to limit fat mobilization. This, however, fails to explain the observations of Dole (1956) and Gordon (1960) of elevated plasma free fatty acid levels in non-diabetic obesity.

LIPID METABOLISM IN DIABETES MELLITUS

FREQUENCY OF HYPERTRIGLYCERIDAEMIA IN DIABETES

As long ago as 1917, Joslin et al noted most diabetics to be hyperlipidaemic. More recently, it has been confirmed that hypertriglyceridaemia occurs commonly in diabetes, especially those with vascular complication (Albrink et al, 1963; Santen et al, 1972). The degree of hypertriglyceridaemia is highly variable; gross lipaemia with an increase in both chylomicrons and VLDL, eruptive xanthoma and lipaemia retinalis is a rare complication of uncontrolled diabetes (Bagdade et al, 1967). More frequently, diabetics are found to have moderate to slight elevations of serum triglyceride levels, due to increased VLDL. Chance et al (1969) found two-thirds of 135 newly diagnosed diabetic children to have hyperlipidaemia mostly due to elevated pre-β lipoproteins, although a high incidence of Type III probably resulted from inclusion of some subjects with elevated β and pre-β lipoproteins under this heading. A similar prevalence was found in untreated diabetics by Hayes (1972). The latter study, however, showed a roughly equal distribution between Types II, III, and IV hyperlipoproteinaemia, although the method of typing, viz. densitometer staining of cellulose acetate electrophoretic strips, may account for the unusually high frequency of the Type III pattern. The situation with maturity onset diabetes is even less clear. Schrade et al (1963) found a large proportion of elderly diabetics to be hypertriglyceridaemic especially if diabetic control was poor. Östman (1965), however, failed to find elevated triglyceride levels in untreated adult type diabetics. Elkeles et al (1971) found less than a quarter of this type of patient to have triglyceride levels above 160 mg/dl. In 160 patients with fasting hyperglycaemia recently reported by Rodger and Dee (1973), 36% had elevated serum triglyceride; age ranged from 5 - 85 and none was previously known to be diabetic. In animal models, hypertriglyceridaemia has been noted in acute alloxan diabetes (Tarrant and Ashmore, 1965; Brown, 1967), after pancreatectomy (Gibbs and Chaikoff, 1941; Chernick and Scow, 1959) and after administration of anti-insulin serum (Gross and Carlson, 1968).

FFA MOBILIZATION

Plasma FFA derives almost entirely from lipolysis of adipose tissue. The role of insulin in inhibiting lipolysis has already been discussed on Page 98. Some plasma FFA may, however, come from intravascular hydrolysis of triglyceride fatty acids (Shames et al, 1970).

Plasma FFA concentrations are commonly elevated in uncontrolled insulin dependent diabetics (Laurell, 1956; Bierman et al, 1957). Maturity onset diabetics show lesser degrees of FFA elevations (Östman, 1965). Plasma FFA turnovers tend to be increased in parallel with plasma FFA levels in untreated diabetics (Bierman et al, 1957; Sailer et al, 1967; Miller, 1967), although Csorba et al (1966) showed no increase in FFA turnover in insulin-treated and mild maturity onset diabetics. Ford et al (1963) also failed to demonstrate an increased FFA turnover in diabetes. The low mean blood glucose level of the diabetic patients in the latter study suggests that the diabetic state was mild at the time of study. In view of this divergence of opinion, the present work includes measurements of FFA turnovers in both insulin dependent and diet-treated diabetics, measurements being repeated after diabetic control was achieved.

In diabetes in animals, FFA concentrations have also been shown to be elevated (Kovacev and Scow, 1966); a rapid fall to normal occurs immediately on administering insulin. In vitro studies in adipose tissue from pancreatectomized animals show lipolysis and FFA release to be much greater than in tissue from control fasted animals (Scow and Chernik, 1970). Based on the finding of a reduced conversion of FFA to triglyceride in human adipose tissue in vitro, Östman (1965) has suggested that reduced uptake of fatty acids in addition to enhanced mobilization could explain the increased net release of FFA in juvenile diabetes; though this defect was most apparent in juvenile diabetes, maturity onset diabetes showed a similar, however, non-significant trend. The concept of increased lipolysis together with decreased re-esterification being of importance in diabetes, was further developed by Björntorp and Hood (1966); they showed that large amounts of insulin correct the re-esterification defect.

Stimulation of hormone sensitive lipase by insulin lack, is the most likely cause of the increased FFA mobilization. Other hormones such as the catecholamines (Christensen, 1972), growth hormone (Hansen, 1970) and glucagon (Unger et al, 1970), which have been shown to be elevated in diabetes, may also be contributing to the enhanced lipolysis.

FATTY ACID SYNTHESIS

Inhibition of fatty acids synthesis in the presence of insulin deficiency has been demonstrated in vitro in liver slices (Chernick and Chaikoff, 1951), homogenized tissue (Abraham et al, 1962) and perfused livers (Haft, 1968). Because acetate lipogenesis (Brady and Gurin, 1950) as well as glucose lipogenesis is depressed, the site of the defect must be in the conversion of acetyl CoA to FFA. This defect was found not to be corrected by the addition of insulin in vitro (Brady and Gurin, 1950; Renold et al, 1955) and that in vivo fatty acid synthesis was restored only some hours after insulin administration (Renold et al, 1955; Haft, 1968); this suggests one or more enzymes of lipogenesis to be deficient. The slow return of lipogenesis after insulin administration is in sharp contrast to the rapid inhibition of lipolysis (Vaughan and Steinberg, 1963). Kalkhoff and Kipnis (1966) demonstrated depressed lipogenesis from ^{14}C acetyl CoA, but not from ^{14}C malonyl CoA in anti-insulin serum treated rats, suggesting the inhibition to be at the acetyl CoA carboxylase step. Inhibition of the insulin induced rise in hepatic lipogenesis by actinomycin (Gillhorn and Benjamin, 1964) suggests the mechanism to be one of repression of enzyme synthesis.

As citrate is known to be a positive effector of acetyl CoA carboxylase, it could play a role in the control of lipogenesis in diabetes. However, despite an increase in lipogenesis in homogenates from livers of diabetic rats on addition of citrate, the rate of lipogenesis was still far below that of normal controls (Abraham et al, 1962). Long chain fatty acids are also known to inhibit lipogenesis by a negative feedback mechanism on acetyl CoA carboxylase (Masaro, 1962); the increased FFA concentrations in diabetes could thus be the primary defect leading to an inhibition of lipogenesis. FFA and long chain fatty acyl CoA have also been shown to inhibit lipogenesis from malonyl CoA (Korchak and Masaro, 1964). However, in anti-insulin serum induced diabetes, lipogenesis from malonyl CoA was not substantially reduced (Kalkhoff and Kipnis, 1966). Furthermore, stimulation of lipolysis by adrenalin (Swaney and Ashmore, 1965) does not reduce lipogenesis, nor does inhibiting lipolysis with nicotinic acid enhance lipogenesis (Nikkilä, 1969).

The above-mentioned studies are all on experimental diabetes in the rat. Because of obvious difficulties in measuring hepatic lipogenesis directly in man, little is known of the effect on lipogenesis of human diabetes.

TRIGLYCERIDE SYNTHESIS AND SECRETION

The main substrate for triglyceride formation by the liver is normally FFA. As previously mentioned, plasma FFA levels are increased in uncontrolled diabetics and hepatic uptake of plasma FFA is proportionately to the plasma concentration within the range encountered physiologically and in most pathological states (Van Harken et al, 1967; 1969). This chain of events could conceivably result in enhanced triglyceride synthesis and secretion.As has been previously discussed (see Page 102), insulin has little definite effect on the esterification of FFA with glycerol -3- phosphate to form triglyceride and on fatty acid synthesis.

Studies of hepatic uptake of FFA in diabetes are conflicting; normal (McElroy et al, 1962), increased (Heimberg et al, 1966) and reduced (Kuhfahl et al, 1967) fractional extraction of FFA have been reported.

Hepatic FFA are esterified with glycerol -3- phosphate. There appears to be no impairment in glycerophosphate formation in liver slices from diabetic rats (Chernick and Scow, 1964) and the conversion of ^{14}C palmitate to hepatic triglyceride does not appear to be diminished in diabetes in both in vitro (Heimberg et al, 1967) and in vivo experiments (Nikkilä, 1969). Van Harken et al (1967) have, however, shown esterification in perfused livers from diabetic rats to be reduced. Only in a cell free supernatant from a diabetic liver has increased esterification been reported (Corder and Kalkhoff, 1969).

Hepatic triglyceride combines with apoprotein, cholesterol and phospholipid at the endoplasmic reticulum leading to the formation of VLDL which is then secreted into plasma (Robinson, 1973). Incorporation of labelled aminoacid precursors into the apoprotein moeity of lipoproteins has been shown by Wilcox et al (1968) and Van Harken et al (1967) to be reduced in diabetics. Availability of apoprotein could conceivably be the rate-limiting factor in hepatic lipoprotein secretion; triglyceride secretion into the medium by the perfused rat liver has indeed been shown to be markedly impaired in alloxan diabetic insulin-deprived rats (Heimberg et al, 1966; Van Harken et al, 1967; Eaton and Kipnis, 1969) at all levels of medium FFA (Heimberg et al, 1966; Van Harken et al, 1967). Against this concept is the observation that triglyceride secretion is partly restored by insulin before a similar effect is seen with aminoacid incorporation (Wilcox et al, 1968).

An alternate possibility to account for the reduced triglyceride secretion in diabetes would be enhanced lipolysis of intrahepatic triglyceride. In support of this idea, increased hepatic FFA uptake has been shown to be insufficient to account for the increased ketogenesis observed in diabetes, (Mayes and Felts, 1967; Van Harken et al, 1967). Triglyceride fatty acid might thus act as an important precursor of ketone bodies in the liver at the expense of synthesis and secretion of lipoproteins into plasma.

In support of impaired triglyceride secretion in vivo, is the finding of reduced relative incorporation of labelled palmitate into plasma triglyceride in insulin-dependent diabetics deprived of insulin for at least twenty-four hours (Sailer et al, 1967). These studies suggest that insulin is required for the secretion of triglyceride by the liver.

Despite reduced incorporation of FFA into secreted triglyceride, the increased FFA flux seen in diabetes might tend to result in an overall increase in the rate of hepatic triglyceride secretion. The net effect of these opposing influences has been directly measured in pancreatectomized dogs by Basso and Havel (1970) who found no evidence that VLDL hypersecretion accounted for the demonstrably high plasma triglyceride levels. This implies that defective removal accounts for the hypertriglyceridaemia. Later work from Havel's laboratory, however, shows a contrasting situation in the acute diabetic state accompanying the short term deficiency of insulin produced by an injection of anti-insulin serum (Balasse et al, 1972). In this situation, enhanced secretion appears to be the major factor leading to hypertriglyceridaemia. The finding by Gross and Carlson (1968) that nicotinic acid could reduce plasma triglycerides in the acutely diabetic rat, suggests enhanced availability of FFA to the liver to be of importance in determining hepatic triglyceride output in this situation. The reason for the different mechanisms of hypertriglyceridaemia in these acute and chronic situations is not clear: it is possible that some insulin remains available to the hepatocytes for a short while following the administration of the antibody. However, if the reduced triglyceride secretion seen in the chronic diabetic state is due to an inability of the liver to produce enough apoprotein, a more likely explanation for the difference observed between acute and chronic diabetes, is that in the acute situation, apoprotein supply has not had sufficient time to become rate-limiting, while FFA is already being mobilized at an increased rate.

Havel (1972) has suggested that in chronic diabetes, the large energy requirement for gluconeogenesis may take precedence over that for synthesis of apoprotein and for lipoprotein secretion, leading to diminished triglyceride output.

In contrast to the findings in chronic diabetic dogs, studies of triglyceride turnover in man suggests that triglyceride hypersecretion accounts for the hypertriglyceridaemia seen in most types of human diabetes. Using endogenous labelling of triglyceride with glycerol, Nikkilä and Kekki (1973) showed increased triglyceride turnover to accompany hypertriglyceridaemia especially in ketoacidosis. The increased triglyceride production was, however, not correlated with plasma FFA. Only in a small group of non-ketotic diabetics with severe lipaemia did they fail to demonstrate triglyceride over-production. The reason for the discrepancy between their findings, and the studies discussed above which suggest reduced triglyceride secretion, is not clear.

REMOVAL OF TRIGLYCERIDE FROM PLASMA

Although some evidence, notably the turnover data of Nikkilä and Kekki (1973), is in favour of excess triglyceride production being of prime importance in the pathogenesis of diabetic hypertriglyceridaemia, an alternative possibility is that the defect is one of impaired removal of triglyceride from the circulation. It is for this reason that removal of triglyceride from plasma was investigated in the present study.

As previously discussed (see Page 19), triglyceride removal occurs largely by hydrolysis of lipoprotein triglyceride in tissues, with re-esterification of the liberated FFA. Much evidence exists in experimental diabetes in support of the concept of impaired removal being of importance in the production of diabetic hyperlipidaemia.

Orally administered fat loads have been shown to have a delayed clearance in diabetics compared with controls. Sandberg et al (1960) showed removal to be impaired in diabetic patients on therapy, the most marked changes occured in those with atherosclerosis and alimentary lipaemia is well-known to be delayed in such subjects (Barritt, 1956; Bouchier and Bronte-Stewart, 1961; Denborough, 1963). Sandberg et al (1960) fed I^{131} labelled triolein together with a standard fat meal, but did not attempt to separate exogenous from endogenous components of plasma triglycerides. Recycled triglyceride fatty acids could thus lead to an apparent slowing of removal.

Kallio (1967), however, showed clearance of a fatty meal to be delayed in diabetics by measurements of optical density of plasma and triglyceride concentration. Although non-specific, the increase in optical density does rely on the presence of larger particles in plasma (Denborough, 1963). The clearance of intravenously injected triglyceride from plasma in diabetes has yielded some conflicting results. Whereas some studies have suggested delayed removal (Waddel and Geyer, 1957; Kessler, 1962; Bagdade et al, 1968b), others have not (Balodimos et al, 1962).

Waddel and Geyer (1957) showed that clearance of an injected triglyceride emulsion was severely impaired in pancreatectomized dogs; insulin treatment corrected the defect. Similarly, Kessler (1962) demonstrated a reduced fractional removal rate of triglyceride following the intravenous injection of large doses of triglyceride emulsion into pancreatectomized and alloxan diabetic dogs compared with insulin treated controls. It has been suggeste (Nestel, 1964) that an inverse relationship exists between plasma triglyceride levels and removal rates of triglyceride. The latter variable has been claimed merely to reflect triglyceride pool sizes. However, in both studies cited above, the dose of triglyceride injected was large, exceeding the endogenous pool size to such an extent that the results are unlikely to have been substantially influenced by the latter. Triglyceride removal from plasma has been shown to be saturable (Boberg et al, 1969; Brunzell et al, 1973); in these experiments, it is likely that maximal removal rates were being measured and shown to be reduced in uncontrolled diabetic animals. Some studies have, however, failed to confirm these results; Brown and Olivecrona (1966) found chylomicron removal to be similar in insulin-deprived and insulin-treated diabetic rats, although less of the injected label was recovered in adipose tissue triglycerides of the diabetic group than of the controls. This was confirmed by Brown (1967) and also by others, (Naidoo et al, 1967; Gries et al, 1967); these studies imply triglyceride removal to be reduced in adipose tissue, whereas removal in other tissues may be normal or even enhanced. Both Naidoo et al (1967) and Gries et al (1967) showed the uptake to be increased in skeletal and cardiac muscle in alloxan diabetic rats; these results being in sharp contrast to the situation in adipose tissue. The reduced recovery of injected triglyceride radioactivity in adipose tissue triglyceride suggests impaired removal of lipoprotein triglyceride from the circulation, but as pointed out by Nikkilä (1969), could also be explained by excessive lipolysis of newly formed adipose tissue triglyceride or of reduced adipose tissue mass, both of which are known to occur in experimental diabetes.

Studies in which lipoprotein lipase has been assayed either directly in adipose tissue or in plasma after its release from tissue sites by the injection of heparin, suggest that deficiency of this enzyme is, in fact, responsible for the reduced triglyceride uptake by diabetic adipose tissue.

LIPOPROTEIN LIPASE

As this enzyme is of major importance in the removal of lipoprotein triglyceride from plasma, assay of its activity is of obvious interest. The enzyme is found in most tissues which utilize plasma triglyceride, the level of activity reflecting the capacity of that tissue to remove the lipid. Most work has been done on rat adipose tissue and post-heparin plasma, although skeletal and cardiac muscle has also been studied. The level of activity depends on various hormonal and nutritional influences Adipose tissue lipoprotein lipase activity for instance, increases on feeding and insulin administration, and decreases with starvation (Bezman et al, 1962; Kessler, 1963). Another factor which is of importance is the tissue site; in cardiac and skeletal muscle, levels of activity respond to nutritional influences in a manner different to that in adipose tissue. Whereas in the latter organ, fasting reduces lipoprotein lipase activity, it results in an elevation of the enzyme in cardiac muscle (Borensztajn and Robinson, 1970). As previously discussed, the factors influencing lipoprotein lipase activity in different tissues are of physiological importance by giving direction to the utilization of lipoprotein triglyceride (Robinson, 1973).

Despite the physiological importance of lipoprotein lipase in tissue, paradoxically, much of the literature refers to studies of the enzyme in blood after release from tissue sites by heparin. Under normal circumstances, the enzyme is barely detectable in blood, but rapidly appears following intravenous heparin administration (Robinson, 1963). In human subjects, this source has the obvious advantage of ease of sampling, tissue samples being more difficult to obtain. Furthermore, activity of the enzyme in post-heparin plasma is easily assayed in both animals and humans, whereas activity of the enzyme in the tissues of man is of an extremely low order. It is only recently that techniques for studying human adipose tissue lipoprotein lipase are appearing (Persson,1972; Nilsson-Ehle, 1974); most earlier studies merely showed the existence of the enzyme in this tissue (Havel et al, 1961; Nestel and Havel, 1962; Diengott and Kerpel, 1965; Chlouverakis, 1965).

PHLA determinations do, however, have serious disadvantages. Firstly, heparin not only releases lipoprotein lipase from adipose tissue, but also from other tissue such as skeletal and cardiac muscle (Robinson, 1963), liver and lung; other lipases such as monoglyceride lipase, esterase and phospholipases are also released (Shore and Shore, 1961; Vogel et al, 1968; Greten et al, 1969). The substrate used in the assay is thus of prime importance. Recently, Huttunen and Nikkilä (1973) have shown endogenous lipoproteins to be a better substrate for the determination of PHLA, than artificial emulsions. Furthermore, PHLA does not seem to show the response to nutritional influences (Schotz and Garfinkel, 1972) seen with tissue sources of the enzyme; physiological effects are thus probably concealed. Lastly, attention has recently been drawn to qualitative differences in lipolytic activities originating in different organs (Ganesan et al, 1971; La Rosa et al, 1972; Zieve and Zieve, 1972). Further difficulties in comparison of PHLA values measured by different investigators, arises due to the lack of standardization of dose of heparin injected and assay procedure (Boberg and

Carlson, 1964). PHLA thus probably represents lipolytic activity from a number of ill-defined sources and interpretation of the enzyme activity in relation to physiological variables and disease states should be made with caution.

Most studies of lipoprotein lipase activity in diabetic animals have shown a deficiency of the enzyme whether assayed directly or in post-heparin plasma. PHLA was first shown to be reduced in pancreatectomized and alloxan-diabetic rats by Meng and Goldfarb (1959). The activity was restored by insulin. A similar effect of insulin was found in diabetic dogs (Kessler, 1962). Adipose tissue lipoprotein lipase activity has also been shown to be substantially reduced in alloxan diabetic rats (Kessler, 1963; Schnatz and Williams, 1963; Brown, 1967, Elkeles and Williams, 1974), but to be increased in cardiac muscle.

Estimation of lipoprotein lipase activity in clinical diabetes are, however, less clear-cut. This may be due to difficulties in the interpretation of PHLA, already mentioned. No impairment of PHLA was found in most diabetics, whether hypertriglyceridaemic or not (Denborough and Paterson, 1962; Jones et al, 1966; Perry, 1967; Wilson et al, 1969). Insulin treatment did not alter the activities. Bagdade et al (1968b) have, however, shown PHLA to fall after withdrawing insulin from diabetic patients. The same group (Bagdade et al, 1967) have also shown insulin reversible, sub-normal PHLA in a group of diabetics with the relatively uncommon pattern of gross lipaemia due to a combination of chylomicronaemia and elevation of VLDL. These intensely lipaemic diabetics were similar to those studied by Wilson et al (1969), who failed to show an impairment of PHLA. The latter study, however, comprised only five patients, two of whom had subnormal levels.

Triglyceride uptake from plasma could conceivably be impaired other than by reduced lipoprotein lipase activity; a reduction in fatty acid re-esterification in the adipocyte has already been referred to on Page 27 ; this might inhibit inward transport of FFA liberated by hydrolysis of lipoprotein triglyceride. The glycerol -3- phosphate required for re-esterification in the adipocyte, is derived from glycolysis from the glycerophosphate dehydrogenase reaction, glycerokinase being virtually absent from human adipose tissue (Steinberg, 1962; Margolis and Vaughan, 1962). Galton and Wilson (1970a) have shown that adipose tissue from diabetic patients cannot utilize glucose and glucose -6- phosphate as effectively as glycerol -3- phosphate for lipogenesis from ^{14}C palmitate when compared with tissue from non-diabetics. They suggest the most likely site of the defect to be at the phosphofructokinase stage. Indeed, they have shown the activity of phosphofructokinase to be reduced in adipose tissue of adult diabetics (Galton and Wilson, 1970b); this could possibly contribute to the impaired glucose utilization by the adipocyte which occurs in such patients. Although Östman (1965) failed to show impaired uptake of glucose by adipose tissue from juvenile diabetics in vitro, decreased assimilation and esterification of FFA was apparent.

MATURITY ONSET DIABETES

Much of the evidence which has been referred to throughout this Chapter has been on experimental diabetes in animals. These animal models are of obvious relevance to the insulin dependent type of diabetes. Their relationship to maturity onset diabetes is, however, less clear, in view of the frequent occurence of hyperinsulinism in the latter condition (Nikkilä and Taskinen, 1970). Many of the metabolic defects may thus differ from those seen in juvenile diabetics. The hereditary obese-hyperglycaemic mouse is probably the animal model closest to the maturity onset diabetic. The chief disturbance of lipid metabolism in these animals appears to be increased hepatic lipogenesis from acetyl CoA (Bates et al, 1955; Winand et al, 1968), citrate (Havard and Lowenstein, 1965) and glucose (Jansen et al, 1967). Fasting also fails to reduce lipogenesis. Winand et al (1968) have also demonstrated an accelerated rate of hepatic esterification of fatty acids in vitro.

In contrast to the increased lipolysis seen in insulin deficiency, lipolysis is not enhanced in obese hyperglycaemic mice (Westman, 1965). A similar situation may occur in human maturity onset diabetes; Östman (1965) showed normal lipolysis in vitro, in adipose tissue from adult diabetics, compared with increased breakdown of tissue triglyceride in a group of juvenile diabetics. Nonetheless, lipolysis has been shown to be elevated in some studies on maturity onset diabetes (Bierman et al, 1957; Björntorp and Hood, 1966), although not in others, (Östman, 1965). The reason for these discrepancies is not clear, but lipolysis of hepatic triglyceride could conceivably elevate plasma FFA levels despite normal release of FFA from adipose tissue.

THE PRESENT WORK

Although much has been written on the disorders of lipid metabolism in diabetes (Nikkilä, 1969, 1973; Havel, 1972; Woodside and Heimberg, 1972), many questions remain unanswered. This is especially so with regard to human diabetes. One fundamental issue which still remains contentious is whether the main defect resulting in hypertriglyceridaemia in diabetes is one of enhanced triglyceride secretion, a decreased removal, or are both factors perhaps operative? Much of the data on experimental diabetes in animals suggests some impairment of triglyceride uptake by adipose tissue; a reduction in lipoprotein lipase activity of adipose tissue appears to be the likely mechanism. Many of these studies, however, are on isolated organs or tissues in vitro; direct measurements of triglyceride input into or removal from plasma are few. From kinetic studies in human diabetes (Nikkilä and Kekki, 1973), over-secretion of triglyceride is suggested to be the primary defect in most subgroups of diabetes including some maturity onset cases. By contrast, direct measurement of splanchnic triglyceride production in chronically diabetic dogs has shown reduced triglyceride production (Basso and Havel, 1970); impaired removal of triglyceride from plasma is implied to be the cause of the hypertriglyceridaemia seen in this situation. As pancreatectomized dogs may not be the ideal model for all types of human diabetes, the present study was undertaken in order to assess removal of triglyceride from plasma in diabetic patients. Two groups of patients have been investigated; one insulin requiring, the other responding to diet alone. Patients who, in addition, required oral anti-diabetic agents were not included. As little is known of triglyceride removal in diet responsive cases, this group was of particular interest. Each subject acted as his own control, measurements being made once in the uncontrolled state, and again when diabetic control had been achieved. Using such an experimental design, it would be preferable if in some cases, the uncontrolled study could precede the study after achieving diabetic control, while in others, the order be reversed. This was, however, not considered ethically justifiable. A further control group was thus included; the results obtained in the two groups of diabetics were compared with those in a group of non-diabetic obese subjects.

The method chosen with which to assess triglyceride removal from plasma was the intravenous fat tolerance test (Boberg et al, 1969). The background and theoretical considerations relating to this test are more fully discussed in the methods section on Page 58 . Analogous to the intravenous glucose tolerance test, this non-steady state test measures the fractional removal rate of an intravenously injected synthetic triglyceride emulsion. It must be emphasized that the emulsion used (Intralipid, Vitrum, Stockholm) is not regarded as a tracer for lipoprotein triglyceride. Intralipid particles have been shown to have physicochemical and kinetic properties very similar to those of chylomicrons (Carlson and Hallberg, 1963; Hallberg and Wersall, 1964; Hallberg, 1965). As Intralipid, chylomicrons and VLDL are believed to be removed from plasma by a common mechanism (Havel, 1965; Boberg et al, 1969), this test provides a measure of the capacity of the subject to remove triglyceride. Because removal of large particles has been shown to be more rapid than that of small particles, the actual values for fractional removal rate obtained cannot reliably be used for determining actual lipoprotein triglyceride turnover. The fractional removal rate as measured by the intravenous fat tolerance test does, however, correlate significantly with triglyceride turnover measured chemically by splanchnic arterio-venous triglyceride differences (Rössner et al, 1974), although values obtained with the intravenous fat tolerance test are somewhat higher. The possible limitations of the intravenous fat tolerance test are discussed on Page 61. It was decided to use this test in the present study because of its simplicity and minimal discomfort to patients

Plasma FFA is the most important precursor for hepatic triglyceride formation in man (Havel, 1965) and plasma FFA levels have been shown to be commonly elevated in uncontrolled diabetes (Laurell, 1956; Bierman et al, 1957). As changes in the turnover of FFA through plasma may sometimes be more extensive than changes in plasma FFA levels (Lewis et al, 1966), measurements of plasma FFA turnover were performed in the present study both in the uncontrolled state and during diabetic control. Triglyceride input into plasma was not studied directly; in view of the precursor product relationship of plasma FFA and triglyceride, it was attempted to correlate plasma FFA turnover rate with plasma triglyceride concentration. A strongly positive correlation would provide suggestive evidence of plasma FFA turnover, and hence input, being a major determinant of plasma triglyceride concentration in diabetes.

CHAPTER III, SECTION B

Thirty-four diabetics were studied; measurements of serum lipid concentration, plasma free fatty acids turnover, and fractional removal rate of triglyceride were made in the uncontrolled state and again after achievement of diabetic control. Full details of methods used are described in Chapter II.

SUBJECTS

Selection criteria

Studies were performed on patients presenting to the Hammersmith Hospital Diabetic Clinic and diabetic in-patients. Diabetics requiring oral hypoglycaemic drugs were excluded from the study. Fully informed consent was obtained in all cases. Thirty-four diabetics were studied; twelve required insulin and twenty-two responded to diet alone. Ten obese subjects, with normal oral glucose tolerance tests according to the standards of FitzGerald and Keen (1964), were also studied. They were selected from patients attending the Lipid Disorders Clinic of the Hammersmith Hospital for obesity, or from obese in-patients, in hospital for non-metabolic reasons.

Insulin-dependent

The insulin-dependent group consisted of five males and seven female patients ranging from 30 - 58 years in age (mean - 40 years). For the purposes of this study, obesity was regarded as being present if the subject's weight exceeded their ideal body weight by more than 15% (Metropolitan Life Insurance Company Tables). By this criterion, two of this group were obese. Eight, one of whom was mildly ketotic, were studied at time of presentation with diabetes. In the remaining four patients, despite having previously been on insulin therapy, control was considered unsatisfactory. All four had a history of having either keto-acidosis, or having failed to respond to diet, alone or in combination with oral hypoglycaemic agents. In these four subjects, insulin was withdrawn for 24 - 48 hours in hospital under careful observation, after which the investigations were performed and effective insulin therapy reinstituted. A diabetic diet was continued throughout. One patient developed ketonuria during the period of insulin withdrawal. Studies were repeated after adequate diabetic control had been achieved, either within seven days during the same admission, or during a subsequent admission to hospital after one to two months. Control was achieved by dietary measures and insulin therapy as indicated. Control was regarded as satisfactory if the fasting blood glucose was less than 120 mg./dl., and if glycosuria did not occur at any time during the day.

Diet-responsive

In the diet-responsive group, there were ten males and twelve females aged 48 - 70 years, mean 57.5 years. All but five were obese by the above criteria. Ketosis was not detected in any. Treatment was by diet alone, without oral hypoglycaemic agents. Two diets were used; the obese diabetics received a daily intake of 800 calories with 60 grammes carbohydrate and 40 grammes fat, whereas the lean subjects received 1,800 calorie 150 gramme carbohydrate diets containing 90 grammes of fat. The type of fat was largely saturated. In-patients received diets prepared by the hospital diet kitchen; out-patients were carefully instructed by a member of the Dietetic Department and were supplie with diet sheets. Repeat measurements were made when diabetic control, defined as above, had been achieved.

Obese non-diabetic

The ten obese non-diabetic subjects ranged from 15 - 32% above ideal body weight. They received the same diet as the obese diabetics; they were restudied at periods ranging between 10 - 30 days from the onset of caloric restriction.

Predicted weight loss occured in both diabetic and non-diabetic groups, indicating satisfactory dietary adherance. All studies were performed after a 12 - 14 hour overnight fast. Blood was sampled for glucose (see Page 75), serum lipid deterination (see Pages 41 and 44) and lipoprotein electrophoresis (see page 46). Preparative ultracentrifugation (see page 47) to separate the very low density (VLDL) fraction of serum, which was then analysed for cholesterol and triglyceride content, was performed in some cases to confirm the type of hyperlipoproteinaemia. FFA turnover through plasma was measured during a constant infusion of albumin bound ^{14}C palmitic acid. From the equilibrium constant specific activity of palmitate and infusion rate of the labelled fatty acid, plasma FFA turnover was calculated. Full details of the method and calculation are to be found on Page 52 . The fractional removal rate of triglyceride from plasma, was determined by the intravenous fat tolerance test as described on page 65. Most patients were tested with the standard dose of Intralipid (0.1 gramme/Kg. body weight); some patients were re-tested at a dose of 0.25 - 0.3 gramme/Kg.

RESULTS

GLUCOSE

The insulin requiring diabetics had a mean Fasting (12 - 14 hour) blood glucose level of 271 mg./dl. in the uncontrolled state (range 178 - 428 mg./dl.). This fell to a mean of 94 mg./dl. during diabetic control. Lower values were detected in the diet responsive group. In the uncontrolled state levels ranged from 125 - 230 mg./dl. with a mean of 188 mg./dl.; mean blood glucose on the day of the repeat study was 102 mg./dl.

SERUM LIPIDS

Fasting plasma triglyceride concentrations are shown in Fig. 11. Pre-treatment levels of above 160 mg./dl. were observed in twelve of the twenty-two diet responsive (55) and eight of the twelve insulin-requiring diabetics (67%), as well as in two non-diabetic obese subjects.

In both groups of patients, diabetic control reduced fasting serum triglyceride levels significantly. A similar response to diet was seen in non-diabetic subjects.

Four patients were intensely lipaemic when their diabetes was uncontrolled; two were diet-responsive and two insulin-requiring. In view of the possibility that they may represent a distinct sub-group, they are considered separately.

Serum triglycerides fell from a mean of 158 ± 23 mg./dl. (SEM) to 110 ± 15 mg./dl. ($p = 0.02$, $n = 10$) and from 161 ± 23 mg./dl. (SEM) to 104 ± 12 mg./dl. ($p = 0.002$, $n = 18$) in the insulin-requiring and diet-responsive groups respectively. If the intensely lipaemic subjects are included, the means value before and during control are 1,060 and 116 mg./dl., and 556 and 110 mg./dl. for these two groups of patients.

The two insulin dependent patients with intense lipaemia had initial triglyceride levels of 5,000 mg./dl. and 6,175 mg./dl. respectively. Triglyceride concentration fell dramatically to 150 mg./dl. in both patients, on achieving control. The two diet-responsive diabetics with severe lipaemia had triglyceride levels

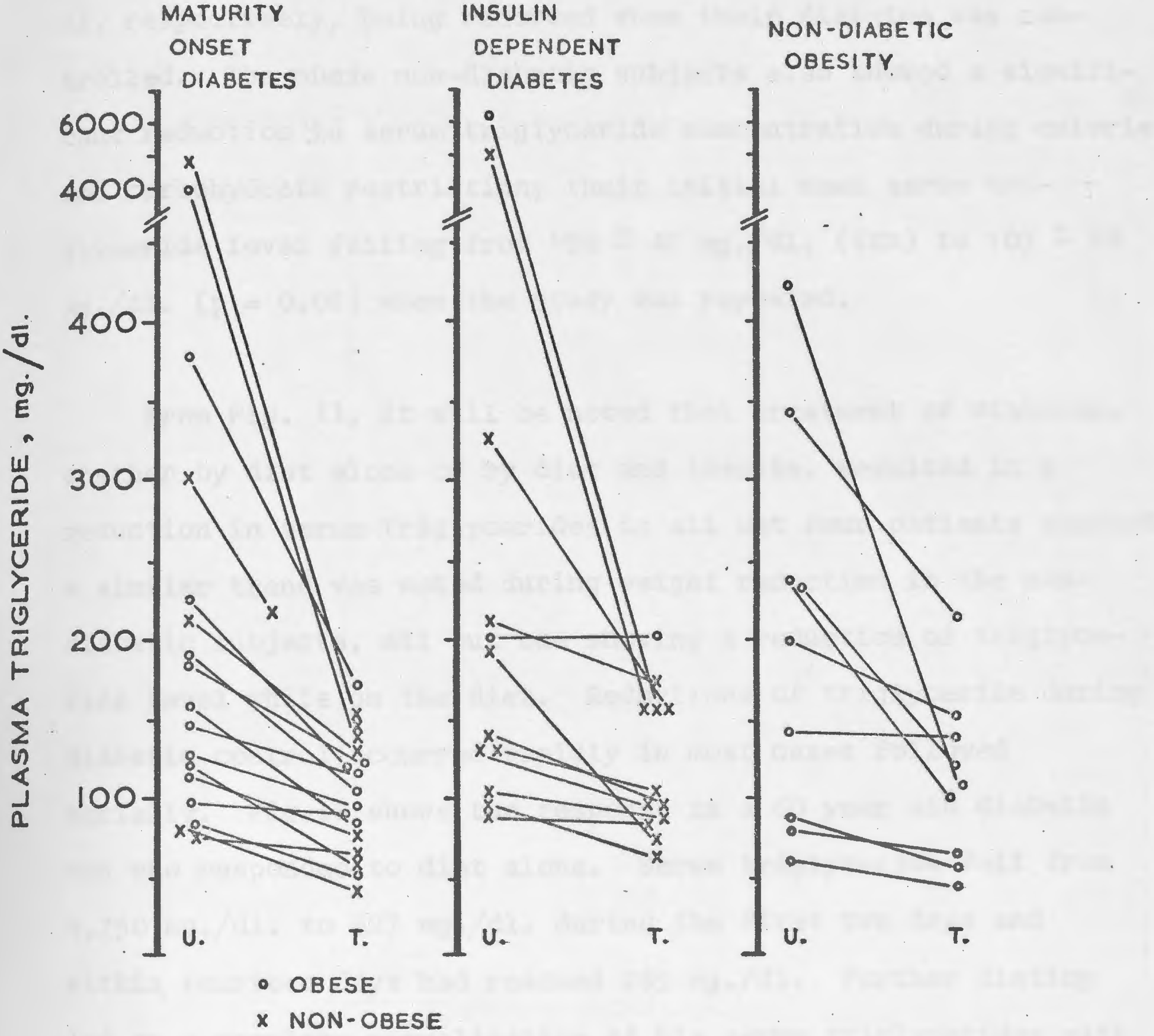

Fig. 11. Serum triglyceride in diabetes – effect of treatment

U = untreated

T = treated

of 4,000 and 4,750 mg./dl. initially, levels of 140 and 150 mg./dl. respectively, being recorded when their diabetes was controlled. The obese non-diabetic subjects also showed a significant reduction in serum triglyceride concentration during calorie and carbohydrate restriction; their initial mean serum triglyceride level falling from 198 ± 42 mg./dl. (SEM) to 107 ± 18 mg./dl. ($p = 0.02$) when the study was repeated.

From Fig. 11, it will be noted that treatment of diabetes, whether by diet alone or by diet and insulin, resulted in a reduction in serum triglycerides in all but four patients studied. A similar trend was noted during weight reduction in the non-diabetic subjects, all but one showing a reduction of triglyceride level while on the diet. Reductions of triglyceride during diabetic control occurred rapidly in most cases followed serially. Fig.12 shows the response in a 60 year old diabetic man who responded to diet alone. Serum triglyceride fell from 4,750 mg./dl. to 627 mg./dl. during the first two days and within fourteen days had reached 285 mg./dl. Further dieting led to a complete normalization of his serum triglycerides with a level of 131 mg./dl. one month later. Associated with this was a rapid disappearance of his eruptive xanthomata.

LIPOPROTEINS

Triglyceride levels of above 160 mg./dl. were associated with an increased prebeta band on lipoprotein electrophoresis in all cases studied. An excess of this lipoprotein was confirmed by ultracentrifugal separation and lipid analysis of the

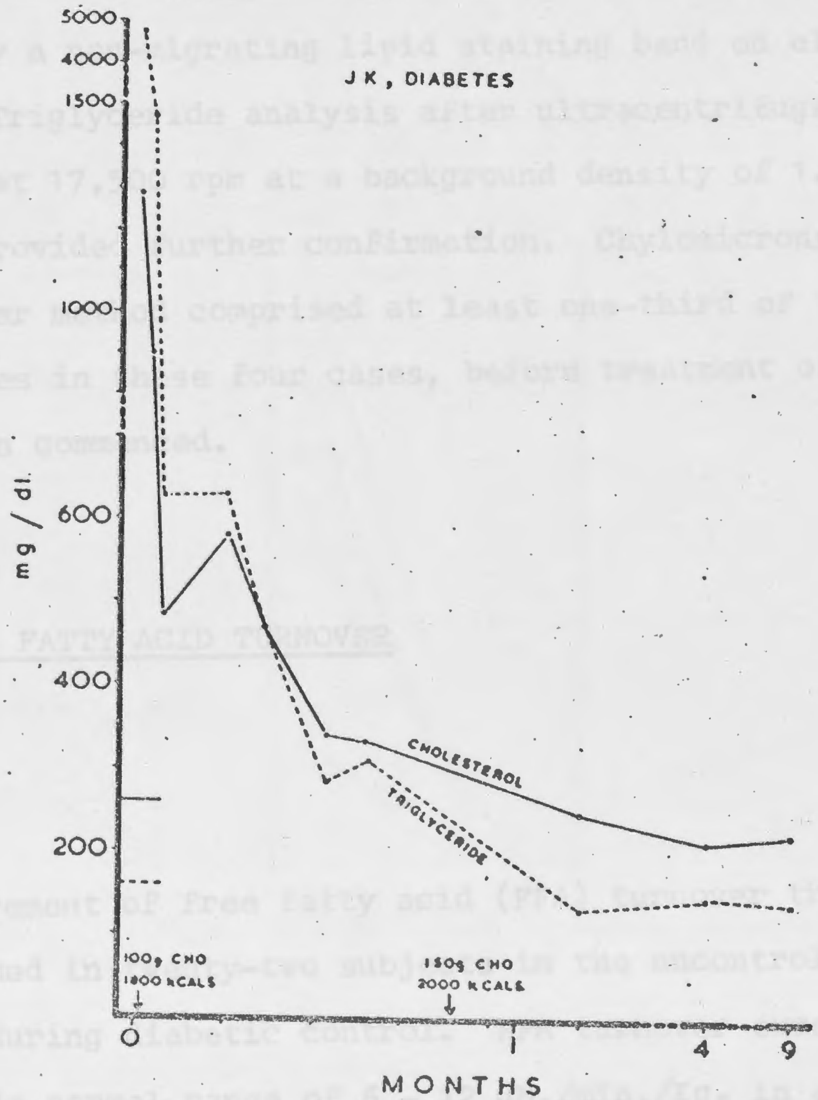

Fig. 12. Serum triglyceride and cholesterol in a 60 year old diabetic treated by dietary measures only

VLDL fraction in some cases. The four intensely lipaemic subjects had fasting chylomicronaemia, in addition to excess VLDL. This was demonstrated by the appearance of a creamy supernatant after overnight storage at 4° C (Cramp, 1973) and confirmed by a non-migrating lipid staining band on electrophoresis. Triglyceride analysis after ultracentrifugation for 30 minutes at 17,500 rpm at a background density of 1.006 (see Page 47) provided further confirmation. Chylomicrons as defined by the latter method comprised at least one-third of the total triglycerides in these four cases, before treatment of the diabetes was commenced.

PLASMA FREE FATTY ACID TURNOVER

Diabetes

Measurement of free fatty acid (FFA) turnover through plasma was performed in twenty-two subjects in the uncontrolled state and again during diabetic control. FFA turnover exceeded the laboratory's normal range of 6 - 12 µM./min./Kg. in all but one subject studied.

In seventeen diet responsive cases, it was 24 ± 3 µM./min./Kg. (mean ± SD), and in five insulin dependent cases 69 ± 27 µM./min./Kg. The highest rates thus occurred amongst insulin-requiring diabetics (see Fig. 13).

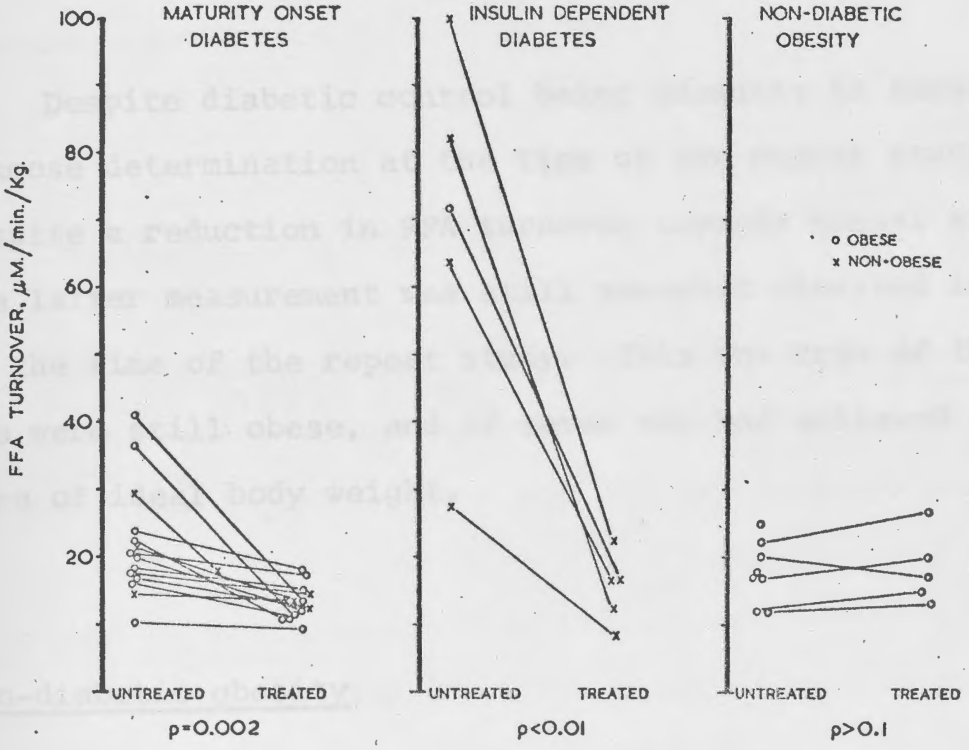

Fig. 13. Plasma FFA turnover in diabetes - effect of treatment

Turnover was reduced towards normal in all insulin-requiring cases when diabetic control was achieved, reaching a mean of 15 ± 5 (SD) μM./min./Kg. ($p < 0.01$ by the paired t-test). In fourteen diet-responsive diabetics in whom repeat measurements were made, turnovers fell from 22 ± 8 to 13 ± 3 μM./min./Kg. ($p = 0.002$). All but two patients showed a reduction in FFA turnover during dieting.

Despite diabetic control being adequate in terms of blood glucose determination at the time of the repeat studies, and despite a reduction in FFA turnover towards normal at this time, the latter measurement was still somewhat elevated in many cases at the time of the repeat study. This was true of three patients who were still obese, and of seven who had achieved or virtually were of ideal body weight.

Non-diabetic obesity

FFA turnover through plasma was measured in nine obese non-diabetic subjects, five of whom underwent second studies during dietary restriction. The second study was carried out after an interval of 10 - 30 days, the diet being identical to that prescribed for the obese diet-responsive. FFA turnover was initially 17 ± 5 μM./min./Kg. (mean ± SD). There was no significant change in the five subjects who had the study repeated. Mean values were 16 ± 5 (SD) and 18 ± 5 before and during treatment respectively, in these patients ($p > 0.1$).

Correlations between plasma FFA turnover and serum triglyceride concentration before diabetic control are shown in Fig. 14. Positive correlations were observed in both groups of diabetics; these were, however, of low order and were not statistically significant. (Diet treated group - $r = +0.21$, $p > 0.1$; insulin-requiring diabetics - $r = +0.62$, $p > 0.1$). The obese non-diabetic subjects showed a non-significant negative correlation ($r = -0.32$, $p > 0.1$).

INTRAVENOUS FAT TOLERANCE TEST

The rate constant, K_2, for the fractional removal rate of exogenous triglyceride, has been determined in uncontrolled diabetics and repeated after control had been achieved.

Results are shown in Fig. 15. In two of the intensely lipaemic subjects, no difference in nephelometric reading was discernible during the period of sampling; no detectable triglyceride removal had occurred and their rate constants were regarded as zero. In all other cases, disappearance of the fat emulsion was exponential when a dose of 0.1 gramme/Kg. was injected. Rate constants can be seen to vary widely from patient to patient. K_2 values for both groups of diabetics are shown in Fig. 16. Although absolute values were higher in females, fractional removal rates were in the low normal or sub-normal range, for each sex.

Treatment of the diabetes, whether by diet alone or by diet and insulin, resulted in an increase in the rate constant, often

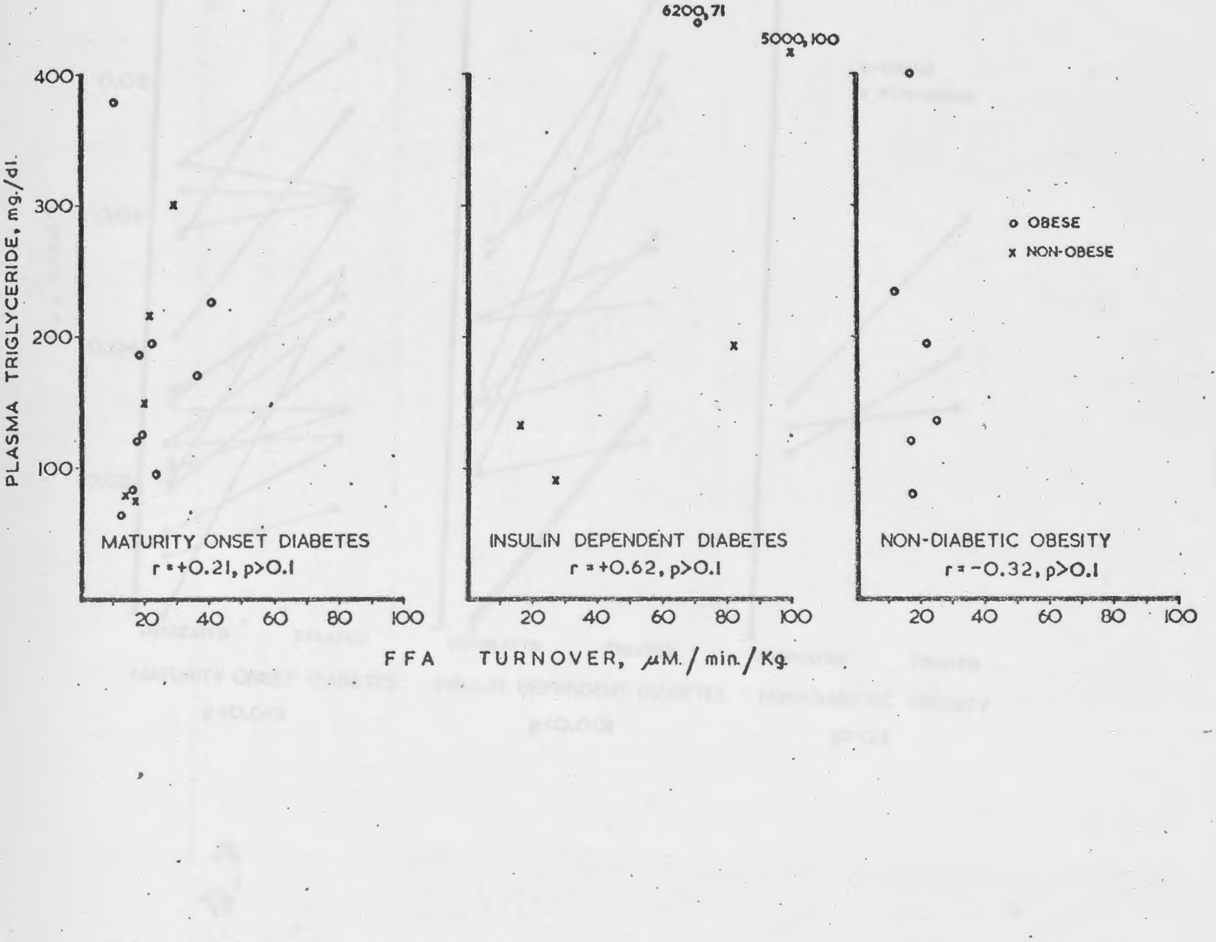

Fig. 14. Correlations between serum triglyceride and FFA turnover

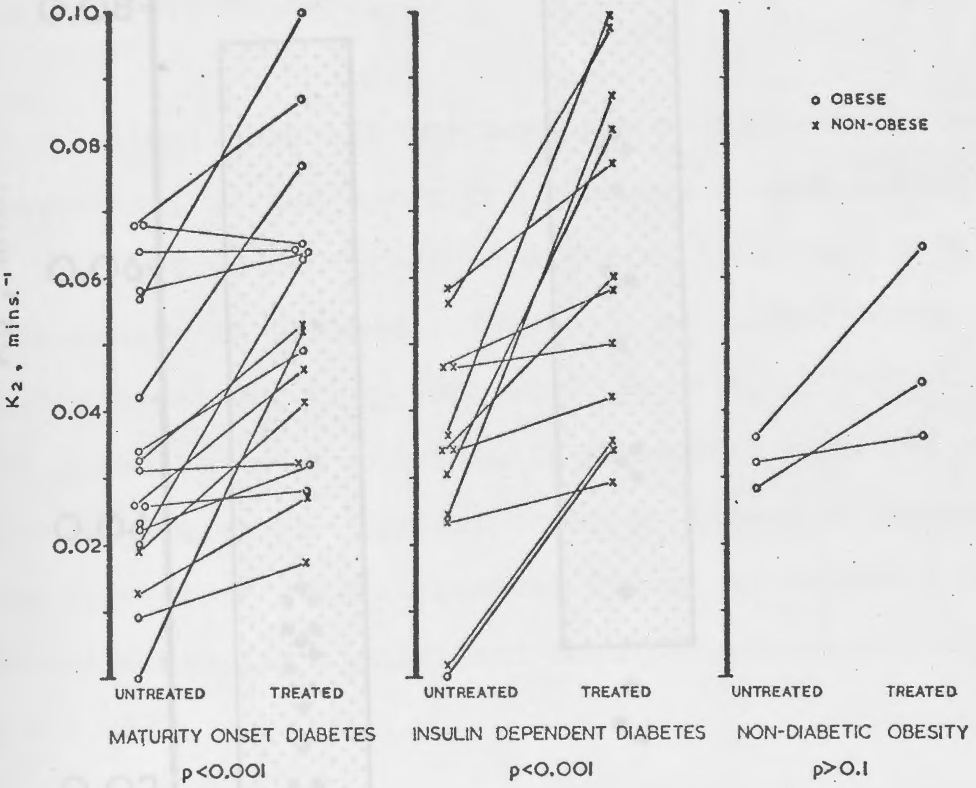

Fig. 15. K_2 in diabetes - effect of treatment

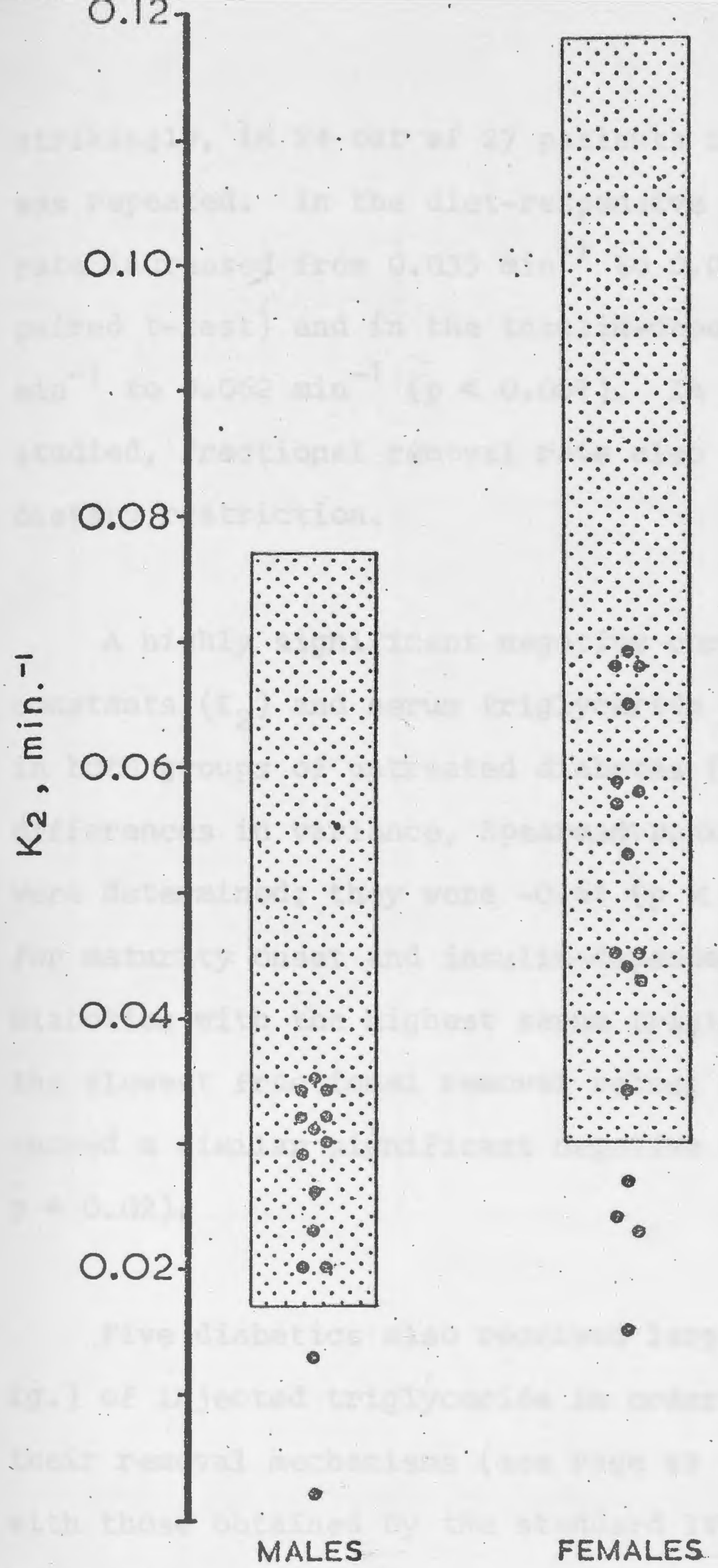

Fig. 16. K_2 in diabetes
(Hatched area represents the normal range)

strikingly, in 24 out of 27 patients in whom this measurement was repeated. In the diet-responsive group, fractional removal rate increased from 0.035 min^{-1} to 0.053 min^{-1} ($p < 0.001$ by the paired t-test) and in the insulin-dependent group, from 0.032 min^{-1} to 0.062 min^{-1} ($p < 0.001$). In the few obese subjects so studied, fractional removal rate also tended to increase with dietary restriction.

A highly significant negative correlation between rate constants (K_2) and serum triglyceride concentration was observed in both groups of untreated diabetes (see Fig. 17). Because of differences in variance, Spearman's rank correlation coefficients were determined; they were −0.61 ($p < 0.01$) and −0.70 ($p < 0.02$) for maturity onset and insulin-dependent diabetes respectively. Diabetics with the highest serum triglyceride values thus had the slowest fractional removal rates; non-diabetic obese subjects showed a similar significant negative correlation ($r = -0.68$, $p < 0.02$).

Five diabetics also received larger doses (0.25 - 0.3 gramme Kg.) of injected triglyceride in order to attempt to saturate their removal mechanisms (see Page 69) and compare these results with those obtained by the standard IVFTT. The larger injection resulted in linear disappearance during the first 60 minutes (by zero order kinetics), followed by exponential disappearance. The serum triglyceride concentration immediately following the injection of the larger dose was calculated to be at least 1,000 mg./dl., the initial linear removal implying saturation of peripheral removal mechanisms. The slope of the line gives the maximal removal rate (K_1). Table 3 shows that in four out

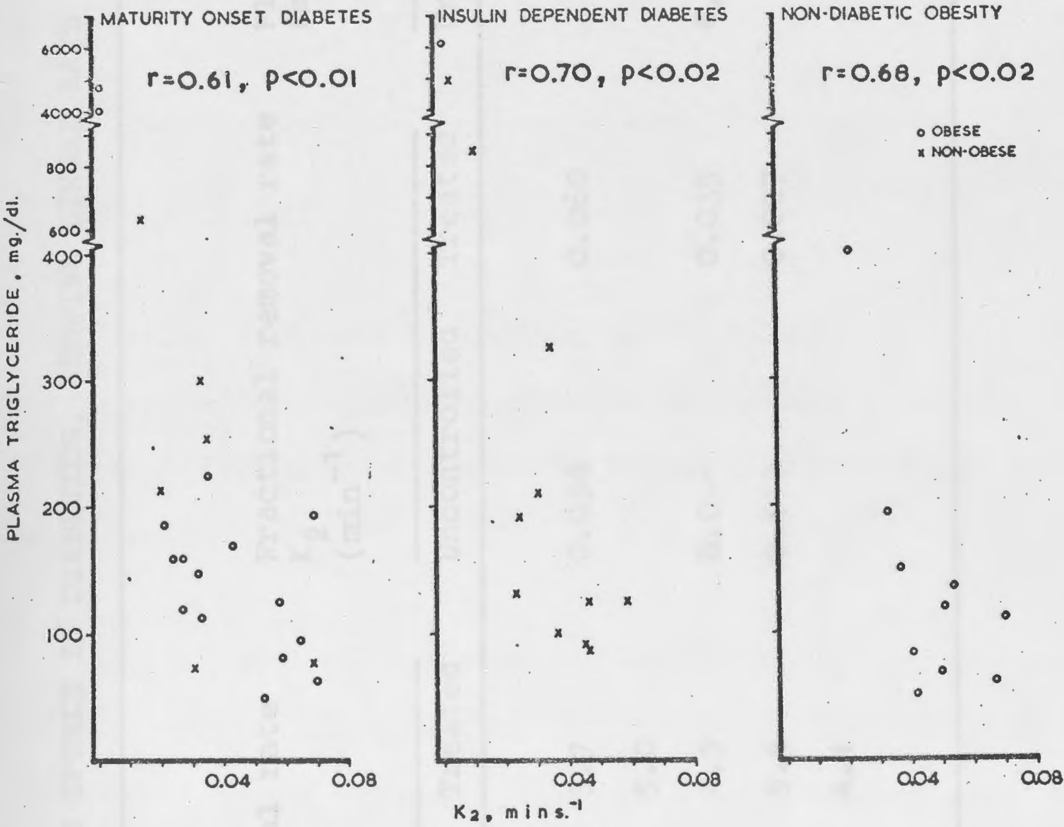

Fig. 17. Correlations between serum triglyceride and K_2

TABLE 3

PLASMA TRIGLYCERIDE UPTAKE IN DIABETICS, SHOWING LINEAR RATE K_1

Treatment	Maximal removal rate K_1 (mg/dl/min)		Fractional removal rate K_2 (min^{-1})		Plasma triglyceride (mg/dl)	
	Uncontrolled	Treated	Uncontrolled	Treated	Uncontrolled	Treated
1. Insulin	2.1	3.7	0.034	0.060	324	153
2. Insulin	5.3	5.0			158	198
3. Insulin	0.0	4.5	0.0	0.035	6,175	150
4. CH restriction	2.2	5.6	0.013	0.027	631	180
5. 800 kcals	3.5	4.1			379	173

of five patients, K_1 increased during diabetic control associated with a reduction in serum triglyceride. The fifth patient showed neither a fall in serum triglyceride nor an improvement in K_1. Diabetic control thus tends to cause maximal removal rate to change in the same direction as does fractional removal rate, which has been shown to be negatively correlated with serum triglyceride concentration.

SUMMARY OF RESULTS

Diabetic control, whether by diet alone or by diet and insulin, reduced fasting serum triglyceride concentrations. A similar effect was observed in obese non-diabetics during dietary restriction.

Plasma FFA turnover was elevated in diabetics, especially in those requiring insulin. Diabetic control reduced the FFA turnover towards normal, although normal values were often not achieved during the period of study. The mildly elevated FFA turnover rates observed in the obese non-diabetics showed no tendency to fall with weight reduction.

Subjects with the lowest fractional removal rates of injected triglyceride had the most marked elevation of serum triglyceride. This rate constant increased markedly with diabetic control, whether by diet alone or by diet and insulin. When removal mechanisms were saturated, the same trend was apparent.

CHAPTER III, SECTION C

SERUM LIPIDS AND LIPOPROTEINS

Serum triglyceride in excess of 160 mg./dl., the arbitary cut-off point in use in this laboratory at the time of the study, was found in rather more than half the diabetics in the uncontrolled state. The findings were similar whether the diabetes was of the insulin-dependent or diet-responsive type. Because of small numbers and of exclusion of diabetics requiring oral hypoglycaemic drugs, no adequate conclusion can be made from the present study regarding the prevalence of hyperlipidaemia in diabetes. None the less, a higher percentage of adult onset diabetics were noted to be hyperlipidaemic than in the study of Elkeles et al (1971). A slightly lower percentage of insulin requiring diabetics were found to be hyperlipidaemic than reported by Chance et al (1969) in newly diagnosed diabetic children. The mean age of the subjects in the latter study was, however, far lower than in the present investigation; direct comparison is thus not valid. Also, in this study, serum triglyceride concentrations were not measured, hyperlipidaemia being diagnosed by an elevated serum cholesterol, total lipid concentration, or an abnormal lipoprotein pattern on paper electrophoresis of serum.

The majority of diabetics with abnormal serum lipids were found in the present study to have elevated serum triglyceride levels associated with an increase in pre-beta lipoprotein; they would thus be classified as having the Type IV lipoprotein pattern in their plasma (Beaumont et al, 1970). Although Chance et al (1969) also found Type IV hyperlipoproteinaemia to be the most frequent abnormal pattern in diabetic children, they also reported a fairly high frequency of Type III hyperlipoproteinaemia; no example of this abnormal lipoprotein pattern was found in the present study. Hayes (1972) found a roughly equal frequency of Types II, III and IV lipoprotein patterns. Some caution must be exerted in the interpretation of these studies. In that of Chance et al (1969), blood was sampled in the non-fasted state. Further doubt is cast by their definition of Type III and that ultracentrifugal confirmation of this infrequently reported lipoprotein pattern was not obtained. In the study of Hayes (1972), diabetics were typed in the manner of the WHO classification (Beaumont et al, 1970), but using densitometer scanning of cellulose acetate electrophoretic strips, which has not been shown to be quantitative.

Four of the patients in the present study had intense lipaemia of the type described by Bagdade et al (1967) in certain insulin requiring diabetics. Of these, only two proved to require insulin, while two responded fully to diet. The latter two patients are probably of the sub-group which Nikkilä and Kekki (1973) refer to as the 'diabetic lipaemic syndrome'. These four patients had increased VLDL concentrations and chylomicrons in plasma in the fasted state, the so-called Type V pattern (Beaumont et al, 1970). Bagdade et al (1967) have referred to this entity as an acquired form of fat induced lipaemia, because chylomicrons are commonly equated with particles derived from dietary fat (see Chapter VI). Particles of a similar size to chylomicrons may, however, appear in plasma even in the absence of dietary fat; Mancini et al (1973) have shown the presence of such particles during carbohydrate induction in subjects on virtually fat free diets. Chylomicrons and VLDL triglycerides are believed to be removed from plasma by a common mechanism following hydrolysis by lipoprotein lipase (Havel, 1965); this mechanism has been shown to be saturable (Boberg et al, 1969) following an injection of an artificial triglyceride emulsion. Recently, (Brunzell et al, 1973) have presented evidence for a common saturable removal mechanism for both triglyceride bearing lipoprotein categories, chylomicrons and VLDL. A defective removal mechanism in diabetes, as suggested by the present work, could then conceivably result in an accumulation in plasma of both VLDL and larger chylomicron-like particles, whether of dietary or endogenous origin. A stage could be reached where elimination of triglyceride is so slow, that an overnight fast would be insufficient to remove all dietary fat; exogenous and endogenous hyperlipaemia could then coexist.

EFFECT OF DIABETIC CONTROL

Control of the diabetic state resulted in a significant reduction in plasma triglyceride concentration. This was found to occur whichever mode of treatment was used.

Control by calorie and carbohydrate restriction

Of thirteen subjects whose serum triglycerides were measured in the uncontrolled state, and again after calorie and carbohydrate restriction, all but two showed reduced levels after treatment. Both showed adequate weight reduction. Only one diabetic in the group on this diet had a serum triglyceride level above 160 mg./dl. at the time of the second study. In this patient, however, levels had more than halved, falling from 375 mg./dl. before treatment, to 180 mg./dl. during diabetic control. It is possible that serum triglyceride concentration falls more slowly than does blood glucose. If so, had the second study been performed at a later date, a lower triglyceride value may have been obtained. It is worth emphasizing that the fall in serum triglyceride concentration during calorie and carbohydrate restriction is not a specific change related to diabetics; when fed an identical diet, non-diabetic obese subjects also showed a fall in their serum triglycerides of the same order of magnitude as that observed in obese diabetics.

Control by carbohydrate restriction

Of the subjects who received the 1,800 calorie, 150 gramme carbohydrate diet, four out of five showed a reduction in serum triglyceride concentrations. All were below 160 mg./dl. at the time of the second study. In most cases, this diet was approximately isocaloric with their usual diets, although all were carbohydrate restricted compared with the diets consumed at the time of the original investigation. One intensely lipaemic patient showed a fall in his serum triglyceride from 4,750 mg./dl. to 150 mg./dl. on what for him was an isocaloric carbohydrate restricted diet. It thus appears that carbohydrate restriction, as distinct from calorie restriction, results in a reduction of serum triglyceride levels in diabetics.

Control by insulin and diet

As the group of diabetics requiring insulin received dietary therapy in addition, it is impossible to differentiate the effect of diet from that of insulin. Most had approximately isocaloric, carbohydrate restricted diets, only two requiring marked calorie restriction in addition. Of the insulin requiring diabetics, two of the twelve restudied had serum triglyceride concentrations of above 160 mg./dl. at the time of the repeat investigation; the triglyceride level had actually risen slightly in one of these patients despite the low calorie, low carbohydrate diet, and insulin. Triglyceride levels fell in all other cases, often strikingly. The two intensely lipaemic patients who required insulin, both had normal serum triglycerides when restudied.

The findings in the present study, suggest that the most adequately treated diabetics will have serum concentrations within the normal range.

Reports in the literature on the effect of treatment vary, probably largely contributed to by variations in efficacy of control. Several studies suggest that triglyceride levels revert to normal with adequate diabetic control as judged by blood glucose criteria (Aldersberg et al, 1956; Sterky et al, 1963; Östman, 1965; Sterky et al, 1966). Diabetic therapy by diet and insulin or sulphonylureas, resulted not only in an improvement of diurnal blood sugar values, but of diurnal serum triglyceride changes as well (Maruhama et al, 1967). Other studies, however, show diabetics to have higher serum triglycerides than controls, despite diabetic control (Albrink et al, 1963; Schrade et al, 1963; New et al, 1963; Hayes, 1972). The effect of treatment on maturity onset diabetes is even less clear when oral hypoglycaemic agents are used. Sailer (1966) showed that tolbutamide controlled diabetics had higher triglyceride levels than did controls. Although no non-diabetic controls were studied by Elkeles et al (1971), only 10 out of 80 treated diabetics were hypertriglyceridaemic, whether treated by diet alone, or in combination with hypoglycaemic agents.

If, as shown by the present study, serum triglycerides as well as blood glucose levels tend to fall during treatment of the diabetes, an explanation must be sought for those cases in this and other studies (Albrink et al, 1963; Schrade et al, 1963; New et al, 1963; Hayes, 1972) whose serum triglycerides remained elevated despite blood sugar levels achieving normal values. One possibility is that the changes in blood glucose occur more rapidly than those in serum triglycerides; the latter may then fall with the further passage of time. Another explanation may be the coexistence of diabetes and primary hypertriglyceridaemia, both conditions having a high prevalence. The incidence of diabetes appears to be between 1 + 8% (Renold et al, 1972), depending on the method of detection and arbitrarily chosen cut-off points dividing normal from abnormal. The prevalence is undoubtedly somewhat higher. High serum triglycerides also occur with remarkable frequency among so-called normal individuals. As previously mentioned, Lewis et al (1974) found 14% of men and 3% of women to have serum triglycerides in excess of 176 mg./dl. Known diabetics were excluded from this study. Hypertriglyceridaemia commonly occurs as a secondary manifestation of diabetes and conversely, primary hypertriglyceridaemia is frequently associated with abnormalities of glucose tolerance (Albrink and Davidson, 1966; Fredrickson et al, 1967). Some cases may clearly fall under either one or other of these headings; in many, however, difficulty arises in deciding which is the primary disorder. None the less, primary diabetes mellitus and primary endogenous hypertriglyceridaemia are both common, and may, therefore, coexist. The most likely explanation for elevated serum triglycerides despite diabetic therapy, however, is that with time, adherence to diabetic dietary regimes becomes less than adequate to maintain serum triglyceride at normal levels. Some degree of dietary indiscretion, for example carbohydrate excess, might be sufficient to elevate serum triglyceride levels without affecting blood glucose concentrations.

Hypertriglyceridaemia has been shown to be an independent risk factor for atherosclerosis (Carlson and Böttiger, 1972). As hypertriglyceridaemia occurs commonly in diabetics, especially those with atherosclerosis (Keiding et al, 1952; Lowy and Barach, 1958; Albrink et al, 1963), management of diabetic patients should thus attempt to correct both carbohydrate and lipid abnormalities.

criteria of diabetic control ought perhaps to be revised, and to include control of serum lipids in addition to blood glucose.

FREE FATTY ACID TURNOVER IN DIABETES

The turnover of plasma free fatty acid exceeded our normal range of 6 - 12 μM./min./Kg. in all but one of the patients studied. This normal range was derived from measurement of FFA turnover in twelve healthy members of hospital and laboratory staff. There was a tendency for higher FFA turnover rates to occur in insulin-requiring diabetics; this is in conformity with earlier work showing high plasma FFA concentrations in insulin-dependent patients (Laurell, 1956; Bierman et al, 1957). The obese diet-responsive diabetics also had abnormally elevated plasma FFA turnovers, lending support to the findings of Sailer et al (1967). Normal FFA turnover has, however, been reported in diabetics (Ford et al, 1963). In this study, plasma FFA turnover was measured after a pulse injection of labelled fatty acid. This method requires frequent blood sampling which may stress the patient, leading to higher FFA levels and hence lower FFA specific activities than a less stressful method. It also requires calculation of pool size. Its use has largely been superceded by the constant infusion method used in the present study. Furthermore, in the study of Ford et al (1963), case details are poorly described; the mean fasting blood glucose of the diabetic patients was 167 ± 49 mg./dl., suggesting that in some of them, the diabetic state was relatively mild at the time of study.

THE EFFECT OF TREATMENT ON PLASMA FREE FATTY ACID TURNOVER

Treatment of the diabetic state led to a reduction of FFA turnover in most cases studied. Plasma FFA turnover fell in all the insulin-treated group; in two of fourteen diet-responsive patients in whom the study was repeated, no fall was evident. Statistically significant differences were, however, found in both groups of diabetic studied. It is of interest that though FFA turnover was nearly always reduced by diabetic control, it often remained somewhat above normal. The reason for this is not clear. The criteria accepted for diabetic control in this study was a fasting glucose below 120 mg./dl.; this does not necessarily imply normality of glucose metabolism throughout the day. It may be postulated that diabetic control was inadequate in terms of lipid metabolism; conceivably, full diabetic control should include correction of FFA and triglyceride abnormalities, as well as glucose. The residual elevation of FFA turnover occurred both in obese and in lean diet-responsive patients, some of whom were at ideal body weight by the time of the repeat investigation. The degree to which FFA turnover was increased in the uncontrolled state also did not relate to the presence of obesity. Increased FFA concentrations have been recorded in non-diabetic obesity (Dole, 1956; Gordon, 1960; Opie and Walfish, 1963), yet in the present study, highest turnovers occurred in lean, insulin-dependent patients, though only two were mildly ketotic at the time of study.

OBESE NON-DIABETICS

In keeping with other reported studies (Nestel and Whyte, 1968; Miller et al, 1968; Birkenhäger and Tjabbes, 1969), FFA turnover was somewhat elevated in the non-diabetic obese patients. The response to a calorie restricted diet was, however, in sharp contrast to that of obese diet-responsive diabetics who received an identical diet. In fact, four out of five obese non-diabetics in whom repeat measurements were made while on diet, FFA turnover rate showed no significant change, compared with the significant reduction seen in diabetics. No significant difference in weight loss between the two groups was noted. The reason for this apparent difference in behaviour between obese diabetics and non-diabetics is not clear, although the slight increase observed in FFA turnover in the non-diabetic subjects is as would be expected of normal subjects during caloric restriction. Similar elevation in FFA turnover have previously been reported in fasting obese patients (Birkenhäger and Tjabbes, 1969), and Jackson et al (1972) found a rise in plasma FFA concentrations in the majority of obese patients treated by fasting.

Plasma FFA is the major source of plasma triglyceride fatty acid in fasting man (Havel, 1961; 1965). In view of this, the elevated plasma FFA turnover might be responsible for excess triglyceride production, and for the hyperglyceridaemia frequently seen. No significant correlation between FFA turnover and fasting serum triglycerides was found in either type of diabetic. As FFA reaching the liver has many alternate fates, and as hepatic triglyceride may derive from sources other than plasma FFA, hypersecretion of triglyceride by the liver in diabetes cannot be excluded. Although no definite conclusions can be made from the data, the lack of significant correlation between FFA turnover and serum triglyceride levels may none the less suggest that the excess triglyceride concentration in plasma does not depend primarily on excess free fatty acid mobilization and hence, hypersecretion. This would be consistent with findings in the isolated perfused rat liver (Heimberg et al, 1966; Van Harken et al, 1967; Heimberg et al, 1967), the pancreatectomized dog (Basso and Havel, 1970), and that of Sailer et al (1967b) on FFA incorporation into plasma triglyceride in insulin-dependent diabetics; all three studies showed triglyceride secretion to be reduced in diabetes.

Poor correlations were also observed between FFA levels and serum triglyceride in diabetes by Nikkilä and Kekki (1973), yet these authors suggest over-production of triglyceride to be the prime cause of diabetic hypertriglyceridaemia on the basis of their kinetic data. The reason for the difference between their findings and those in experimental diabetes and of Sailer et al (1967b), is for the present not clear. In order to explain their findings, it is necessary to postulate that the reduced triglyceride secretion demonstrated in vitro, is counteracted by the increased FFA transport shown in this study and elsewhere (Sailer et al, 1967a). Despite a reduced capacity for triglyceride formation, the liver of a diabetic animal could, if supplied with sufficient substrate, conceivably produce more triglyceride than its normal counterpart with a lesser supply of FFA. The net effect of these opposing influences in the intact animal was shown to result in a reduced triglyceride secretion in pancreatectomized dogs (Basso and Havel, 1970), although studies from the same laboratory showed enhanced triglyceride secretion in dogs shortly after the administration of anti-insulin serum (Balasse, Bier and Havel, 1972). Extrapolation of these findings to human diabetes poses obvious difficulties. The lack of significant correlation between FFA turnover and serum triglyceride levels observed in the present study, does not exclude the possibility of enhanced triglyceride secretion being a factor in the production of hypertriglyceridaemia in some cases.

TRIGLYCERIDE REMOVAL

Using the intravenous fat tolerance test, the fractional removal rates of triglyceride (K_2 values) in untreated diabetics, tended to be in the low normal and sub-normal range for both sexes. Fractional removal rate was slowest in those diabetics with the highest triglyceride levels whether diet-responsive or insulin sensitive, a highly significant correlation between K_2 and serum triglyceride concentration being observed.

When diabetic treatment was instituted, fractional removal rates rose in almost all cases. Associated with the enhanced removal rates, triglyceride concentrations fell. It is of interest that the pattern of response was the same in both insulin-dependent and diet-responsive cases.

Before interpreting low K_2 values to signify reduced fractional turnover of <u>endogenous</u> triglyceride, it is necessary to consider an alternate explanation, namely, that the slow disappearance of Intralipid is a consequence of an expanded plasma triglyceride pool. Nikkilä and Kekki (1973) have interpreted the reduced K value found in some of their diabetic patients to be a function of triglyceride pool size, on the basis of the same regression line between K values and serum triglyceride concentration in diabetics and non-diabetics. Conversely, it could be argued that the higher triglyceride levels are a result of slower fractional removal rates of triglyceride from plasma. The evidence in favour of the latter argument is discussed on pages .

In the present investigation, the significance of the fractional turnover rate has further been tested by performing the intravenous fat tolerance test at a higher dose in some patients. With the higher plasma concentrations achieved, the emulsion disappears in a linear fashion by zero order kinetics; saturation of removal mechanisms is implied, and maximal removal rate or K_1 is measured in mg./dl./min. (Boberg et al, 1969). In four out of five such studies the K_1 value was found to increase when the diabetes was controlled. Assuming plasma volume not to change between the two studies, K_1 is independent of pool size considerations; these results then lend support to the findings with fractional removal rates. In the single case in which K_1 values failed to increase with diabetic control, triglyceride levels did not decrease between the first and second studies. These observations with the intravenous fat tolerance test suggested that the enhanced rate of disappearance of the fat emulsion when the diabetes is controlled, is not a consequence of a difference in pool size.

A point of some interest is the relationship of K_1 to the fasting serum triglyceride. Using the polyvinylpyrrolidone gradient technique (Gordis et al, 1962), Boberg et al (1969) reported no correlation between K_1 and K_2 or between K_1 and triglyceride concentration. The present studies, utilizing the apparently more sensitive nephelometric technique of detecting exogenous triglycerides, have shown the relationship of K_1 to triglyceride to be analogous to that of K_2 and triglyceride concentration. This suggests that K_1 and K_2 may be measuring aspects of the same process.

As discussed on page 19, tissue lipoprotein lipase activity may be the rate-limiting factor in the removal of triglyceride from plasma. Although in experimental diabetes, this enzyme has been shown to be reduced in adipose tissue of diabetic rats (Kessler, 1963; Brown, 1967; Elkeles and Williams, 1974), evidence for its deficiency in human diabetes is less convincing. Reduced post-heparin lipolytic activites (PHLA) have, however, been reported in insulin-dependent diabetics (Bagdade et al, 1967). Most studies of lipoprotein lipase in humans have measured the enzyme in plasma after its release from tissue stores by heparin. The problems relating to this method have been discussed on page 120. Changesin PHLA may not be sufficiently sensitive to reflect differences in fractional removal rate. As the latter probably depends on lipoprotein lipase activity, it would be of considerable interest to try to relate fractional removal rates of triglyceride to measurements of human adipose tissue and muscle lipoprotein lipase when satisfactory methods become available. It is possible that removal could be limited by other stages in the uptake of triglyceride from plasma, e.g. re-esterification of fatty acids in the adipocyte, as this has been shown to be defective in diabetics (Östman, 1965; Bjöntorp and Hood, 1966).

A question which as yet remains unanswered is why all diabetics do not exhibit hypertriglyceridaemia. Over half the patients studied in the present work had triglyceride levels in excess of 160 mg./dl. Other authors have reported both lower (Elkeles et al, 1971) and higher (Chance et al, 1969) frequencies. The serum triglyceride concentration is determined by both input and removal of triglyceride. The effect of insulin lack at various sites could potentially lead to a wide range of secretion rates. For example, rapid FFA turnover and hence presumably, rapid hepatic uptake, could in the presence of sufficient insulin lead to triglyceride over-production. If removal mechanisms were able to cope with the increased influx, normal serum triglyceride levels would result. On the other hand, a reduced clearance of triglyceride for any of the reasons discussed above, could lead to triglyceride accumulation in plasma especially in the face of over-production. Thus differing secretion rates could interact with impaired removal to produce the wide range of serum triglyceride concentrations encountered in diabetes. The duration of insulin deficiency may be of importance in addition to the degree, as suggested by the markedly contradictory effect on triglyceride production of pancreatectomy (Basso and Havel, 1970) and anti-insulin serum (Balasse et al, 1972) when studied by the same method in the same laboratory.

The effect of other hormones must also be considered. Glucagon levels have been shown to be elevated in some diabetics

(Unger et al, 1970); besides stimulating lipolysis, this hormone is believed to inhibit lipoprotein secretion (Vaughan et al, 1964) by inhibiting apoprotein synthesis (Eaton et al, 1973), although Eaton and Schade (1973) have hypothesized that diabetics may be resistant to the lipid lowering effect of glucagon. A wide range of secretion rates could again be postulated to arise due to the effect of glucagon. These could interact with various removal rates to produce a large spectrum of serum triglyceride concentrations.

It can be seen that the factors affecting serum triglyceride concentration in diabetes are extremely complicated. To a certain extent, this is because of the heterogeneity of the diabetic syndrome, with insulin lack being present at one end of the spectrum and hyperinsulinism at the other. Furthermore, the interaction of constitutional factors, dietary factors such as the amount and type of fat and carbohydrate, and the intake of alcohol with the metabolic defects due to the diabetes, can further complicate the picture. Studies on experimental animals have done much to help our knowledge of the subject, but we are still a long way from fully understanding the many complex metabolic disturbances, and the manner in which they relate to each other, in diabetes mellitus.

CHAPTER III - REFERENCES

Abraham, S., Migliorini, R.F., Bottz, W. and Chaikoff, I.L. (1962) Biochim. Biophys. Acta 62, 27.

Abrams, N.E., Jarrett, R.J., Keen, H., Boyns, D.R. and Crossby, J.R. (1969) Brit. med. J. 1, 599.

Adlersberg, D., Wang, E.J., Rifkin, H., Berkman, J. Ross, G. and Weinstein, L. (1956) Diabetes 5, 116.

Albrink, M.J., Lavietes, P.H. and Man, E.B. (1963) Ann. intern. Med. 58, 305.

Albrink, M.J. and Meigs, J. (1964) Amer. J. clin. Nutrit. 15, 255.

Albrink, M.J. and Davidson, P.C. (1966) J. Lab. clin. Med. 67, 573.

Altman, K.I., Miller, L.L. and Bly, C.G. (1951) Arch. Biochem. Biophys. 31, 329.

Bagdade, J.D., Porte, D.,Jr. and Bierman, E.L. (1967) New Engl. J. Med. 276, 427.

Bagdade, J.D., Porte, D.,Jr. and Bierman, E.L. (1968a) J. clin. Invest. 47, 3-a.

Bagdade, J.D., Porte, D.,Jr. and Bierman, E.L. (1968b) Diabetes 17, 127.

Balasse, E.O., Bier, D.M. and Havel, R.J. (1972) Diabetes 21, 280.

Balasse, E.O. and Ooms, H.A. (1973) Diabetologia 9, 145.

Balodimos, M.C., Ball, J.J. and Williams, R.H. (1962) Metabolism 11, 365.

Barritt, D.M. (1956) Brit. med. J. 2, 640.

Basso, L.V. and Havel, R.J. (1970) J. clin. Invest. 49, 537.

Bates, M.W., Zomzely, C. and Mayer, J. (1955) Endocrinology 57, 505.

Beaumont, J.L., Carlson, L.A., Cooper, G.R., Fejfar, Z., Fredrickson, D.S. and Strasser, T. (1970) Bull. Wld. Hlth. Org. 43, 891.

Bezman, A., Felts, J.M. and Havel, R.J. (1962) J. Lipid Res. 3, 427.

Bierman, E.L., Dole, V.P. and Roberts, N.T. (1957) Diabetes 6, 475.

Bierman, E.L., Bagdade, J.D. and Porte, D.,Jr. (1968) Amer. J. clin. Nutrit. 21, 1434.

Birkenhäger, J.C. and Tjabbes, T. (1969) Metabolism 18, 18.

Birnbaumer, L. and Rodbell, M. (1969) J. biol. Chem. 244, 3477.

Bjöntorp, P. and Hood, B. (1966) Acta med. scand. 179, 221.

Blecher, M., Merlino, N.S. and Ro'ane, J.T. (1968) J. biol. Chem. 243, 3973.

Boberg, J. and Carlson, L.A. (1964) Clin. chin. Acta 10, 420.

Boberg, J., Carlson, L.A. and Hallberg, D. (1969) J. Atheroscler. Res. 9, 159.

Borensztajn, J. and Robinson, D.S. (1970) J. Lipid Res. 11, 111.

Boden, G. and Willms, B. (1966) Klin. Wschr. 44, 579.

Bouchier, I.A.D. and Bronte-Stewart, B. (1961) Lancet I, 363.

Brady, R.O. and Gurin, S. (1950) J. biol. Chem. 187, 589.

Braunsteiner, H., Sailer, S. and Sandhofer, F. (1966) Klin. Wschr. 44, 116.

Bray, G.A. (1972) J. clin. Invest. 51, 537.

Brown, D.F. and Olivecrona, T. (1966) Acta physiol. scand. 66, 9.

Brown, D.F. (1967) Diabetes, 16, 90.

Brunzell, J.D., Hazzard, W.R., Porte, D.,Jr. and Bierman,E.L. (1973) J. clin. Invest. 52, 1578.

Butcher, R.W. and Sutherland, E.W. (1967) Ann. N.Y. Scad. Sci. 139, 849.

Butcher, R.W. (1968) New Engl. J. Med. 279, 1378.

Butterfield, W.J.H., Hanley, T. and Whichelow, M.J. (1965) Metabolism 14, 851.

Butterfield, W.J.H. and Whichelow, M.J. (1968) Lancet II, 785.

Cahill, G.F.,Jr. and Owen, O.E. (1968) In Carbohydrate Metabolism and its Disorders (eds. F. Dickens, P.J. Randle and W.J. Whelan) Academic Press, New York. p.497.

Carlson, L.A. and Hallberg, D. (1963) Acta physiol. scand. 59, 52.

Carlson, L.A. and Wahlberg, F. (1966) Acta med. scand. 180, 307.

Carlson, L.A. and Böttiger, L.E. (1972) Lancet i, 865.

Cerasi, E. and Luft, R. (1967) Diabetes 16, 615.

Chance, G.W., Albutt, E.C. and Edkins, S.M. (1969) Lancet i, 1126.

Chernick, S.S. and Chaikoff, I.L. (1951) J. biol. Chem. 188, 389.

Chernick, S.S. and Scow, R.O. (1959) Amer. J. Physiol. 196, 125.

Chernick, S.S. and Scow, R.O. (1964) J. biol. Chem. 239, 2416.

Cheung, W.Y. (1967) Biochemistry 6, 1079.

Chiles, R. and Tzagournis, M. (1970) Diabetes 19, 458.

Chlouverakis, C. (1965) Proc. Soc. exp. Biol. (N.Y.) 119, 775.

Christensen, N.J. (1972) J. clin. Invest. 51, 779.

Corder, C.N. and Kalkhoff, R.J. (1969) J. Lab. clin. Med. 73, 559.

Csorba, T.R., Matsuda, I. and Kalant, N. (1966) Metabolism 15, 262.

Das, I. and Chain, E.B. (1972) Biochem. J. 128, 95.

Denborough, M.A. and Paterson, B. (1962) Clin. Sci. 23, 485.

Denborough, M.A. (1963) Clin. Sci. 25, 115.

Dingott, D. and Kerpel, S. (1965) Israel J. med. Sci. 1, 1015.

Dole, V.P. (1956) J.clin. Invest. 35, 150.

Eaton, R.P. and Kipnis, D.M. (1969) Amer. J. Physiol. 217, 1153.

Huttunen, J.K. and Nikkilä, E.A. (1973) Europ. J. clin. Invest. 3, 483.

Jackson, R.A., Moloney, M., Lowy, C., Wright, A.D., Hartog, M., Pilkington, T.R.E. and Fraser, T.R. (1972) Diabetes 20, 214.

Jansen, G.R., Zanetti, M.E. and Hutchison, C.F. (1967) Biochem. J. 102, 870.

Jones, D.P. and Arky, R.A. (1965) Metabolism 14, 1287.

Jones, D.P., Plotkin, G.R. and Arky, R.A. (1966) Diabetes 15, 565.

Joslin, E.P., Bloor, W.R. and Grey, H. (1917) J. Amer. med. Assoc. 69, 375.

Joslin, E.P., Dublin, L.I. and Marks, H.H. (1935) Amer. J. med. Sci. 189, 163.

Jungas, R.L. and Ball, E.G. (1962) Fed. Proc. 21, 202.

Jungas, R.L. and Ball, E.G. (1963) Biochemistry 2, 383.

Jungas, R.L. (1966) Proc. Nalt. Acad. Sci. U.S. 56, 757.

Kalkhoff, R.K. and Kipnis, D.M. (1966) Diabetes 15, 443.

Kallio, V. (1967) Acta med. scand. Suppl.467.

Karam, J.H., Grodsky, G.M. and Forsham, P.H. (1965) Ann. N.Y. Acad. Sci. 131, 374.

Keiding, N.R., Mann, G.V. and Root, H.F. (1952) Diabetes 1, 434.

Kessler, J.I. (1962) J. Lab. clin. Med. 60, 747.

Kessler, J.I. (1963) J. clin. Invest. 42, 362.

Korchak, H.M. and Masoro, E.J. (1964) Biochim. Biophys Acta 84, 750.

Kovacev, V.P. and Scow, R.O. (1966) Amer. J, Physiol. 210, 1199.

Kuo, J.F. and de Renzo, E.C. (1969) J. biol. Chem. 244, 2252.

Kuhfahl, E., Mueller, F. and Dettmer, D. (1967) Acta biol. med. Germ. 18, 563.

Eaton, R.P. (1973) J. Lipid Res. 14, 312.

Eaton, R.P. and Schade, D.S. (1973) Lancet 1, 973.

Elkeles, R.S., Lowry, C., Wyllie, A.D.H., Young, J.L. and Fraser, T.R. (1971) Lancet 1, 880.

Elkeles, R.S. and William, E. (1974) Clin. Sci. Mol. Med. 46, 661.

Evans, J.C. and Ostrander, L.D. (1967) Lancet 1, 761.

Fitzgerald, M.G. and Keen, H. (1964) Brit. med. J. 1, 1568.

Ford, C.R., Stevens, R., Bolinger, R.E. and Morris, J.H. (1963) Proc. Soc. exp. Biol. (N.Y.) 113, 177.

Ford, S., Bozian, R.C. and Knowles, H.C. (1968) Amer. J. clin. Nutrit. 21, 904.

Fredrickson, D.S., Levy, R.I. and Lees, R.S. (1967) New Engl. J. Med. 276, 34, 94, 148, 215, 273.

Galton, D.J. and Wilson, J.D.P. (1970a) Clin. Sci. 38, 661.

Galton, D.J. and Wilson, J.D.P. (1970b) Brit. med. J. 2, 444.

Galton, D.J. (1971) The human adipose cell. Butterworth London p.73.

Ganesan, D., Bradford, R.H., Alaupovic, P. and McConathy, W.J. (1971) Febs letters 15, 205.

Gibbs, G.E. and Chaikoff, I.L. (1941) Endocrinology 29, 877.

Gillhorn, A. and Benjamin, W. (1964) Science 146, 1166.

Glueck, C.I., Levy, R.I. and Fredrickson, D.S. (1969) Diabetes 18, 739.

Goldman, J.K. and Cahill, G.R.Jr. (1964) Metabolism 13, 572.

Goldrick, R.B. and Galton, D.J. (1974) Clin. Sci. Mol. Med. 46, 469.

Goodman, D.S. (1958) J. Amer. chem. Soc. 80, 3892.

Gordis, E. (1962) Proc. Soc. exp. Biol. (N.Y.) 110, 657.

Gordon, E.S. (1960) Amer. J. clin. Nutrit. 8, 740.

Greten, H., Levy, R.I. and Fredrickson, D.S. (1969) J. Lipid Res. 10, 326.

Gries, F.A., Potthoff, S. and Jahnke, K. (1967) Diabetologia 3, 311.

Gross, R.C. and Carlson, L.A. (1968) Diabetes 17, 353.

Haft, D.E. (1967) Amer. J. Physiol. 213, 219.

Haft, D.C. (1968) Diabetes 17, 244.

Hagen, J.H. (1963) J. Lipid Res. 4, 46.

Hallberg, D. and Wersall, J. (1964) Acta chim. scand. suppl.325, 231.

Hallberg, D. (1965) Acta physiol. scand. 65, suppl.254.

Hansen, A.P. (1970) J. clin. Invest. 49, 1467.

Havel, R.J. (1961) Metabolism 10, 1031.

Havel, R.J., Felts, J.M. and Bezman, A. (1961) Clin. Res. 9, 72.

Havel, R.J. and Carlson, L.A. (1963) Life Sci. 3, 651.

Havel, R.J. (1965) In Handbook of Physiology V: Adipose Tissue (eds. A.E. Renold and G.F. Cahill) Amer. Physiol. Soc. Washington D.C. p.499.

Havel, R.J. (1972) New Engl. J. Med. 287, 1186.

Hayes, T.M. (1972) Clin. Endocr. 1, 247.

Heimberg, M., Dunkerley, A. and Brown, T.O. (1965) Biochem. Pharmacol. 14, 890.

Heimberg, M., Dunkerley, A. and Brown, T.O. (1966) Biochim. Biophys. Acta 125, 252.

Heimberg, M., Van Harken, D.R. and Brown, T.O. (1967) Biochim. Biophys. Acta 137, 435.

Heimberg, M., Weinstein, I. and Kohout, M. (1969) J. biol. Chem. 244, 2278.

Hirsch, J. and Knittle, J.L. (1970) Fed. Proc. 29, 1516.

Hollenberg, C.H. (1959) Amer. J. Physiol. 197, 667.

Hollister, L.E., Overall, J.E. and Snow, H.L. (1967) Amer. J. clin. Nutrit. 20, 777.

La Rosa, J.C., Levy, R.I., Windmueller, H.G. and Fredrickson, D.S. (1972) J. Lipid Res. 13, 356.

Lassers, B.W., Wahlqvist, M.L., Kaijser, L. and Carlson, L.A. (1971) Lancet II, 448.

Laurell, S. (1956) Scand. J. clin. Lab. Invest. 8, 81.

Letarte, J. and Fraser, T.R. (1969) Diabetologia 5, 358.

Lewis, B., Wittman, W., Krut, L.H., Hansen, J.D.L. and Brock, J.F. (1966) Clin. Sci. 30, 371.

Lewis, B., Chait, A., Wootton, I.D.P., Oakley, C.M., Krikler, D.M., Sigurdsson, G., February, A., Maurer, B. and Birkhead, J. (1974) Lancet I, 141.

Lowy, A.D.,Jr. and Barach, J.H. (1958) Circulation 17, 14.

Mahler, R., Stafford, W.S., Tarrant, M.E. and Ashmore, J. (1964) Diabetes 13, 297.

Mancini, M., Mattock, M., Rabaya, E., Chait, A. and Lewis, B. (1973) Atherosclerosis 17, 445.

Mansour, T.E., Clague, M.E. and Beernink, K.D. (1962) Fed. Proc. 21, 238.

Margolis, S. and Vaughan, M. (1962) J. biol. Chem. 237, 44.

Maruhama, Y., Goto, Y. and Yamagata, S. (1967) Metabolism 16, 985.

Masoro, E.J. (1962) J. Lipid Res. 3, 149.

Masoro, E.J. (1968) Physiological Chemistry of Lipids in Mammals. Saunders, Philadelphia, London, Toronto. p.58.

Masri, M.S., Lyon, I. and Chaikoff, I.L. (1952) J. biol. Chem. 197, 621.

Mayes, P.A. and Felts, J.M. (1967) Nature 215, 716.

Medes, G., Thomas, A. and Weinhouse, S. (1952) J. biol. Chem. 197, 181.

Meng, H.C. and Goldfarb, J.L. (1959) Diabetes 8, 211.

Miettinen, T.A., Taskinen, M., Pelkonen, R. and Nikkilä,E.A. (1969) Acta med. scand. 186, 247.

Miller, H.I. (1967) Metabolism 16, 1096.

Miller, H.I.,Bortz, W.M. and Durham, B.C. (1968) Metabolism 17, 515.

Mueller, P.S. and Evans, W.H. (1963) J. Lab. clin. Med. 61, 953.

McElroy, W.T.,Jr., Siefert, W.L. and Spetzer, J.J. (1960) Proc. Soc. exp. Biol. (N.Y.) 104, 20.

Naidoo, S.S., Lossow, W.J. and Chaikoff, I.L. (1967) Experientia 23, 829.

Nestel, P.J. and Havel, R.J. (1962) Proc. Soc. exp. Biol. (N.Y.) 109, 985.

Nestel, P.J. and Steinberg, D. (1963) J. Lipid Res. 4, 461.

Nestel, P.J. (1964) J. clin. Invest. 43, 943.

Nestel, P.J. (1967) Metabolism 16, 1123.

Nestel, P.J. and Whyte, H.M. (1968) Metabolism 17, 1122.

New, M.I., Robets, T.N., Bierman, E.L. and Reader, G.L. (1963) Diabetes 12, 208.

Nikkilä, E.A. and Ojala, K. (1966) Life Sci. 5, 89.

Nikkilä, E.A. and Pykälistö, O. (1968) Biochim. Biophys. Acta 152, 421.

Nikkilä, E.A. (1969) Advanc. Lipid Res.

Nikkilä, E.A. and Taskinen, M.R. (1970) In Atherosclerosis: Proceedings of 2nd International Symposium (ed. R.J. Jones) Springer-Verlag, Berlin, p.200.

Nikkilä, E.A. and Kekki, M. (1973) Metabolism 22, 1.

Nikkilä, E.A. (1973) Progr. biochem. Pharmacol. 8, 271.

Nilsson-Ehle, P. (1974) M.D. Thesis. Univ. Lund.

Ogilvie, R.F. (1933) J. Path. Bact. 37, 473.

Opie, L.N. and Walfish, P.G. (1963) New Engl. J. Med. 268, 757.

Östman, J. (1965) Acta med. scand. 177, 639.

Owen, O.E. and Reichard, G.A.,Jr. (1971) Progr. biochem. Pharmacol. 6, 177.

Passonneau, J.V. and Lowry, O.H. (1962) Biochem. Biophys. Res. Comm. 7, 10.

Penhos, J.C., Wu, C.H., Lemberg, A., Daunas, J., Brodoff, B., Sodero, A. and Levine, R. (1968) Metabolism 17, 246.

Perley, M. and Kipnis, D.M. (1966) Diabetes, 15, 867.

Perry, W.F. and Bowen, H.F. (1962) Canad. J. Biochem. Physiol. 40, 749.

Perry, W.F. (1967) Clin. chim. Acta 16, 189.

Persson, B. (1972) M.D. Thesis. Univ Göteborg.

Randle, P.J., Garland, P.B., Hales, C.N. and Newsholme, E.A. (1963) Lancet I, 785.

Randle, P.J., Garland, P.B., Newsholme, E.A. and Hales, C.N. (1965) Ann. N.Y. Acad. Sci. 131, 324.

Reaven, G., Calciano, A., Codey, R., Lucas, C. and Miller, R. (1963) J. clin. Endocr. 23, 1013.

Reaven, G.M., Lerner, R.L., Stern, M.P. and Farquhar, J.G. (1967) J. clin. Invest. 46, 1756.

Renold, A.E., Hastings, A.B., Nesbett, F.B. and Ashmore, J. (1955) J. biol. Chem. 213, 135.

Renold, A.E., Stauffacher, W. and Cahill, G.F., Jr. (1972) In The Metabolic Basis of Inherited Disease (eds. J.B. Stanbury, J.B. Wyngaarden and D.S. Fredrickson), McGraw-Hill, New York. p.83.

Rifkind, B.M., Gale, M. and Lawson, D. (1968) Cardiovasc. Res. 2, 143.

Robinson, D.S. (1963) Advan. Lipid Res. 1, 133.

Robinson, D.S. (1973) J. clin. Path. 26, suppl. 5, 5.

Robinson, G.A., Butcher, R.W. and Sutherland, E.W. (1968) Ann. Rev. Biochem. 37, 149.

Rodger, N.W. and Dee, E.L. (1973) Canad. med. Assoc. J. 109, 363.

Rony, H.R. and Ching, T.T. (1930) Proc. Soc. exp. Biol. (N.Y.) 27, 533.

Rössner, S., Boberg, J., Carlson, L.A., Freyschuss, U. and Lassers, B.W. (1974) Europ. J. clin. Invest. 4, 109.

Rubenstein, B. and Rubenstein, D. (1964) Canad. J. Biochem. 44, 129.

Sailer, S., Sandhofer, F. and Braunsteiner, H. (1966) Metabolism 15,135.

Sailer, S., Sandhofer, F. and Braunsteiner, H. (1967a) Klin. Wschr. 45, 86.

Sailer, S., Sandhofer, F., Belzano, K. and Braunsteiner, H. (1967b) Klin. Wschr. 45, 918.

Salaman, M.R. and Robinson, D.S. (1960) Biochem. J. 99, 640.

Salans, L.B., Knittle, J.L. and Hirsch, J. (1968) J. clin. Invest. 47, 153.

Salans, L.B. and Dougherty, J.W. (1971) J. clin. Invest. 50, 1399.

Sandberg, H., Sok Min, B., Feinberg, L. and Bellet, S. (1960) Arch. intern. Med. 105, 866.

Santen, R.J., Willis, P.W.M. and Fajans, S.S. (1972) Arch. intern. Med. 130, 833.

Schlierf, G. and Kinsell, L.W. (1965) Proc. Soc. exp. Biol. (N.Y.) 120, 272.

Schnatz, J.D. and Williams, R.M. (1963) Diabetes 12, 174.

Schotz, M.C. and Garfinkel, A.S. (1972) Biochim. Biophys. Acta 270, 472.

Schrade, W., Boehle, E.,Biergler, R. and Harmuth, E. (1963) Lancet I, 285.

Scow, R.O. and Chernick, S.S. (1970) In Comprehensive Biochemistry Vol. 18: Lipid Metabolism (eds. M. Florkin and E.H. Storte) Elsevier, Amsterdam.

Shames, D.M., Frank, A., Steinberg, D. and Biermen , M. (1970) J. clin. Invest. 49, 2298.

Shore, B. and Shore, V. (1961) Amer. J. Physiol. 201, 915.

Steinberg, D. (1962) In Fat as a Tissue (eds. K. Rodahl and B. Issekutz) McGraw-Hill, New York. p.127.

Sterky, G., Larsson, Y. and Persson, B. (1963) Acta pacd. scand. 52, 11.

Sterky, G., Persson, B. and Larsson, Y. (1966) Diabetologia 2, 14.

Sutherland, E.W., Oye, I. and Butcher, R.W. (1965) Recent Progr. Horm. Res. 21, 623.

Tarrant, M.S. and Ashmore, J. (1965) Diabetes 14, 179.

Topping, D.L. and Mayes, P.A. (1972) Biochem. J. 126, 295.

Unger, R.H., Aguilar-Parada, E., Müller, W.A. and Eisentraut, A.M. (1970) J. clin. Invest. 49, 837.

Van Harken, D.R., Brown, T.O. and Heimberg, M. (1967) Lipids 2, 231.

Van Harken, D.R., Dixon, C.W. and Heimberg, M. (1969) J. biol. Chem. 244, 2278.

Vaughan, M. (1961) J. Lipid Res. 2, 293.

Vaughan, M. and Steinberg, D. (1963) J. Lipid Res. 4, 193.

Vaughan, M., Steinberg, D. and Pittman, R. (1964) Biochim. Biophys. Acta 84, 154.

Vogel, W.C., Brunzell, J.D. and Bierman, E.L. (1971) Lipids 6, 805.

Waddel, W.R. and Geyer, R.B. (1957) Proc. Soc. exp. Biol. (N.Y.) 96, 251.

Wakil, S.J. (1961) J. Lipid Res. 2, 1.

Weber, G. and Hird Convery, H.J. (1966) Life Sci. 5, 1139.

West, C.E. and Passey, R.F. (1967) Biochem. J. 102, 58.

Westman, S. (1965) Metabolism 14, 1027.

Wichelow, M.J. and Butterfield, W.J.H. (1971) Quart. J.Med. 40, 261.

Wilcox, H.G., Dishman, G. and Heimberg, M. (1968) J. biol. Chem. 243, 665.

Wilson, D.E., Schreibman, P.H. and Arky, R.A. (1969) Diabetes 18, 562.

Winand, J., Furnell, J. and Christopher J. (1969) Biochim. Biophys. Acta 152, 280.

Windmueller, H.G. and Spaeth, A.E. (1967) Arch. Biochem Biophys. 122, 362.

Wing, D.R., Salaman, M.R. and Robinson, D.S. (1966) Biochem. J. 99, 648.

Woodside, W.F. and Heimberg, M. (1972) Israel J. med. Sci. (1972) 8, 309.

Zieve, F.J. and Zieve, L. (1972) Biochem. Biophys. Res. Comm. 47, 1480.

CHAPTER IV

ALCOHOL AND SERUM TRIGLYCERIDES

World spirits consumption varies from country to country. In Britain, for example, the annual consumption is from 1.6 litres per head compared with 14.8 litres per head in the Soviet Union (Johnson, 1971). In addition to this, enough wine is produced to supply every one of the three thousand million inhabitants of the globe with twelve bottles a year. Distribution of alcohol consumption is not even; the more 'civilized' communities partake of more than their fair share of the ethanol available. France is perhaps the worst offender with an average of 18.1 litres pure alcohol per person, per year (Brunt, 1973).

The association between alcohol over-indulgence and high blood lipids has long been known; since the realization that an elevated serum triglyceride concentration is a risk factor for the development of IHD, the entity of alcoholic hypertriglyceridaemia has assumed a new importance. Because alcohol over-indulgence may thereby be aiding the development of atherosclerosis, the relationship between alcohol consumption and IHD warrants further investigation.

Much research has been devoted to the effect of alcohol on lipid metabolism. This has resulted not only in the elucidation of a number of specific consequences of alcohol administration, but has also helped shed light on the understanding of certain aspects of the metabolism of lipids.

Perhaps the best known effect of alcohol on lipid metabolism is the development of fatty liver. The effect of alcohol on blood lipids is also long known. Feigl (1918) was the first to document an increase in blood lipids in association with alcoholism. In 1958, Zieve described intense lipaemia as a direct consequence of alcoholism (Zieve, 1958). In this original description, the lipaemia was associated with fatty liver, jaundice and haemolytic anaemia. Such intense lipaemia in association with ethanolic over-indulgence is a rare occurrence; by contrast, more moderate elevations of serum triglyceride seem to occur relatively frequently.

For this reason, patients presenting to the Hammersmith Hospital Lipid Disorders Clinic with hypertriglyceridaemia are carefully questioned about their ethanolic consumption; hypertriglyceridaemic subjects with substantial alcohol intakes were studied in order to try to determine whether their high alcohol intakes were related to their hypertriglyceridaemia.

Despite marked progress in the field, some uncertainties still remain regarding the mechanism of fatty liver and alcohol hyperlipaemia. The metabolism of alcohol produces metabolic changes, which may in themselves directly influence biochemical pathways involved in lipid metabolism. The major site of alcohol metabolism is the liver; the role played by the liver in the synthesis and secretion of lipoprotein makes it a likely site for the interaction between metabolism of alcohol and lipids to occur. It has also been suggested (Losowsky et al, 1963) that alcoholic hyperlipidaemia may be a consequence of impaired peripheral uptake of alcohol from plasma. This process has been assessed in the present study using the IVFTT as a measure of triglyceride removal from plasma.

METABOLISM OF ALCOHOL

Absorption of alcohol occurs readily from the stomach and the rest of the gastrointestinal tract. Following absorption, about 2 - 10% is eliminated unchanged by the kidneys and lungs; the remainder is oxidised (Thompson, 1956). Experimental hepatectomy has shown the liver to be the major site of alcohol oxidation (Thompson, 1956), although a small amount of extrahepatic oxidation occurs both in vitro (Lieber, 1967) and in vivo (Larson, 1959).

The initial stage in the metabolism of alcohol is its oxidation to acetaldehyde (Lieber, 1968). This reaction is catalyzed by alcohol dehydrogenase, a zinc-containing enzyme. Reduced nicotinamide adenine dinucleotide (NADH) is generated. Although abundant in liver, this enzyme has also been found in small quantities in other tissues (Lieber, 1967). In liver cells, it has been isolated in pure form from the cytosol (Nyberg et al, 1953; Wartberg et al, 1964) which is believed to be the major site of alcohol oxidation. Oxidation of alcohol in the liver may also occur via other pathways. Lieber and De Carli (1968; 1972) have described a microsomal ethanol oxidising system (MEOS) with co-enzyme requirements similar to those of the drug detoxifying enzyme systems; the reduced form of nicotinamide adenine dinucletide phosphate (NADPH), and oxygen are thus required. This system has been considered to be distinct from alcohol dehydrogenase, to play a significant role in the in vivo oxidation of alcohol, and to adapt to chronic ethanol administration by increasing its activity (Lieber and De Carli, 1970). The oxidation of ethanol by alcohol dehydrogenase and by the hepatic microsomal oxidizing system can respectively be represented as follows:

$$\mathrm{CH_3CH_2OH + NAD^+ \xrightarrow[\text{alcohol dehydrogenase}]{} CH_3CHO + H^+ + NADH}$$

$$\mathrm{CH_3CH_2OH + NADPH + H^+ + O_2 \longrightarrow CH_3CHO + NADP^+ + 2H_2O}$$

It can be seen that the oxidation of ethanol by alcohol dehydrogenase results in the generation of NADH, which can be used by the mitachondria to conserve chemical energy in the form of adenosine triphosphate. On the other hand, the microsomal ethanol oxidizing system utilizes NADPH and oxygen, producing heat and not conserving chemical energy. The metabolic effects thus caused by ethanol oxidation, depend in part on the proportions oxidised by these two systems; the regular use of alcohol by inducing the latter system may enhance its own oxidation (Lieber and De Carli, 1972). This system has been regarded as responsible for about 20 - 25% of the oxidation of ethanol both in vitro and in vivo (Lieber and De Carli, 1970; Papenberg et al, 1970; Lieber and De Carli, 1972).

One further mechanism of alcohol oxidation has also been suggested. Microsomal NADPH oxidase generates H_2O_2; it has been postulated that the H_2O_2 could lead to catalytic oxidation of ethanol to acetaldehyde. If this is correct, any source of H_2O_2, for example hypoxanthine - xanthine oxidase should be capable of having the same effect. While this has been shown to occur in vitro, this NADP dependent microsomal ethanol oxidising system appears to play an insignificant part in the in vivo oxidation of alcohol (Carter and Isselbacher, 1972).

The first specific product of ethanol oxidation is acetaldehyde; levels increase in blood (Forster, 1956) and alveolar air (Freund and O'Hollaren, 1965) following alcohol administration. Blood levels, however, remain low, suggesting that further metabolism occurs rapidly. The exact fate of acetaldehyde is uncertain; it is most widely believed to be oxidised to acetate, acetyl CoA and CO_2, with the generation of NADH (Lieber, 1968). ^{14}C ethanol has been found in a number of metabolites of acetyl CoA such as fatty acids and cholesterol, suggesting acetyl CoA to be an intermediate (Snyder et al, 1964). Blood levels of acetate have also been shown to be elevated after the administration of alcohol (Lundquist et al, 1962; Crouse et al, 1968), thus raising the possibility that acetyl CoA could derive from acetate. Alternatively, the possibility that ethanol is converted to acetyl CoA which may act as a precursor for substances such as fatty acids, or be converted to acetate, could equally apply.

NADH is thus generated by two reactions; firstly by the oxidation of alcohol to acetaldehyde, and also during oxidation of acetaldehyde to acetate. The net result of ethanol oxidation is generation of an excess of reducing equivalents in the liver, thus altering the redox potential. The increased NADH/NAD ratio results in a change in the ratio of those metabolites dependent for reduction on the NADH/NAD couple.

THE EFFECT OF ALCOHOL ON SERUM LIPIDS

Of the plasma lipids, triglyceride concentrations are most affected (Losowsky et al, 1963; Lieber, 1968), although elevations of cholesterol, phospholipid and free fatty acids have also been described (Lieber et al, 1963). In addition to elevating fasting serum triglyceride concentration, alcohol appears also to result in an enhanced alimentary lipaemia (Talbott and Keating, 1962; Wilson et al, 1970). Furthermore, different effects have been documented after 'acute' alcohol administration and in the chronic state.

Ethanol, when ingested alone after an overnight fast, causes a slow elevation of serum triglyceride level to about 30% above fasting levels (Wilson et al, 1970). Also, when administered by continuous intravenous infusion, serum triglycerides showed a progressive and significant rise when compared with fasted controls (Jones et al, 1963; Nestel and Hirsch, 1965). The rise in triglyceride levels observed in these three studies must have been due to endogenous triglycerides, as the subjects were fasting in all cases. The effect appears to be due to alcohol per se, and not to calorie overload, as no comparable triglyceride increment was noted after feeding isocaloric quantities of either carbohydrate or fat (Losowsky et al, 1963; Nestel and Hirsch, 1965). Nestel and Hirsch (1965) showed the triglyceride elevation to be due to an increase in VLDL triglyceride.

The ingestion of alcohol also appears to potentiate alimentary lipaemia both in man (Talbott and Keating, 1962; Brewster et al, 1966; Barboriak and Meade, 1968; Wilson et al, 1970) and in rats (Di Luzio and Poggi, 1963; Barboriak and Meade, 1968). Jones et al (1970) have shown that when ethanol and corn oil are ingested simultaneously, the increment in triglyceride levels above baseline values, far exceeds that which might be expected from a simple summation of that due to the ethanol and the fat. By studying the composition of the fatty acids in plasma, they showed the enhanced postprandial lipaemia to be due to triglycerides with the fatty acid composition of corn oil; little dilution from endogenous fatty acids was noted. Unfortunately, no data is available on the effect of alcohol alone on fatty acid composition. A further point of interest from this study is that the postprandial lipaemia was due largely to an increase in VLDL; chylomicrons contributed little. The dose of fat fed was 100 grammes. In Chapter VI of this thesis, experiments are described in which the same dose of butterfat was fed in the absence of alcohol; the resultant lipaemia was largely due to chylomicrons. It is conceivable that the type of lipoprotein produced by the gut in response to fat feeding may be modified by simultaneous ingestion of alcohol.

More prolonged intake of alcohol also leads to an elevation of serum triglyceride. Although no data is available as to the frequency of hyperlipidaemia amongst alcoholics and those ingesting large amounts of alcohol, the association between chronic alcohol over-indulgence and hypertriglyceridaemia is well documented (Losowsky et al, 1963; Lieber et al, 1963; Jones et al, 1963; Kudzma and Schonfeld, 1971). Similar observations have been made on rats chronically fed with alcohol (Baraona and Lieber, 1970); after a 24 day period the lipid content of all plasma lipoprotein fractions were increased in comparison with control rats. This phenomenon appears to differ from carbohydrate inducibility, Kudzma and Schonfeld (1971) showing carbohydrate and ethanol inducibility to be distinct entities. Alternating high carbohydrate and ethanol diets resulted in carbohydrate, but not ethanol induction of triglyceride levels in non-alcoholic subjects; the alcoholic group appeared to be very sensitive to the effect of ethanol in raising their triglyceride levels, but less so to carbohydrate. Fry et al (1973) have described a case in which alcohol and carbohydrate produced a similar hypertriglyceridaemic response; they suggest that a single mechanism may be responsible for the triglyceride elevation in both situations. If alcohol and carbohydrate inducibility are separate entities, as suggested by Kudzma and Schonfeld (1971), then the finding in the patient of Fry et al could be explained by both phenomena being present.

Recently, Mendelson and Mello (1973) showed the serum triglyceride response to alcohol to be much greater in subjects with primary endogenous hypertriglyceridaemia than in normal men or those with carbohydrate induced hypertriglyceridaemia before the onset of drinking.

In susceptible subjects, ethanol usually results in an elevation of VLDL; fasting chylomicronaemia may, however, ensue if the diet contains moderate amounts of fat as well (Kudzma and Schonfeld, 1971).

Serum phospholipids and cholesterol concentrations have also been shown to increase in response to chronic alcohol administration in man, although to a lesser degree than triglyceride (Lieber et al, 1963). Given over a period of a week, Grande et al (1960) have shown small increases in serum cholesterol concentrations to be evident in both men and dogs. Both free and ester cholesterol, however, remained constant in a case studied by Jones et al (1963), although triglycerides rose by 340% and phospholipids by 90%.

In view of the precursor-product relationship between FFA and triglyceride in the liver, the effect of alcohol on plasma free fatty acid concentrations has been studied by a number of investigators. Findings vary according to the dose and duration of alcohol administration.

Alcohol administered over a short period of time has been shown to reduce plasma FFA concentrations whether administered orally (Jones et al, 1963; Jones et al, 1965; Feinman and Lieber, 1967) or intravenously by infusion (Lieber et al, 1962a; Bouchier and Dawson, 1964; Nestel and Hirsch, 1965). The reduced plasma levels after acute ethanol administration are associated with a reduction in the turnover rates of plasma FFA (Jones et al, 1965; Nestel and Hirsch, 1965). As glycerol levels are also reduced (Feinman and Lieber, 1967), a reduced mobilization of FFA from adipose tissue is more likely than increased FFA utilization. Administered for longer periods (up to 18 days), in modest doses (up to 300 grammes a day), no change has been observed in plasma FFA concentrations (Lieber et al, 1963; Schapiro et al, 1965). An elevation of plasma FFA levels in response to alcohol has only been reported when the dose of alcohol has been exceedingly high, far greater than likely to be encountered in most subjects with alcoholic hypertriglyceridaemia. In man, plasma FFA have been shown to increase only at the exceedingly high alcohol dose of 400 grammes per day (Lieber et al, 1963). In fasted rats fed alcohol by gastric tube, circulating FFA levels have also been shown to increase following the administration of a very large dose (Brodie et al, 1961; Mallov, 1961), although this finding has not been confirmed (Elko et al, 1961). It is possible that the reduction of FFA levels observed at lower doses of alcohol is offset by increased FFA mobilization resultant on high doses of ethanol (Lieber, 1972).

A direct effect of alcohol in inhibiting lipolysis seems unlikely, as concentrations of ethanol similar to that found after consumption of alcohol by animals and man, had no effect on glycerol release when incubated with adipose tissue in vitro (Bizzi and Carlson, 1965). Recently, however, Curtiss-Prior (1972) has shown ethanol to inhibit lipolysis which was stimulated by the presence of fasted rat serum in the incubation medium. A more likely possibility is that lipolysis is inhibited by a metabolite of alcohol, of which acetate appears the most likely. Crouse et al (1968) showed plasma FFA concentration to fall in response to the administration of acetate in doses which resulted in plasma levels similar to that found after alcohol administration to man.

MECHANISM OF THE PRODUCTION OF FATTY LIVER AND HYPERTRIGLYCERIDAEMIA BY ALCOHOL

While it has become widely accepted that serum triglyceride concentrations may become elevated in response to acute and chronic ethanol consumption, agreement has still not been reached as to the mechanism. An early hypothesis for the mechanism of production of alcohol fatty liver was of defective synthesis or secretion of lipoprotein by the liver (Shapiro et al, 1962). The simultaneous production of steatosis and hypertriglyceridaemia (Losowsky et al, 1963; Baraona and Lieber, 1970), however, makes this possibility unlikely. More likely is the possibility that the triglyceride accumulation in both liver and the circulation, are the result of a single mechanism; an increased production of triglyceride by the liver could lead to the development of a fatty liver and an enhanced secretion of triglyceride into plasma.

Alternatively, the elevated serum triglyceride level accompanying alcoholic excess could be explained by impaired peripheral removal of triglyceride from plasma. This possibility has been tested - results are presented in this Chapter.

EFFECT OF ALCOHOL ON TRIGLYCERIDE REMOVAL FROM PLASMA

Some suggestions have been put forward in support of the hypothesis that removal of triglyceride from plasma is depressed by alcohol. As has been discussed in Chapter I, hydrolysis of lipoprotein-borne triglycerides by lipoprotein lipase appears to be the chief means whereby triglyceride is removed from plasma.

Losowsky et al (1963) showed sub-normal post-heparin lipolytic activities in six out of eight subjects with alcoholic hyperlipidaemia, implying that reduced uptake of triglyceride from plasma might account for the raised lipid level. However, the lipolytic activity increased to normal in only one of four patients re-studied after alcohol was withdrawn, though all patients showed a fall in triglyceride levels. This suggests a cause of low post-heparin lipolytic activity other than alcoholism. Indeed, two of their subjects with low lipoprotein lipase levels were diabetic; the relationship of diabetes to post-heparin lipolytic activity has been discussed earlier (see page 120). Reduced plasma lipoprotein lipase activity has also been described in liver disease (Datta, 1965; 1966) and all eight subjects had liver disease of varying degree. Yet some of their patients and controls had fairly marked evidence of liver disease without reductions in lipoprotein lipase activities.

Post-heparin lipolytic activity was not shown to fall during the development of hypertriglyceridaemia during ethanol administration in subjects studied by Kudzma and Schonfeld (1971), suggesting that the elevation of serum triglyceride concentration was not due to diminished triglyceride removal from plasma. Further evidence against impaired removal being an important mechanism in the development of alcohol hypertriglyceridaemia is the observation by Baraona and Lieber (1970) that the rate of clearance of injected triglyceride did not fall in rats made hypertriglyceridaemic by chronic feeding with alcohol.

EFFECT OF ALCOHOL ON METABOLIC PATHWAYS OF TRIGLYCERIDE SYNTHESIS AND SECRETION

As impaired removal of triglyceride from plasma seems unlikely to play a major role in the pathogenesis of alcohol hypertriglyceridaemia, the alternative possibility of enhanced triglyceride secretion into plasma would appear to be of importance. Enhanced triglyceride synthesis by the liver could also account for the development of fatty liver due to alcohol.

Various mechanisms could result in enhanced synthesis. Increased mobilization of FFA from adipose tissue has been shown to be an unlikely factor (see page 186). Reduced hepatic fatty acid oxidation or increased lipogenesis could provide an excess of fatty acid to be utilized for triglyceride synthesis in liver. These possibilities will be considered in more detail later. A decreased release of lipoprotein by the liver has also been proposed as a possible mechanism for the development of fatty liver. This suggestion followed the demonstration that the addition of ethanol resulted in an increase in hepatic triglyceride associated with a fall in perfusate triglyceride concentration in the isolated perfused rat liver (Shapiro et al, 1962); the co-development of steatosis and hypertriglyceridaemia in rats chronically fed alcohol (Baraona and Lieber, 1970), however, renders this possibility unlikely.

Alcohol has effects at many sites in the pathways of hepatic lipid metabolism which would tend to favour an increased production of triglyceride being of importance in the pathogenesis both of steatosis and hypertriglyceridaemia. Many of the effects of alcohol on lipid metabolism can, in fact, be explained on the basis of altered redox potential in the liver, consequent on the oxidation of alcohol. As discussed previously, the net effect of oxidation of alcohol by alcohol dehydrogenase in the cytoplasm, is the generation of an excess of reducing equivalents resulting in an increased NADH/NAD ratio (Lieber, 1967). This results in a change in the ratio of those metabolites dependent on this couple for reduction. As alcohol dehydrogenase occurs in the soluble cytoplasm, measurements of the total NADH/NAD ratio fails to take into account cellular compartmentalization; this is obviously of importance in view of the major site of fatty acid oxidation being the mitachondrion. Any hypothesis of the effect of an increased NADH/NAD ratio will thus have to take this into account and will have to consider mechanisms of 'shuttling' cytoplasmic reducing equivalents to the mitochondrion.

The increase of reduced NAD relative to the oxidised form appears to be coupled to the reduction of dihydroxyacetone phosphate to glycerol -3- phosphate (Nikkilä and Ojala, 1963a).

This could have a two-fold effect. Firstly, by the provision of the glycerol moeity of triglyceride by glycerol -3- phosphate (Nikkilä and Ojala, 1963a), trapping of fatty acids could lead to enhanced triglyceride formation. The relative role of glycerol -3- phosphate from this source and that from extra-hepatic glycerol is, however, not clear (Nikkilä and Ojala, 1963b). In addition, the shuttling of cytoplasic reducing equivalents into mitochondria is known to occur via glycerol -3- phosphate (Lieber, 1967); accumulation of NADH intra-mitochondrially is well-known to inhibit the citrate cycle. Indeed, citrate cycle activity has been shown to be slowed by alcohol (Lieber, 1969). The significance of this will be discussed later.

Another extramitochondrial function which has been shown to be enhanced by alcohol in vitro, is fatty acid synthesis (Lieber et al, 1966). The mechanism is not clear as NADPH is required for this process. Transhydrogenation of NADH to NADPH is a possibility; another is mitochondrial elongation, which is NADH dependent.

Probably the most important effect of ethanol on lipid metabolism is that related to fatty acid oxidation. Decreased oxidation of fatty acids consequent on alcohol has been demonstrated in rat liver slices (Lieber and Schmid, 1961), in the isolated perfused rat liver (Lieber et al, 1967), as well as in isolated mitochondria from ethanol fed animals (Rubin et al, 1972). As previously mentioned, some of the excess reducing equivalent generated by the oxidation of alcohol in the cytoplasm may be shuttled into the mitochondria by glycerol

-3- phosphate. This may slow citrate cycle activity leading to a diminished production of NADH by the cycle. The respiratory chain would thus be fed by reducing equivalents derived directly from ethanol rather than from the citrate cycle. Fatty acid oxidation, the main source of substrate for the citrate cycle in the liver (Fritz, 1961), would thus be inhibited in the presence of alcohol, which could supplant it for the provision of reducing equivalents (Lieber, 1973). In the absence of ethanol, most of the oxygen consumed by mitochondria is for fatty acid oxidation, but in its presence, the oxidation of ethanol to acetate appears to utilize more oxygen than the oxidation of FFA (Lundquist et al, 1962).

Accumulation of hepatic fatty acid may, therefore, ensue; this would increase their availability for triglyceride synthesis and could thereby explain both the development of fatty liver and excessive triglyceride secretion by the liver. The source of the fatty acids could be dietary or endogenous, either from adipose tissue stores or from de novo synthesis. In fact, fatty acids from all these sources have been shown to accumulate in hepatic triglyceride of ethanol treated animals, although both in ethanol fed rats and human volunteers, prolonged ethanol feeding resulted in a fatty acid pattern in hepatic triglyceride, different to that found in adipose tissue. When ethanol was fed with a fat-containing diet, a large proportion of the hepatic triglyceride fatty acids have been shown to be of dietary origin (Lieber and Spritz, 1966; Lieber et al, 1966; Wilson et al, 1970). These experiments were performed by prelabelling adipose tissue triglyceride with fatty acids contained in certain oils; ethanol administration was accompanied by a switch to dietary fatty acids differing from those of adipose tissue, thereby allowing differentiation between the fatty acids from these two sources. When administered, with a low fat diet, the hepatic triglyceride accumulation due to ethanol also had a fatty acid pattern different both from that of adipose tissue and dietary fatty acids, suggesting the source to be endogenously synthesized fatty acids (Lieber and Spritz, 1966; Lieber et al, 1966). Inhibition of lipolysis with a β-blocking agent also failed to prevent the accumulation of triglyceride in the liver during ethanol infusion in rhesus monkeys (Nair et al, 1973). The only circumstances under which adipose tissue triglyceride fatty acids have been shown to accumulate in liver triglyceride is after a single sub-lethal dose of ethanol fed to rats (Lieber et al, 1966; Scheig and Isselbacher, 1965).

Decreased fatty acid oxidation has been shown to persist long after cessation of alcohol ingestion (Rubin et al, 1972). It is, therefore, of interest that marked ultrastructural changes (Iseri et al, 1966; Ruben and Lieber, 1967) have been demonstrated in the mitochondria in association with these functional changes. Increased fragility of mitochondria from ethanol treated animals has also been described (French and Todoroff, 1970; Rubin et al, 1972).

Ultrastructural changes other than those involving the mitochondria have also been described; smooth endoplasmic reticulum proliferation (Iseri et al, 1966; Lane and Lieber, 1966; Rubin and Lieber, 1968) and a reduction in the rough endoplasmic reticulum (Ishii et al, 1973) have been described.

The smooth endoplasmic reticulum is the main site of fatty acid esterification and lipoprotein formation. These structural changes may have functional counterparts. Baraona and Lieber (1970) have shown ^{3}H palmitate and ^{14}C lysine incorporation into serum lipoprotein to be increased by feeding ethanol; enhanced lipoprotein production due to alcohol is thus suggested. The same group of workers (Baraona et al, 1973) have also shown increased lipoprotein production in response to chronic ethanol feeding despite the absence of alcohol administration at the time of the measurement of the lipoprotein production. This suggests an increased capacity for lipoprotein formation in these animals. The activity of the enzyme responsible for the esterification of fatty acids to form triglycerides, 1 - α - glycerophosphate acyl transferase has also shown to be increased by ethanol feeding (Lieber, 1973; Joly et al, 1973).

These studies all favour increased lipoprotein formation being of importance in the pathogenesis of fatty liver. As discussed earlier, reduced fatty acid oxidation or enhanced fatty acid synthesis consequent on alcohol administration could provide the fatty acid for the lipoprotein triglyceride. The glycerol moeity could result from glycerol -3- phosphate due to the change in redox potential consequent on alcohol oxidation. Protein synthesis occurs chiefly in the rough endoplasmic reticulum. The reduction in this sub-cellular fraction, noted on electron microscopy and by chemical fractionation (Ishii et al, 1973), is somewhat difficult to explain in view of the increased incorporation of labelled amino acid into the apoprotein moeity of serum lipoproteins

(Baraona and Lieber, 1970). Further study of this aspect would thus seem warranted. It is, however, conceivable that when triglyceride production at the smooth endoplasmic reticulum exceeds the supply of apoprotein, more triglyceride could be incorporated into the lipoprotein molecule per unit of protein. This might explain the appearance of chylomicra in fasting serum of subjects with intense lipaemia such as those described by Zieve (1958).

Attention thus far has centred around the role of the liver in lipoprotein production. Recent evidence, however, has shown the intestine to be a source of non-dietary VLDL in plasma (Ockner et al, 1968; Ockner et al, 1969; Ockner and Jones, 1970; Jones and Ockner, 1971). The endogenous triglyceride-rich lipoprotein formed, is indistinguishable from that of hepatic origin. The intestine not only absorbs much of the alcohol ingested (Cooke, 1970), but is also able to metabolize alcohol via alcohol dehydrogenase (Mistilis and Birchall, 1969); an inter-relationship between the metabolism of alcohol and lipid by the gut is thus conceivable. It is of interest that Carter et al (1971) have shown intestinal lipogenesis from palmitate, to be stimulated during alcohol feeding in rats, compared with isocalorically glucose fed controls. Increased triglyceride production by the intestine in response to alcohol feeding could result in increased production of lipoprotein destined for plasma. Indeed, intraduodenal administration of ethanol to rats was shown to cause an increased intestinal lymph output of VLDL, compared with isocaloric amounts of carbohydrate (Mistilis and Ockner, 1972). The time of the appearance of the endogenous triglyceride in intestinal lymph coincided with an increase in serum triglyceride in rats without intestinal fistula; this would support the concept of intestinal lymph lipoproteins contributing to ethanol induced hyperlipidaemia of endogenous origin. The relative contribution of the liver and gut to the production of alcohol induced hypertriglyceridaemia is, however, not clear, and warrants further study. That the contribution of the gut is minor, is suggested by the finding that in chronically alcohol fed rats, whereas hyperlipaemia resulted, intestinal lymph lipid output was no greater than in a control group of rats given a diet containing an equal amount of fat, but devoid of alcohol (Baraona et al, 1973). Studies in which hepatic triglyceride secretion was blocked with orotic acid, also suggest a minor contribution of extrahepatic tissues (Hernell and Johnson, 1973).

The bulk of evidence thus favours increased production of VLDL mainly in the liver in response to ethanol administration. A direct effect of alcohol on removal of triglyceride from plasma has little evidence in its support. The main effect of alcohol leading to over-production of triglyceride by the liver, appears to be diminished fatty oxidation, alcohol being oxidised in preference to fatty acids. This would increase the availability of the free fatty acid for triglyceride synthesis; accumulation of triglyceride in the liver, or secretion into plasma as VLDL could thereby account for both disturbances of lipid metabolism commonly encountered in alcoholics, viz. fatty liver and hypertriglyceridaemia.

ALCOHOL AND ATHEROSCLEROSIS

The effect of alcohol on the development of atherosclerosis and IHD has for some years been a controversial issue. The most widely held view, arising mainly from post-mortem studies, is that alcohol tends to protect against the development of atherosclerosis. This view point goes back as far as 1904 when Calbot presented data indicating atherosclerosis to be uncommon prior to the age of 50 years in alcoholics. In support of this, Eberhard (1936) showed alcohol to protect against the development of atherosclerosis in the cholesterol fed rabbit.

More recently, further data supporting this concept has been presented, although most studies suggest alcohol to be of preventative benefit only in the presence of cirrhosis. The life shortening effect of cirrhosis is, of course, well-known. Post-mortem studies have shown the incidence of atherosclerotic heart disease to be considerably lower in patients with hepatic cirrhosis than in general population (Berrios and Rodriguez, 1959; Grant et al, 1959; Howell and Manion, 1960; Ruebner et al, 1961). In a prospective study, Hirst et al (1965) found cirrhotics to have less atheromatous arteries than controls; alcoholics without cirrhosis, however, had the same frequency of severe coronary narrowing, coronary occlusion and myocardial infarction, as did controls. Myocardial infarction in male alcoholics occurred only one-fifth as frequently as in non-alcoholics. They suggest that the protective effect of cirrhosis on the development of atherosclerosis, may relate to the effect of cirrhosis on oestrogen metabolism, blood coagulation, or both.

Caution must, however, be exerted in interpreting post-mortem data. Because of the extremely high prevalence of atherosclerosis amongst Western communities, control groups will undoubtedly be heavily weighted by such cases, and are unlikely to be representative of the population studied. Control groups selected to eliminate this bias are thus desirable. In one such study where victims of road traffic accidents were used as controls, no significant difference in the degree of atherosclerosis was found between cirrhotics and the accident victims (Parish and Eberly, 1961). Thus, although cirrhosis may protect against atherosclerosis, there is little evidence that alcohol per se does.

On the other hand, there is little to suggest that alcoholism increases the predisposition to atherosclerosis, despite the evidence cited in both animals and man with regard to alcohol and serum lipids. Most studies have failed to show more atherosclerosis in alcohol treated than in control animals (Grande et al, 1959; Nikkilä and Ollila, 1959), although Gottlieb et al (1959) observed more extensive atheroma in the aortas of ethanol fed rats than in controls. Despite large post-mortem studies having failed to show an increased incidence of atherosclerosis in alcoholics compared with the general population (Wilens, 1947; Hirst et al, 1965), certain individuals who are predisposed to develop an alcohol-induced elevation of serum triglyceride, could thereby be at risk of developing premature vascular disease. Identification of such susceptible individuals would, therefore, be desirable.

SCOPE OF THE PRESENT WORK

Amongst identifiable causes of hypertriglyceridaemia, alcohol seems to be remarkably common (see table 4, page 197). Accordingly, the clinical features have been studied in thirteen patients with alcoholic hyperlipidaemia. Experiments were also performed in order to determine the mechanism whereby alcohol over-indulgence raises the serum triglyceride concentration. The bulk of evidence from studies in both man and experimental animals favours over-production of triglyceride; some evidence supporting impaired triglyceride removal from plasma has also been presented (Losowsky et al, 1963). The present study was undertaken in order to determine the role of triglyceride removal in the pathogenesis of alcoholic hypertriglyceridaemia using the IVFTT. The findings, which are in keeping with alcohol-induced hypersecretion of triglyceride being of prime importance do, however, suggest a possible basis for individual susceptibility to the serum triglyceride raising effect of alcohol.

CHAPTER IV, SECTION B

Amongst the identifiable causes of hypertriglyceridaemia, alcohol over-indulgence appears to be remarkably common. During the investigation of a patient with hypertriglyceridaemia, it is important to identify known secondary causes (Fredrickson et al, 1967), as treatment of the primary disorder frequently leads to correction of the serum lipid abnormality as well. Primary hypertriglyceridaemia tends frequently to be familial (Fredrickson et al, 1967); follow-up of the families of such cases is thus of importance, and where possible, routinely practised by the Hammersmith Hospital Lipid Disorders Unit. In 1971, 211 patients with hypertriglyceridaemia of identifiable cause were seen by members of our unit. The causes are listed in Table 4, together with the number of cases of each seen.

TABLE 4

NUMBER OF PATIENTS WITH HYPERTRIGLYCERIDAEMIA OF IDENTIFIED CAUSE AT HAMMERSMITH HOSPITAL IN 1971

Cause	No.
Familial	62
Diabetes mellitus	43
Alcoholism	31
Chronic renal disease	20
Hypothyroidism	14
Gout	11
Hypopituitarism	10
Acromegaly	7
Cholestasis	4
Malabsorption	3
Oral contraceptives	3
Cushing's syndrome	1
Addison's disease	1
Gram-negative septicaemia	1
Total	211

Alcoholic hypertriglyceridaemia can be seen to occur remarkably commonly. Amongst the non-familial causes, it was found to be second only to Diabetes Mellitus.

Studies will be described on thirteen of these patients. In order to examine the effect of alcohol on the peripheral removal of triglyceride from plasma, the IVFTT was performed twice on each patient. The first occasion was during a period of alcohol consumption; the second was after alcohol withdrawal had resulted in a substantial reduction in serum triglyceride concentration. It is conceivable that in some cases the presence of alcoholic over-indulgence and hypertriglyceridaemia could merely be a chance association. For this reason, it was decided to include in the study, only those patients whose serum triglyceride levels fell substantially during alcohol withdrawal. Those patients whose triglycerides fell by at least 25% were included; in many cases, concentrations became entirely normal. The choice of a 25% reduction defining 'alcoholic hypertriglyceridaemia' was arbitrary. In those with a lesser reduction of serum triglycerides, alcohol was considered probably not to be playing a significant role in the pathogenesis of the hypertriglyceridaemia.

EXPERIMENTAL DESIGN

Serum triglyceride concentration and fractional removal rate of triglyceride were determined during alcohol consumption and following its withdrawal. Patients were admitted to the ward for the study. All had been consuming substantial amounts of alcohol for some weeks prior to admission. The intake of ethanol was at least 140 grammes/day, a variety of beverages being responsible. Patients were instructed not to modify their drinking and eating habits in any way prior to admission. On the day of admission, the patient's usual daily intake of alcohol was provided on the ward. The following day, serum lipids and lipoproteins, and intravenous fat tolerance were measured after an overnight fast. No further alchol in any form was permitted during the remainder of the admission. As surreptitious drinking could invalidate the results, occasional checks for blood alcohol were performed at random intervals; in no cases was any detected. Only one patient developed withdrawal symptoms; these responded satisfactorily to treatment with Chlorpromazine.

During the period of alcohol withdrawal, the patients were fed a diet, isocaloric with that ingested before admission. Calorie intake was calculated for each individual by a detailed dietary history taken by a trained dietitian. Calories previously consumed as alcohol were replaced by increasing the amount of food eaten. The composition of the diet was that of a typical British hospital ward, with protein providing 12%, carbohydrate 48% and fat 40% of caloric intake. The fat was predominantly saturated with a P/S ratio of 2.0. Body weight was measured daily; the mean change between the first and the second studies was +0.3Kg. In no subject did body weight change by more than 1 Kg.

Measurements were repeated after a period ranging from seven days in patients with mild and modest lipaema, to twelve days in patients with intense lipaemia at the time of the first study.

During the early part of the admission, blood was sampled for determination of lactic dehydrogenase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase and uric acid. Liver biopsy, when performed, was carried out early in the admission.

In two patients, alcohol over-indulgence and diabetes mellitus could both have contributed to the hypertriglyceridaemia. In one of these subjects, the period of alcohol withdrawal was followed by treatment of his diabetes by an 800 calorie, 60 gramme carbohydrate diet. Measurements of serum lipids and fractional removal rate of triglyceride were performed during diabetic control in addition to before and after alcohol withdrawal.

One patient was admitted to the surgical ward with an acute abdomen, thought to be associated with alcoholic hyperlipaemia. His serum triglycerides were grossly elevated at the time of admission with the presence of excess pre-beta lipoprotein and chylomicrons in a fasting sample. Following alcohol restriction, his serum triglycerides fell rapidly. By the fourth day of his admission, serum triglyceride level had fallen sufficiently for the initial measurement of fractional removal rate and triglyceride to be made; by this time his intravenous fluid therapy had been stopped and he was ingesting a normal ward diet. Further studies were performed after four and ten days off alcohol.

SUBJECTS AND CLINICAL ASPECTS

The patients were eleven men and two women; ages ranged from 29 - 59 years. Alcohol intake ranged from 140 grammes per day to 320 grammes per day. Mean serum triglyceride at the time of the initial study was 746 ± 180 mg./dl. (SEM). Values ranged from 200 mg./dl. to 1,800 mg./dl.

Some clinical features of the patients are shown in Table 5.

TABLE 5

CLINICAL FEATURES

Age	Sex	Liver	Liver enzymes	Vascular disease	Plasma-triglycerides (mg./100 ml.)	
					On alcohol	Treated
39	M	-	Normal	-	662	213
44	F	-	Normal	-	244	148
59	M	Fatty	Normal	P.V.D.	200	102
43	F	-	Normal	I.H.D.	252	166
47	M	Fatty	Raised	-	1,430	262
49	M	Fatty	Normal	I.H.D.	283	214
29	M	Fatty	Raised	-	1,150	236
45	M	Normal	Normal	P.V.D.	276	179
42	M	Fatty ?	Raised	-	1,400	114
51	M	Fatty	Raised	I.H.D. + P.V.D.	1,820	285
52	M	-	Normal	I.H.D. + P.V.D.	180	131
40	M	-	Normal	-	1,480	158
57	M	Fatty	Raised	I.H.D.	294	183

P.V.D. = peripheral vascular disease.
I.H.D. = ischaemic heart disease.

Many had evidence of vascular disease. None had a history of previous myocardial infarction. Ischaemic heart disease (IHD) was considered to be present if a classical history of angina pectoris was obtained and/or there was ECG evidence of ischaemia. Peripheral vascular disease (PVD) was diagnosed by a classical history of intermittent claudication, with absent pulses. In one case, this diagnosis had previously been confirmed by arteriography. Three patients had IHD, two had PVD, and in a further two, both were present. One patient presented with acute abdominal pain for which he was admitted to a surgical ward; alcohol restriction was associated with the disappearance of his pain. The same patient had moderate bilateral parotid enlargement which subsided on withdrawing alcohol; another had Dupuytren's contractures. In no case was xanthelasma, xanthomata or corneal arcus detected.

One patient had diet-responsive diabetes mellitus, a diabetic glucose tolerance curve being detected in another. No other cause of secondary hypertriglyceridaemia was discovered nor, did any patient give a family history of early-onset vascular disease or xanthomata. Five of the thirteen patients had elevations of one or more of the serum enzymes assayed. Serum amylase activity was raised in two patients, including the patient who presented with an acute abdomen.

Liver biopsies were obtained from eight subjects; six showed fatty change, one was normal, and one showed non-specific hepatic granuloma for which no cause was subsequently detected. A further patient had a 7 cm. smooth non-tender hepatomegaly, but refused to undergo liver biopsy. Cirrhosis was not detected in any subject.

EFFECT OF WITHDRAWING ALCOHOL ON SERUM LIPIDS AND IVFTT

At the time of the first study, the mean serum triglyceride was 746 ± 180 mg./dl. (SEM). The elevated serum triglyceride levels were shown to be due to the pre-β lipoprotein by lipoprotein electrophoresis, confirmation being obtained in some cases by ultracentrifugation. The patient who presented with abdominal pain also had chylomicrons present after a 14 hour overnight fast; this was assessed by the appearance of serum after overnight storage at $4^{0}C$, and by lipid-staining material remaining as the origin on electrophoresis. Fasting chylomicronaemia was not present in any other case.

Serum triglyceride concentrations at the time of the first study plotted against fractional removal rates of triglyceride are seen in Figure 18. No significant correlation was detected ($r = -0.31$; $p > 0.1$). Figure 20 shows the distribution of K_2 values in these patients; it can be seen that most lie towards the lower end of the normal range. The mean K_2 for males with alcoholic hyperlipidaemia was 0.025 $min.^{-1}$, compared with 0.047 $min.^{-1}$ in normal subjects (see page 69).

The effect of withdrawing alcohol on serum triglycerides and triglyceride fractional removal rate is shown in Table 6 and illustrated in Figure 19.

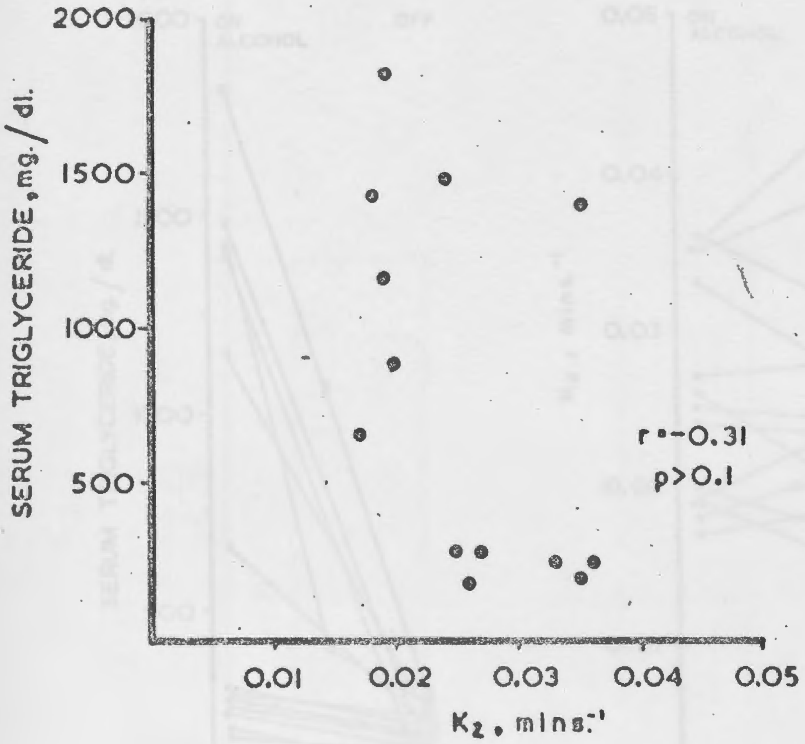

Fig. 18. Correlation between triglyceride and K_2 during alcohol ingestion

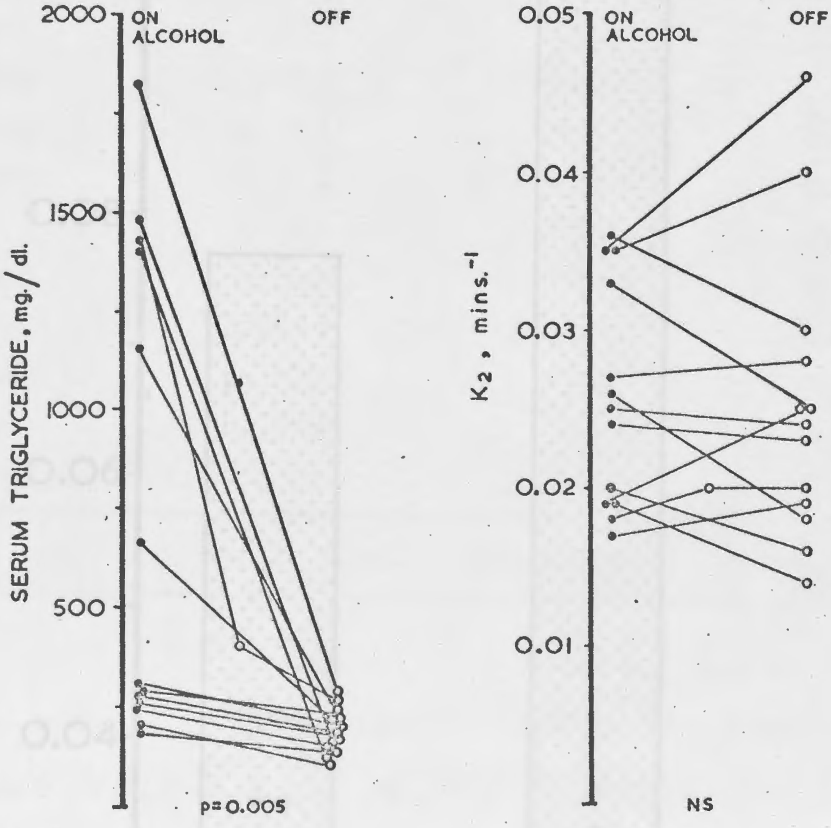

Fig. 19. Serum triglyceride and K_2 - effect of withdrawing alcohol

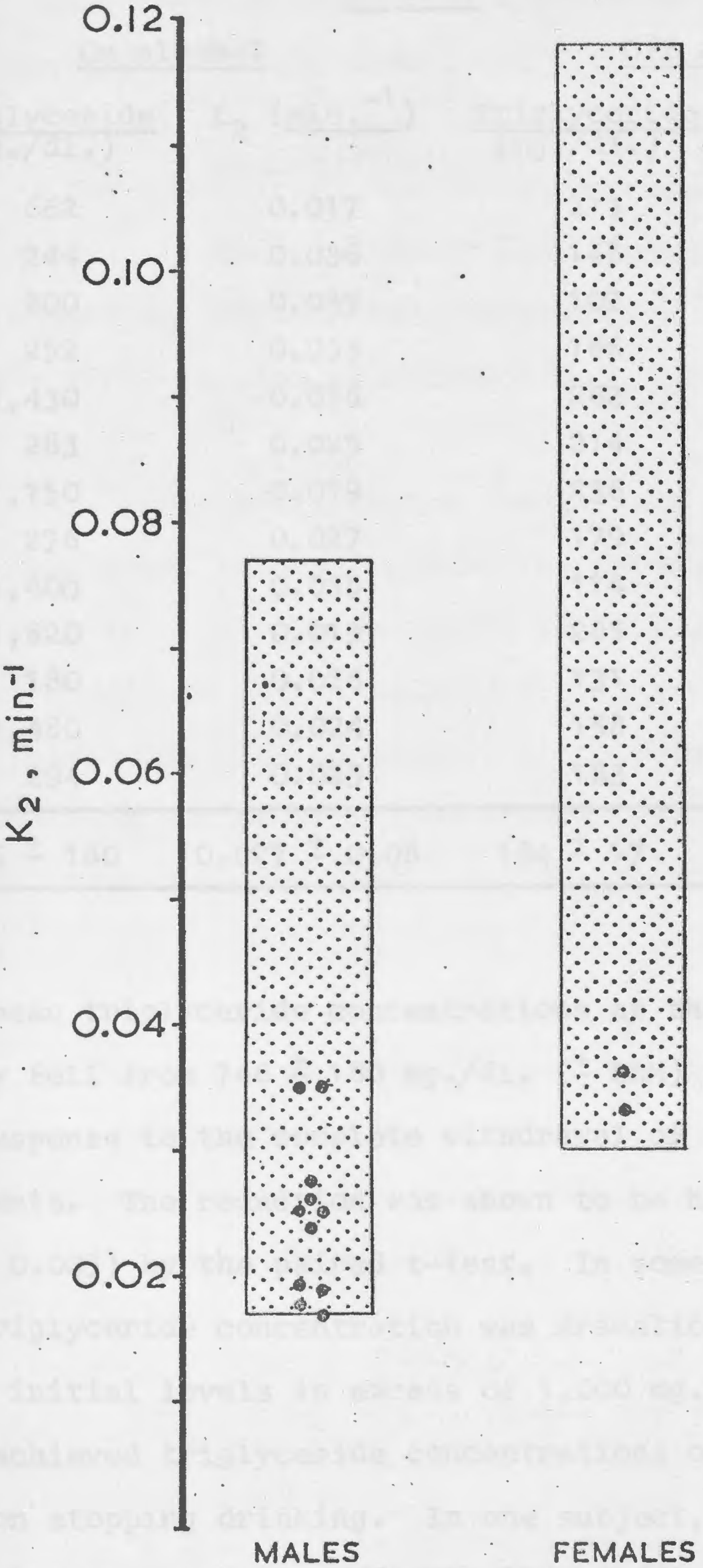

Fig. 20. K_2 in alcoholic lipaemia
(Normal ranges represented by hatched areas)

TABLE 6

	On alcohol		Off alcohol	
Sex	Triglyceride (mg./dl.)	K_2 (min.$^{-1}$)	Triglyceride (mg./dl.)	K_2 (min.$^{-1}$)
M	662	0.017	213	0.019
F	244	0.036	148	0.030
M	200	0.035	102	0.040
F	252	0.033	166	0.025
M	1,430	0.018	262	0.020
M	283	0.025	214	0.024
M	1,150	0.019	236	0.025
M	276	0.027	179	0.028
M	1,400	0.035	114	0.046
M	1,820	0.019	285	0.014
M	180	0.026	131	0.018
M	1,480	0.024	158	0.023
M	294	0.025	183	0.023
± SEM	746 ± 180	0.027 ± 0.08	184 ± 17	0.028 ± 0.01

The mean triglyceride concentrations at the time of the initial study fell from 746 ± 180 mg./dl. (± SEM) to 184 ± 17 mg./dl. in response to the complete withdrawal of alcohol in these patients. The reduction was shown to be highly significant ($P < 0.005$) by the paired t-test. In some cases the reduction in triglyceride concentration was dramatic; the five patients with initial levels in excess of 1,000 mg./dl., for example, all achieved triglyceride concentrations of less than 300 mg./dl. on stopping drinking. In one subject, values fell by more than 90% from 1,400 mg./dl. to 114 mg./dl. It is noteworthy that at the time of the second study, about half the subjects still had some elevation of their serum triglyceride concentrations.

Table 6 also shows that the change in serum triglyceride concentration was not associated with any significant change in fractional removal rate of triglyceride from plasma; mean values for the initial and repeat examination were 0.027 ± 0.08 min.^{-1} (± SEM) and 0.028 ± 0.01 respectively ($p > 0.1$). The effect on triglyceride concentration and rate constant in the IVFTT is also illustrated in Figure 19.

Thus serum triglyceride levels were not correlated with the fractional removal rate of triglyceride from plasma, and the fall in triglyceride levels which followed the withdrawal of alcohol, was not associated with a significant increase in the fractional rate of removal of triglyceride from plasma. However, as can be seen from Figure 20, the distribution of K_2 values was in the lower part of the normal range.

In two patients, diabetes may also have contributed to the hypertriglyceridaemia. The response of one such patient to withdrawal of alcohol and to subsequent control of his diabetes is shown in Table 7.

TABLE 7

PLASMA-TRIGLYCERIDE CONCENTRATION AND FRACTIONAL REMOVAL RATE IN A PATIENT WITH ALCOHOLISM AND DIABETES

	Before treatment	Off alcohol	Off alcohol, diabetic diet
Plasma-triglyceride (mg./dl.)	635	213	68
K_2 (min.^{-1})..	0.017	0.019	0.041
Plasma-glucose (fasting) (mg./dl.)	215	191	82
Body-weight (kg.).. ..	77.0	76.5	73.1

when the calories consumed as alcohol were replaced by food, his triglyceride fell substantially from 635 mg./dl to 213 mg./dl. The rate constant in the IVFTT was virtually unchanged, being 0.017 min.$^{-1}$ and 0.019 min.$^{-1}$ on and off alcohol respectively. The reduction in serum triglyceride concentration was thus unaccompanied by an increased fractional rate of removal of triglyceride from plasma; body weight fell by only 0.5 Kg. The diabetes remained uncontrolled off alcohol, with a fasting blood glucose of 191 mg./dl. Subsequently, institution of a 60 gramme carbohydrate 800 calorie diet, led to weight loss and correction of the diabetes. The patient was restudied after 21 days on this diet, while in addition remaining off alcohol completely. By this time, he had lost nearly 3 Kg. in weight and his fasting blood glucose had fallen to 82 mg./dl. In addition, a marked further reduction in triglyceride concentration to 68 mg./dl. had occurred; this was accompanied by an increased rate of removal of triglyceride from plasma, the K_2 value having increased to 0.041 min.$^{-1}$.

Several triglyceride estimations in the patient who presented with an acute abdomen are shown in Figure 21 . A non-fasting triglyceride concentration measured on admission was 14,000 mg./dl. Within 36 hours of alcohol abstention and intravenous fluid therapy, his level had fallen dramatically to 4,000 mg./dl. Both this and the initial sample with the level of 14,000 mg./dl. contained chylomicrons in addition to excess VLDL. This was assessed by inspection of serum stored overnight at 4^{o}C and by the demonstration of non-migrating lipid staining material on electrophoresis of serum. At that time,

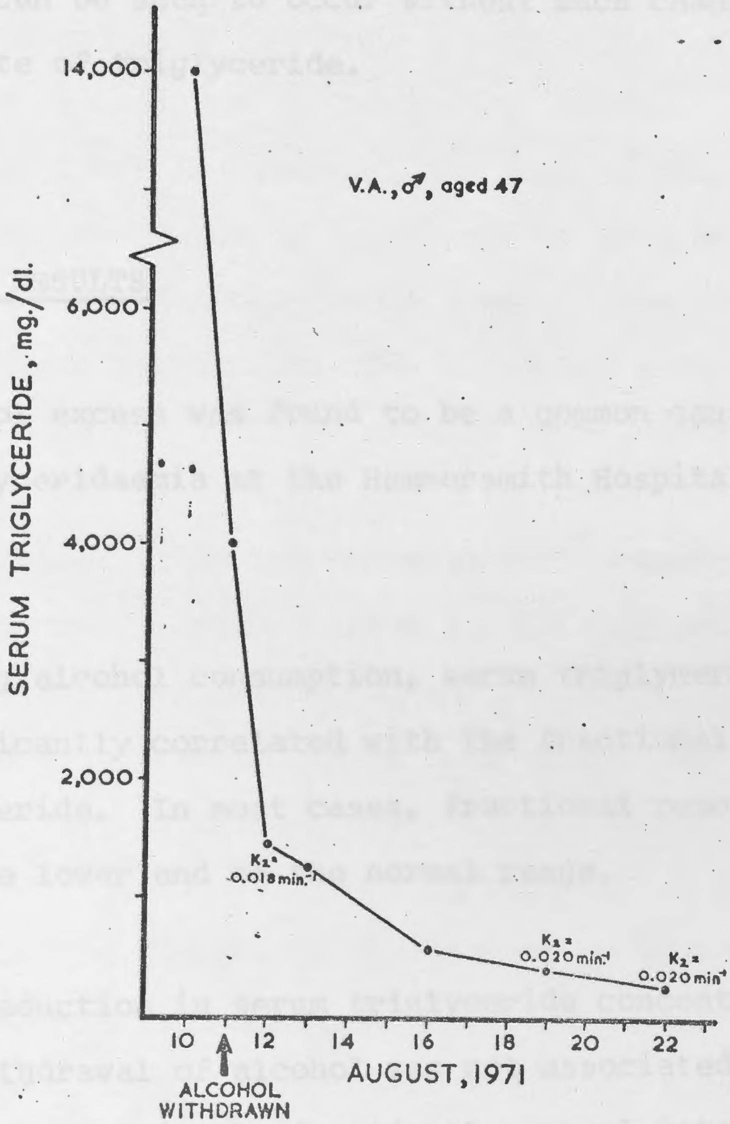

Fig. 21. Effect of withdrawing alcohol from a patient with alcoholic hypertriglyceridaemia

his abdominal pain had virtually disappeared and oral feeding was recommenced. Fasting values over the next eleven days are shown, including K_2 values on the three days on which he had intravenous fat tolerance tests. The reduction in his triglyceride can be seen to occur without much change in fractional removal rate of triglyceride.

SUMMARY OF RESULTS

Alcohol excess was found to be a common cause of secondary hypertriglyceridaemia at the Hammersmith Hospital Lipid Disorders Clinic.

During alcohol consumption, serum triglyceride levels were not significantly correlated with the fractional removal rate of triglyceride. In most cases, fractional removal rates were towards the lower end of the normal range.

The reduction in serum triglyceride concentration following the withdrawal of alcohol was not associated with a significant increase in the fractional removal rate of triglyceride from plasma.

CHAPTER IV, SECTION C

The mechanism of alcoholic lipaemia has previously been studied both in man and in laboratory animals. Although no consistent pattern has emerged, the bulk of the evidence favours over-production of triglyceride; some support for impaired peripheral triglyceride removal from the circulation also exists, in particular, the sub-normal post-heparin lipolytic activities in subjects studied by Losowsky et al (1963).

The present study was undertaken in order to determine the role of triglyceride removal in the pathogenesis of alcoholic hypertriglyceridaemia, using the IVFTT. In this condition, serum triglyceride levels have previously been shown to fall within days of ceasing alcohol consumption (Kudzma and Schonfeld, 1971); alcohol was thus regarded as being the cause of hypertriglyceridaemia in the present investigation when triglyceride levels fell substantially on withdrawing alcohol. In most cases, normal values were achieved, although an arbitrary cut-off point of 25% reduction was used for inclusion in the study. In view of the psychological upsets accompanying alcoholism, it was felt necessary to exclude surreptitious drinking by performing occasional spot checks for alcohol in the blood stream; on no occasion was any detected. It is also of interest that withdrawal symptoms were experienced in only one case. This suggests a low degree of dependence in the group of patients studied. Many would not, in fact, have been classified as alcoholics, but as heavy drinkers.

During alcohol withdrawal, serum triglyceride fell significantly from a mean of 746 mg./dl. to 184 mg./dl. In many cases, this reduction was very marked and occurred rapidly. This change was associated with almost identical mean K_2 values before and after alcohol withdrawal. The lack of increase in the fractional removal rate of triglyceride, in association with the fall in serum triglyceride which followed alcohol withdrawal, suggests that the reduction in triglyceride levels is unlikely to be due to enhanced peripheral triglyceride removal. The absence of a change in intravenous fat tolerance when the hypertriglyceridaemia is corrected by withdrawing alcohol is, therefore, compatible with the view that alcohol produces a lipaemia by increasing plasma-lipoprotein secretion. The lack of significant correlation between fractional removal rate and triglyceride concentration while consuming alcohol, is further evidence against triglyceride removal being the most important determinant of serum triglyceride levels in these cases. These findings are in sharp contrast to the comparable studies in diabetics described in Chapter III. Triglyceride levels in the uncontrolled diabetic state showed a significant negative correlation with fractional removal rate of triglyceride; treatment of the diabetes also resulted in pronounced speeding up of fractional removal of triglyceride and simultaneous reduction in serum triglyceride levels. Whereas in diabetes, treatment thus appears to affect the removal mechanism of triglyceride from plasma, the present studies fail to show a direct effect of alcohol on removal of triglyceride from plasma in keeping with in vitro studies on adipose tissue (Bizzi and Carlson, 1965). Although Losowsky et al (1963) described low PHLA values in six out of eight patients with alcoholic hypertriglyceridaemia, it is of note that of four cases restudied after the withdrawal of alcohol, lipolytic activity increased in only one, despite triglyceride levels falling in all. Kudzma and Schonfeld (1971) also failed to show a change in lipolytic activity with the development of hypertriglyceridaemia during alcohol administration. The reason for the low lipolytic activities in the cases described by Losowsky et al (1963) is not clear; two may have been influenced by the fact that they were diabetic; no cause was obvious in the others. The present findings are thus in agreement with both these studies, despite the suggestion by Losowsky et al (1963) that impaired removal may be the dominant factor.

The present findings are consistent with the view that the hypertriglyceridaemia induced by alcohol is due to triglyceride over-production. VLDL formation has been shown to be stimulated by alcohol consumption in rats (Baraona and Lieber, 1970; Baraona et al, 1973). As discussed earlier, the most attractive hypothesis for enhanced triglyceride production relies on the demonstration of reduced fatty acid oxidation consequent on alcohol administration (Lieber and Schmid, 1961; Lieber et al, 1967). Increased quantities of fatty acids would thus be made available for triglyceride synthesis which could lead to the development of fatty liver and provide the lipid portion of the lipoprotein secreted by the liver. The role of the gut in such an hypothesis is not clear, although intestinal lipogenesis (Carter et al, 1971) and intestinal lipoprotein production (Mistilis and Ockner, 1972) have been shown to be increased in response to alcohol feeding.

The simultaneous presence of fatty liver and hypertriglyceridaemia in six of the cases currently studied, mitigates strongly against early postulates that impaired release of triglyceride from the liver as lipoprotein could contribute to the development of steatosis. Fatty liver and hypertriglyceridaemia have also been shown to develop simultaneously in rats fed with alchol (Baraona and Lieber, 1970).

An important question which arises from these considerations is that if alcohol results in enhanced triglyceride secretion and lipoprotein formation, why then do all heavy drinkers not have hypertriglyceridaemia? In some instances, this could be due to severe liver disease with consequent impairment of lipoprotein synthesis. This is, however, clearly not the entire answer, as one does not have to search hard to find examples of heavy drinkers having normal or low triglyceride concentrations, and normal liver function. Alcohol consumption in such cases may be many times higher than that encountered in the subjects in the present investigations, many of whom would be considered only 'moderate' drinkers by some standards. A more general explanation may follow from the observation that the K_2 values in all thirteen cases were at the lower end of the normal range. The mean value was also considerably lower than the mean value in normal subjects. It is conceivable that alcohol induced hypersecretion of triglyceride by the liver (and probably the gut), might only lead to hyperlipidaemia in individuals with a relatively limited capacity for removal of triglyceride from plasma, while those with more rapid removal rates might be able to deal with an excessive triglyceride secretion with impunity. Such a hypothesis would be tenable in relationship to both exogenous and endogenous triglycerides in view of the belief that both are removed from plasma by a similar mechanism (Havel, 1965; Brunzell et al, 1973). The enhanced alimentary lipaemia when alcohol and fat are ingested simultaneously (Wilson et al, 1970) would be explained on a similar basis. This hypothesis is also in agreement with the observation by Losowsky et al (1963) of persistently sub-normal post-heparin lipolytic activities after withdrawing alcohol from some of their patients; some other underlying lesion resulting in reduced lipolytic activity is thus likely to have co-existed in these patients. If a low capacity for removal of triglycerides from plasma makes one susceptible to develop hypertriglyceridaemia following alcohol consumption, it is likely that normal females with their faster fractional removal rates as measured by the IVFTT (see page 69) would be better protected against this eventuality than their male counterparts, and may be yet another factor in the relative protection that premenopausal females enjoy against the development of atherosclerosis and IHD.

The reason for the low K_2 values found in this study, and the low PHLA values observed by Losowsky et al (1963) is not immediately apparent. Any condition unrelated to the intake of alcohol which is associated with impaired or limited triglyceride removal from plasma may be postulated. One such is diabetes mellitus (see Chapter III), although subjects whose removal rates are at the lower end of the normal range for constitutional or other reasons, may also be at risk. It is perhaps relevant that two of the patients of Losowsky et al (1963) were diabetics, as were two out of thirteen in the present studies. Findings in the patient with alcoholism and diabetes, in whom measurements were made before and after alcohol withdrawal, and again after diabetic control, are consistent with this hypothesis. Alcohol withdrawal together with an increased intake of food, to make good the calories not consumed in the form of ethanol, resulted in a substantial reduction in serum triglyceride concentration, despite the fractional removal rate remaining unchanged. Persistence of an elevated triglyceride level and low K_2 value suggests inadequate triglyceride removal despite a reduced input of triglyceride into plasma, consequent on stopping drinking. When in addition to remaining off alcohol, his diabetes was brought under control by appropriate dietary measures, a further reduction in serum triglyceride concentration, to well within the normal range resulted, which on this occasion was accompanied by an increased rate of triglyceride removal from plasma. Interaction between the capacity for triglyceride removal from plasma and alcohol-induced hypersecretion by the liver could also explain the case described by Fry et al (1973); excessive alcohol and carbohydrate inducibility of triglyceride were both found in the same patients with endogenous hypertriglyceridaemia. A similar situation may pertain to the cases recently described by Mendlesohn and Mello (1973) in whom alcohol administration led to a far more pronounced elevation of serum triglyceride levels in the presence of primary endogenous hypertriglyceridaemia than in normolipaemic or carbohydrate-induced men. As impaired triglyceride removal has been claimed to underly many cases of endogenous hypertriglyceridaemia (Ryan and Schwartz, 1965; Porte and Bierman, 1969; Boberg, 1971), alcohol-induced triglyceride secretion in excess of the capacity for triglyceride removal, could result in an expanded plasma triglyceride pool. Carbohydrate-induction has also been shown to be associated with reduced triglyceride removal (Mancini et al, 1973); the reason for the difference in the response to alcohol between carbohydrate-induced subjects and those with endogenous hypertriglyceridaemia is not clear, but may relate to the degree of impairment of peripheral triglyceride removal.

It is also noteworthy that in the present study despite a significant lowering of serum triglyceride by alcohol withdrawal, levels were still elevated in about half the cases at the time of the second study. This may be due to the short period of time between the two investigations; alternatively, it may suggest an underlying disorder such as endogenous hypertriglyceridaemia. Indeed, on long-term follow-up, mild elevations of serum triglyceride were persistently found in some cases despite staying off alcohol.

Data is not available on the incidence of hyperlipidaemia amongst alcoholics; if a relatively limited capacity for triglyceride from plasma is a prerequisite to the development of alcoholic hyperlipidaemia, the incidence might be expected to be fairly low. Alcohol as a cause of secondary hyperlipidaemia presenting at a clinic specializing in clinical disorders of lipid metabolism is, however, fairly common (see Table 4). It was perhaps, therefore, somewhat unexpected that a positive correlation was found between alcohol intake and serum triglyceride concentration in a survey of lipid and lipoprotein concentrations amongst a 'healthy' London population (Lewis et al, 1974), albeit the correlation was of low order.

The plasma lipoprotein pattern in all patients in the present investigation was an elevation of pre-beta lipoprotein. One patient with intense lipaemia also had chylomicron particles in fasting plasma; this does not necessarily imply that these particles were of dietary origin as endogenous particles with characteristics similar to chylomicrons have been shown to be produced during carbohydrate induction (Mancini et al, 1973). Lipoprotein formation in the liver occurs at the smooth endoplasmic reticulum where triglyceride and apoprotein combine to form lipoprotein. The exact derivation of the apoprotein moeity is not clear although it is likely to derive from the rough endoplasmic reticulum, the major site of protein formation in the cell (Lieber, 1973). Although under normal circumstances, the lipoprotein produced by the liver is VLDL, in conditions in which the supply of triglyceride exceeds that of protein, triglyceride deposition in the liver could ensue. Some of the excess triglyceride available could conceivably result in the formation of lipoproteins with more triglyceride per unit of protein, and characteristics similar to those of chylomicrons. A similar situation can be envisaged with regard to the production of lipoprotein by the intestine. In Chapter VI, it will be seen that the lipoprotein appearing in plasma following ingestion of a fatty meal depends to a large extent on the dose of fat ingested; high fat loads result in the appearance mainly of chylomicrons in plasma whereas lower, more physiological doses result in a substantial contribution to the alimentary lipaemia by VLDL. A similar situation could exist in relation to alcohol. Intestinal lipogenesis has been shown to be stimulated by ethanol (Carter et al, 1971); it is possible that a stage may be reached where the triglyceride supply relative to the availability of apoprotein leads to the formation of large chylomicron-like particles. On reduction of the availability of triglyceride for lipoprotein formation in these two organs, reversion to the more usual VLDL production might occur. Consistent with this hypothesis, is the observation that on withdrawing alcohol in the patient with intense lipaemia, chylomicrons rapidly disappeared. This was followed by a reduction in serum triglyceride concentration presumably due to reduced VLDL. An alternative explanation of this finding could be that removal mechanisms for triglyceride were saturated by VLDL; exogenous triglyceride removal could then be inhibited.

Serum uric acid was found to be elevated in two subjects out of eight in whom it was measured. Hyperuricaemia in association with alcohol excess is well recognized (Lieber et al, 1962; Gebbie and Prior, 1967; Newcombe, 1972), uric acid levels tending to decrease rapidly on alcohol withdrawal (Lieber et al, 1972). Inhibition of renal tubular excretion of urate by lactate, which accumulates as a result of the increased NADH/NAD ratio in the cytosol, is believed to cause the hyperuricaemia (Yü et al, 1957; Lieber et al, 1972). About half the subjects studied by Gebbie and Prior (1967) had hyperuricaemia in association with alcoholic hypertriglyceridaemia, a somewhat higher proportion than that found in the current investigation. Numbers in both studies are small, however, and in neither was lactate simultaneously measured. Furthermore, in the present study, uric acid determinations were also not performed until at least the third day of admission. Had this been estimated before alcohol withdrawal, a higher frequency of hyperuricaemia may have been detected.

The relationship between alcoholism and the development of atherosclerosis has been discussed on page 197. It appears that cirrhosis 'protects' against the development of atherosclerosis; no evidence of cirrhosis was, however, obtained in any of the subjects studied. The clinical incidence of atherosclerosis in the group studied was exceedingly high considering the young ages of many of the patients (see Table 5). Vascular disease was present in somewhat more than half the patients; three had IHD, two had PVD and in a further two both were present. Other risk factors for the development of atherosclerosis have not been carefully investigated in the present study and it may be relevant that many of the patients smoked cigarettes. The association between hypertriglyceridaemia and atherosclerosis (Albrink et al, 1961), and the recent demonstration of triglyceride as an independent risk factor for the development of IHD (Carlson and Böttiger, 1972), does however, tend to suggest that alcohol may have played an indirect role in the development of the premature atherosclerosis in these subjects by elevating their serum triglyceride levels. It may be with much foresight that Allbutt in 1915, said of atherosclerosis: 'Alcohol then is not a cause in eminent domain, but is a potent ally of any other poison which may be in co-operation with it.'

CHAPTER IV - REFERENCES

Albrink, M.J., Meigs, J.W. and Man, E.B. (1961) Amer. J. Med. 31, 4.

Allbutt, C. (1915) In Diseases of the Arteries, London p.249.

Baraona, E. and Lieber, C.S. (1970) J. clin. Invest. 49, 769.

Baraona, E., Pirola, R.C. and Lieber, C.S. (1973) J. clin. Invest. 52, 296.

Barboriak, J.J. and Meade, R.C. (1968) Amer. J. med. Sci. 255, 245.

Berrios, J.B. and Rodriguez, R.C. (1959) Bol. Assoc. Med. Puerto Rico 51, 205.

Bizzi, A. and Carlson, L.A. (1965) Life Sci. 4, 2123.

Boberg, J. (1971) M.D. Thesis, Acta Univ. Upsal. 105.

Bouchier, I.A.D. and Dawson, A.M. (1964) Clin. Sci. 26, 47.

Brewster, A.C., Lankford, H.G., Schwartz, M.G. and Sullivan, J.F. (1966) Amer. J. clin. Nutrit. 19, 255.

Brodie, B.B., Butler, W.M., Horning, M.G., Maickell, R.P. and Mailing, H.M. (1961) Amer. J. clin. Nutrit. 9, 432.

Brunt, P.W. (1973) J. Roy. Coll. Phycns. 8, 45.

Brunzell, J.D., Hazzard, W.R., Porte, D.,Jr. and Bierman, E.L. (1973) J. clin. Invest. 52, 1578.

Cabot, R.C. (1904) J. Amer. med. Assoc. 43, 774.

Carlson, L.A. and Böttiger, L.E. (1972) Lancet 1, 865.

Carter, E.A., Drummey, G.D. and Isselbacher, K.J. (1971) Science, 174, 1245.

Carter, E.A. and Isselbacher, K.J. (1972) Lab. Invest. 27, 283.

Cooke, A.R. (1970) Amer. J. dig. Dis. 15, 449.

Crouse, J.R., Gerson, C.D., De Carli, L.M. and Lieber, C.S. (1968) J. Lipid Res. 9, 509.

Curtiss-Prior, P.B. (1972) Experientia 28, 1430.

Datta, D.V. (1965) Gastroenterology 49, 515.

Datta, D.V. (1966) J. Lab. clin. Med. 67, 461.

Di Luzio, H.R. and Poggi, M. (1963) Life Sci. 2, 751.

Eberhard, T.P. (1936) Arch. Path. 21, 616.

Elko, E.E., Wooler, W.R. and Di Luzio, N.R. (1961) Amer. J. Physiol. 201, 923.

Feigl, J. (1918) Biochem. Ztschr. 92, 282.

Feinman, L. and Lieber, C.S. (1967) Amer.J.clin. Nutrit. 20, 400.

Forster, B. (1956) Deutsche Ztschr. 45, 221.

Fredrickson, D.S., Levy, R.I. and Lees, R.S. (1967) New Engl. J. Med. 276, 34, 94, 148, 215, 273.

French, S.W. and Todoroff, T. (1970) Arch. Path. 89, 329.

Freund, G. and O'Hollaren, P. (1965) J. Lipid Res. 6, 471.

Fritz, I.B. (1961) Physiol. Rev. 41, 52.

Fry, M.M., Spektor, A.A., Connor, S.L. and Connor, W.E. (1973) Amer. J. clin. Nutrit. 26, 798.

Gebbie, T. and Prior, I.A.M. (1967) Med. J. Aust. 2, 769.

Gottlieb, L.S., Broitman, S.A., Vitale, J.J. and Zamchek, N. (1959) J. Lab. clin. Med. 53, 433.

Grande, F., Heupel, H.W., Amatuzio, D.S. and Hay, L.J. (1959) Circulation 20, 985.

Grande, F., Hay, L.J., Heupel, H.W. and Amatuzio, D.S. (1960) Circulat. Res. 8, 810.

Havel, R.J. (1965) In Handbook of Physiology V (Adipose Tissue) (eds. A.E. Renold and G.F. Cahill) Amer. Physiol. Soc. Washington D.C. p.499.

Hernell, O. and Johnson, O. (1973) Lipids 8, 503.

Hirst, A.E., Hadley, G.G. and Gore, I. (1965) Amer. J. med. Assoc. 249, 143.

Howell, W.L. and Manion, W.C. (1960) Amer. Heart J. 60, 341.

Iseri, O.A., Lieber, C.S. and Gottlieb, L.S. (1966) Amer. J. Path. 48, 535.

Ishii, H., Joly, J.G. and Lieber, C.S. (1973) Biochim. Biophys. Acta 291, 411.

Johnson, H. (1971) In World Atlas of Wine, Mitchell Beazley, London.

Joly, J.G., Feinman, L., Ishii, H. and Lieber, C.S. (1973) J. Lipid Res. 14, 337.

Jones, A.L. and Ockner, R.K. (1971) J. Lipid Res. 12, 580.

Jones, D.P., Losowsky, M.S., Davidson, C.S. and Lieber, C.S. (1963) J. Lab. clin. Med. 62, 675.

Jones, D.P., Perman, E.S. and Lieber, C.S. (1965) J. Lab. clin. Med. 66, 804.

Kudzma, D.J. and Schonfeld, G. (1971) J. Lab. clin. Med. 77, 384.

Lane, B.P. and Lieber, C.S. (1966) Amer. J. Path. 49, 593.

Larsen, J.A. (1959) Nature 184, 1236.

Lieber, C.S. and Schmid, R. (1961) J. clin. Invest. 40, 394.

Lieber, C.S., Leevy, C.M. and Stein, S.W. (1962a) J. Lab. clin. Med. 59, 826.

Lieber, C.S., Jones, D.P., Losowsky, M.S. and Davidson, C.S. (1962b) J. clin. Invest. 41, 1863.

Lieber, C.S., Jones, D.P., Mendelsohn, J.H. and De Carli, L.M. (1963) Trans Assoc. Amer. Phycns. 76, 289.

Lieber, C.S. and Spritz, H. (1966) J. clin. Invest. 45, 1400.

Lieber, C.S., Spritz, N. and De Carli, L.M. (1966) J. clin. Invest. 45, 51.

Lieber, C.S. (1967) Ann. Rev. Med. 18, 35.

Lieber, C.S., Lefevre, A., Spritz, N., Feinman, L. and De Carli, L.M. (1967) J. clin. Invest. 46, 1451.

Lieber, C.S. and De Carli, L.M. (1968) J. clin. Invest. 47, 620.

Lieber, C.S. (1968) Advanc. int. Med. 14, 151.

Lieber, C.S. and De Carli, L.M. (1970) J. biol. Chem. 245, 2505.

Lieber, C.S. (1972) In Chemical and Biological Aspects of Drug Dependence (eds. S.J. Mule and H. Brill) Chemical Rubber Co. Press, Cleveland, p.135.

Lieber, C.S. and De Carli, L.M. (1972) J. Pharmacol. exp. Ther. 181, 279.

Lieber, C.S. (1973) New Engl. J. Med. 288, 356.

Losowsky, M.J., Jones, D.P., Davidson, C.S. and Lieber, C.S. (1963) Am. J. Med. 35, 794.

Lundquist, F., Tygstrup, N., Winkler, K., Mellemgaard, K. and Munck-Petersen, S. (1962) J. clin. Invest. 41, 955.

Mallov, S. (1961) Quart. J. Stud. Alcohol 22, 250.

Mancini, M., Mattock, M., Rabaya, E., Chait, A. and Lewis, B. (1973) Atherosclerosis 17, 445.

Mendelsohn, J.H. and Mello, N.O. (1973) Science 180, 1372.

Mistilis, S.P. and Birchall, A. (1969) Nature 223, 199.

Mistilis, S.P. and Ockner, R.K. (1972) J. Lab. clin. Med. 80, 34.

Nair, C.R., Saha, S., Chakravarti, R.N., Chhuttani, P.N. and Datta, D.V. (1973) Clin. Sci. 44, 505.

Nestel, P.J. and Hirsch, F.Z. (1965) J. Lab. clin. Med. 66, 357.

Newcombe, D.S. (1972) Metabolism 21, 1193.

Nikkilä, E.A. and Ollila, O. (1959) Circulation 7, 588.

Nikkilä, E.A. and Ollila, O. (1963a) Proc. Soc. exp. Biol. (N.Y.) 113, 814.

Nikkilä, E.A. and Ollila, O. (1963b) Life Sci. 2, 717.

Nyberg, A., Schuberth, J. and Auggard, L. (1953) Acta chem. scand. 7, 1170.

Ockner, R.K., Bloch, K.J. and Isselbacher, K.J. (1968) Science 162, 1285.

Ockner, R.K., Hughes, F.B. and Isselbacher, K.J. (1969) J. clin. Invest. 48, 2079.

Ockner, R.K. and Jones, A.L. (1970) J. Lipid Res. 11, 284.

Papenberg, J., van Wartburg, J.P. and Aebi, H. (1970) Enzymol. Biol. Clin. 11, 237.

Parish, H.M. and Eberley, A.L.,Jr. (1961) Indiana State Med. Ass. 54, 341.

Porte, D.,Jr. and Bierman, E.L. (1969) J. Lab. clin. Med. 73, 631.

Rubin, E. and Lieber, C.S. (1967) Gastroenterology 52, 1.

Rubin, E. and Lieber, C.S. (1968) New Engl. J. Med. 278, 869.

Rubin, E., Beattie, D.S. and Lieber, C.S. (1972) Fed. Proc. 31, 131.

Ruebner, B.H., Migai, K. and Abbey, H. (1961) Lancett ii, 1435.

Ryan, W.G. and Schwartz, T.B. (1965) Metabolism 14, 1243.

Schapiro, R.H., Shimizu, Y., Drummey, G.D. and Isselbacher, K.J. (1962) Clin. Res. 10, 234.

Schapiro, R.H., Drummey, G.D., Scheig, R., Mendelsohn, J.H. and Isselbacher, K.J. (1965) Gastroenterology 44, 849.

Scheig, R. and Isselbacher, K.J. (1965) J. Lipid Res. 6, 269.

Snyder, R., Schulman, M.P. and Westerfeld, W.W. (1964) Arch. Biochem. Biophys. 108, 215.

Talbott, G.D. and Keating, B.M. (1962) Geriatrics 17, 802.

Thompson, G.N. (1956) In Alcoholism, Charles C. Thomas, Springfield.

Von Wartburg, J.P., Bethune, J.L. and Vallee, B.L. (1964) Biochemistry 3, 1775.

Wilens, S.L. (1947) J. Amer. med. Assoc. 135, 1136.

Wilson, D.C., Schreibman, P.H., Brewster, A.C. and Arky, R.A. (1970) J. Lab. clin. Med. 75, 264.

Yü, T.F., Sirota, J.H., Berger, L., Halpern, M. and Gutman, A.B. (1957) Proc. Soc. exp. Biol. (N.Y.) 96,809.

Zieve, L. (1958) Ann. intern. Med. 48, 471.

CHAPTER V

DIETARY POLYUNSATURATED FAT
AND
SERUM TRIGLYCERIDES

CHAPTER V, SECTION A

An association between IHD and serum cholesterol concentration has been demonstrated in many epidemiological studies (e.g. Keys et al, 1963; Gordon and Verter, 1969; Keys, 1971). Serum cholesterol, and factors influencing it, have thus for many years assumed a role of central importance in studies on the pathogenesis of atherosclerosis.

Both the quantity and nature of dietary fat have long been recognized as major determinants of serum cholesterol concentration. In 1952, ingestion of vegetarian diets was shown to lead to a reduction in serum cholesterol concentration (Groen et al, 1952; Kinsell et al, 1952). This effect was shown largely to be due to the presence of vegetable oils of polyunsaturated fatty acids (Ahrens et al, 1955;Malmros and Wigard, 1957). Polyunsaturated fatty acids are fatty acids with more than one double bond in the hydrocarbon chain. One of the most abundant unsaturated fatty acids to occur in mammalian lipids is linoleic acid, an eighteen carbon diunsaturated fatty acid, with double bonds at the 9 and 12 positions. It cannot be synthesized by mammals, yet is required for normal functioning of the organism; for this reason, it is termed an essential fatty acid (Nilsson et al, 1957).

For the past two decades, most investigations into the relationship between dietary fat and atherosclerosis have centred around the presumed link between them, namely serum cholesterol and cholesterol metabolism. The effect of dietary fat on other classes of circulating lipids and lipoproteins has received relatively little attention.

An association between the concentration of triglyceride in plasma and IHD, has more recently been suggested. Unfortunately, compared with the abundant epidemiological data available with regard to serum cholesterol, relatively little is known of the association between serum triglyceride and IHD, largely due to methodological considerations. This is because serum triglyceride determinations have only been practised on a wide scale in the past decade; current availability of semi-automated procedures for their measurement has stimulated a recent interest in this field. Another difficulty related to triglyceride determinations in epidemiological studies, is the need for blood to be sampled under fasting conditions, which is not often easy to accomplish in free-living populations. The evidence linking serum triglycerides with IHD is reviewed in Chapter I (see page 10).

In view of the likelihood of serum triglyceride elevation being implicated in the pathogenesis of atherosclerosis, the effect of diet on triglyceride levels is of obvious importance. The influence of dietary fat on serum triglyceride levels is not as clearly defined as its effects on the serum cholesterol, but serum triglyceride reduction in response to unsaturated fat feeding is well documented (Ahrens et al, 1957; Beveridge et al, 1964; Spritz and Mishkel, 1969; Grande et al, 1972).

The relationship between dietary fats and serum triglycerides has been further investigated in the present study.

I will begin this Chapter by discussing the evidence linking dietary fats with IHD. This will be followed by a brief review of studies on the effect of polyunsaturated fats on serum lipids, and on the mechanism whereby they result in a reduction of serum cholesterol and triglyceride concentrations. It will be seen that whereas much is known about the mechanism of the reduction of serum cholesterol in response to feeding polyunsaturated fats, relatively little is known of the means whereby serum triglyceride concentration is reduced. The present investigation was undertaken to further study the mode of action of dietary polyunsaturated fat in reducing serum triglyceride concentration.

THE RELATIONSHIP BETWEEN DIETARY FATS AND IHD

The major determinant of the mean serum cholesterol concentration of a population, appears to be the amount and type of dietary fat ingested. When fourteen groups of men aged 40 - 59 were studied in seven countries, about 80% of the variability of serum cholesterol between populations could be explained by differences in the intake of dietary saturated fat by the different populations (Keys, 1970). Within a community, serum cholesterol concentrations may vary widely among men on similar diets (e.g. Lewis et al, 1974); this variation is probably related to constitutional factors. The Framingham study demonstrated that no statistical association existed between dietary fat intake and serum cholesterol levels within a community on a relatively uniform diet (Kannel and Gordon, 1969). Not only epidemiological data, but also controlled feeding experiments have shown diet to exert a powerful influence on serum cholesterol levels (Ahrens et al, 1954; Bronte-Stewart et al, 1956; Keys et al, 1965a, b and c). Substitution of saturated dietary fat by polyunsaturated fat, is associated with a reduction in serum cholesterol concentration.

The importance of the relationship between dietary fat intake and serum cholesterol is realized when one considers the overwhelming evidence in favour of the latter as a risk factor for the development of IHD. Prospective community studies from various parts of the world have been in agreement that increasing levels of serum cholesterol are associated with a progressively increasing risk of developing IHD, even from concentrations as low as 200 mg./dl. (e.g. Doyle, 1963; Stamler et al, 1960; Keys et al, 1963; Dayton et al, 1970; Kannel, 1971; Carlson and Böttiger, 1972). When the results of the main prospective studies in the USA were combined in the Pooling Project (Stamler, 1971), a similar conclusion was reached. It appears that serum cholesterol is a prominent feature of the coronary profile, and can be used to predict IHD and to estimate risk. Morbidity, from IHD amongst different populations tends to be related to 'average' serum cholesterol level in these populations (Keys, 1957; Gordon, 1959). In countries with a low incidence of IHD, patients with coronary disease tend to have higher levels of serum cholesterol than the population average (Keys and Fidanza, 1960). In countries with a high coronary mortality rate, an individual with a serum cholesterol concentration 50 mg./dl. lower than average, has a risk of developing IHD of approximately 50% of the population average.

As the intake of dietary fat appears to be an important determinant of serum cholesterol concentration, this raises the question of whether dietary fat intake is associated with the development of IHD. In support of this possibility, Masironi (1970) found a strong positive correlation between IHD mortality rates and consumption of both total and saturated fat, in a survey which involved 37 countries. Stamler et al (1970), in a study of 20 developed countries, showed a significant correlation between mortality rate from IHD and several dietary factors in addition to total and saturated fat intake; these included monounsaturated fat, cholesterol, protein and sucrose. Notable among foodstuffs which failed to show a positive correlation were polyunsaturated fat and the total carbohydrate content of the diet.

The association between dietary fat intake and serum cholesterol concentration, and in turn, that between serum cholesterol and IHD incidence and mortality, has resulted in the postulation of a hypothesis linking the intake of dietary fat with IHD. This hypothesis, known as the 'dietary fat hypothesis', attempts to explain the differences in IHD incidence observed in epidemiological and experimental dietary studies, on the basis of the quality and quantity of dietary fat ingested. It is based on the assumption that dietary fat influences plasma lipid concentrations, which in themselves influence the development of atherosclerosis. The strongest points favouring this hypothesis are the epidemiological ones already mentioned. Although many of these studies clearly demonstrated an association between serum cholesterol, dietary fat intake and IHD, little can be said regarding cause and effect. Proof of a direct causal relationship between dietary fat and serum cholesterol in the pathogenesis of IHD is lacking, although studies showing a predictive value of a serum cholesterol, e.g. the Framingham Study (Kannel and Gordon, 1969) tend to reinforce this possibility.

Experimental evidence also tends to support a causal relationship. Diets which are capable of inducing atheroma in experimental animal models tend to result in a simultaneous elevation of serum cholesterol (Blaton et al, 1970; Howard et al, 1972). Such atherogenic diets frequently resemble diets ingested by coronary prone populations in terms of content of saturated fat and cholesterol. It must, however, be remembered that lesions produced in these animals are not indistinguisable from those of human atherosclerosis; certain caution should thus be exerted in interpretation of such data.

Studies on the pathogenesis of atherosclerosis also tend to reinforce a direct causal relationship between serum cholesterol and atheromatous plaques. Cholesterol has been shown to enter the aorta from both the outer (Adams, 1971) and inner surfaces (Adams and Bayliss, 1973). The theory that cholesterol enters the arterial wall directly from plasma in the form of beta lipoprotein has been questioned on two counts. Firstly, whereas the majority of cholesterol in plasma is esterified, the free form predominates in atheromatous plaques (Newman and Zilversmit, 1966; Hashimoto and Dayton, 1966). Secondly, although beta lipoprotein has been identified in the arterial intima (Woolf and Pilkington, 1965; Walton and Williamson, 1968; Smith and Slater, 1972), most proteins, probably lipoproteins included, enter the aorta from the outer surface (Adams et al, 1969). When the intimal surface is damaged, the direction of entry is, however, reversed (Adams et al, 1970).

A further implication of the hypothesis linking dietary fats to IHD, is that modification of the fat intake should result in a reduction of serum cholesterol concentration, and be of positive value in the prevention of atherosclerosis and IHD. Dietary measures which have been shown to result in a lowering of serum cholesterol concentrations are:

(1) a reduction in the intake of saturated fatty acid (Keys et al, 1965a);

(2) and increased intake of polyunsaturated fatty acids (Keys et al, 1965b);

(3) a reduction in cholesterol intake (Keys et al, 1965c).

The evidence for a cholesterol-lowering effect and the relative importance of these three factors will be discussed later in this Chapter (see page 249).

Modified fat diets, whether used experimentally or therapeutically, consist of replacement, or partial replacement of saturated by polyunsaturated fatty acids. The effect of reducing saturated, and of increasing unsaturated fat intake, will then be cumulative. Furthermore, as cholesterol and saturated fats tend to be present in similar foodstuffs, such as dairy products and meat, a reduction in the saturated fat content of the diet is usually accompanied by a reduction in the cholesterol content as well. Interpretation of many studies thus becomes difficult. Further problems of interpretation arise from changes in carbohydrate and caloric content, both of which factors may independently affect serum lipid concentrations.

Despite these shortcomings, accumulating evidence of cholesterol-lowering diets being of positive benefit in the prevention of IHD, further strengthens the argument that diets rich saturated fat and cholesterol are causally associated with IHD. Two main groups of individuals have been studied; men with no prior history of heart disease, and men who have previously suffered myocardial infarction or who have angina pectoris. Studies involving these two groups have been designated primary and secondary prevention trials respectively.

In the three main primary prevention studies reported to-date, a beneficial effect of a modified diet has been demonstrated in all (Christakis et al, 1966; Dayton et al, 1969; Miettinen et al, 1972) in terms of both reduction of serum cholesterol concentration and IHD incidence. The dietary manipulations were similar in all three; the main changes were a reduction in cholesterol intake, and substitution of polyunsaturated vegetable oils for saturated fat.

Most secondary prevention trials also suggest a beneficial effect of modified fat diets (Morrison, 1964; Leren, 1970; Bierenbaum et al, 1973), although some have failed to show a significant lowering of mortality due to IHD (Medical Research Council's Research Committee, 1968). In all these studies, serum cholesterol was reduced by dietary means, sometimes by as much as 50 mg./dl.; it is, therefore, tempting to attribute the beneficial effects of such diets to the lower serum cholesterol concentrations produced.

One of the major criticisms of the hypothesis that dietary fat influences the incidence of IHD by raising the serum cholesterol concentration, is that it fails to take into account the many other factors known to participate in what clearly is a disease of multifactorial aetiology. Furthermore, although the data comparing populations in terms of saturated fat intake, serum cholesterol and IHD is impressive, differences in intake of saturated fat within a population do not show nearly the same correlation with serum cholesterol values (Kannell and Gordon, 1969). Serum cholesterol still remains a useful factor in predicting the susceptibility of an individual to the development of IHD within a population (Kannel and Gordon, 1969; Carlson and Böttiger, 1972). This may be due to the interaction with constitutional factors, dietary factors other than saturated fats, or dietary effects mediated other than via serum cholesterol.

With the ever-increasing evidence of the association between IHD and serum triglyceride (see page 10), dietary induced elevations of serum triglyceride should no doubt be considered as a means whereby diet may have its effect on the development of atherosclerosis. Little epidemiological evidence is as yet available as to the association between dietary fat intake, serum triglyceride levels and IHD incidence. Most incidence linking serum triglycerides and dietary fat comes from dietary experiments in man. Many studies point towards serum triglyceride levels being higher during consumption of saturated fat than when substituted by polyunsaturated fat (Ahrens et al, 1957; Kinsell et al, 1959; Beveridge et al, 1964; Spritz and Mishkel, 1969). This finding is, however, not universal (Arvidson and Malmros, 1972; Anderson, Grande and Keys, 1973). Furthermore, the pathogenetic mechanisms linking serum triglycerides and atherosclerosis are somewhat more difficult to envisage, triglyceride being only a minor component of atherosclerotic plaques. Zilversmit (1973) has, however, presented evidence to support the hypothesis that atheroma may result from liberation of cholesterol rich fragments in close proximity to arterial endothelium following degradation of VLDL and chylomicrons by arterial lipoprotein lipase. High local concentrations of cholesterol, could then conceivably result in enhanced cholesterol uptake by the artery. With increasing knowledge of the relationship of dietary fat to serum triglycerides, and of the latter to atherosclerosis, the dietary fat hypothesis may well require extension to include consideration of serum triglyceride as well as cholesterol. Another criticism of the hypothesis relating the intake of dietary fat to IHD is that populations exist in which the intake of saturated fat is such that high cholesterol levels and a high IHD incidence might be expected, yet the reverse holds. The most studied group are the Masai of East Africa. This tribe eats a diet with a cholesterol and animal fat content similar to that of western societies. The prevalence of IHD is nevertheless virtually nil, and serum cholesterol levels are far below what one would expect for the amount of saturated fat and cholesterol ingested, (Shaper, 1972). However, recently, data has been presented (Eiss et al, 1971) suggesting that the Masai are able to suppress endogenous cholesterol synthesis to a greater extent than most humans. This may be an inherited characteristic, unique to this group of people, which affords them protection against the development of atherosclerosis.

A further criticism is that conclusive evidence is lacking on reduction of IHD mortality in response to modification of the diet. None the less, the primary and secondary prevention studies currently available are certainly encouraging if not conclusive, and a definite answer will depend on further and perhaps better designed long-term studies. It is also important to realize that all these studies have been performed on middle aged or elderly subjects; atherosclerosis will no doubt have been present, if not well advanced in many at the onset of the study. More conclusive answers are likely to be obtained from primary prevention studies in much younger groups of individuals, but results will of necessity take many years to become available.

Despite shortcomings in this hypothesis, much evidence supports it, and dietary fat seems to be at least an important factor in a multifactorial disease process.

POLYUNSATURATED FAT AND SERUM CHOLESTEROL

Cholesterol Metabolism

Due to the association between plasma cholesterol and IHD, cholesterol is frequently regarded as a harmful substance; its essential role for the normal functioning of the body must, however, not be forgotten. Perhaps its most important function is a structural one, where it is an essential element of all animal cells and subcellular particles. It is also an obligatory precursor of the steroid hormones and of the bile acids. As a constituent of plasma lipoproteins, its role is perhaps not clearly defined; it does, however, appear to play a part in the transport of cholesterol from tissues to the liver (Glomset, 1968), from which site it may be removed by catabolism.

Much of the total cholesterol in the body is exchangeable with the plasma pool. A complex interchangeable system exists largely because equilibration between various pools occurs at different rates (Myant, 1971). The exchangeable mass undergoes turnover owing to its participation in an incomplete enterohepatic circulation with net loss via the faeces; replacement of losses is by endogenous synthesis and from cholesterol in the diet. In man, about 40 - 60% of dietary cholesterol is absorbed from the intestine after having mixed with endogenous cholesterol derived partly from bile and partly from de novo intestinal synthesis (Myant, 1973). The bulk of cholesterol is not dietary, but is synthesized endogenously by the liver and intestine. A complex synthetic pathway exists, the enzyme hydroxymethyl glutaryl CoA reductase appears to be the rate limiting step, and is the probably site at which control over synthesis is affected (Siperstein and Guest, 1960).

In the steady state, cholesterol input is balanced by output. Because of its chemical stability, the steroid nucleus is eliminated mainly in the faeces, either unchanged or as bile salts, the main metabolite of cholesterol. Approximately 20 - 30 grammes of bile acids are produced from cholesterol per day; because of an enterohepatic circulation, most of this is reabsorbed by the ilium (Borgström et al, 1963; Lack and Weiner, 1967), although about one gramme is excreted with the neutral steroid in the faeces.

Effect of polyunsaturated fat on serum cholesterol levels

In 1952, a cholesterol lowering effect of a diet in which polyunsaturated fat was substituted for saturated fat, was described (Groen et al, 1952; Kinsell et al, 1952). This has been confirmed in numerous studies in both man and experimental animals (Ahrens et al, 1954; Ahrens et al, 1955; Bronte-Stewart et al, 1956; Beveridge et al, 1956; Keys et al, 1957a; Malmros and Wigard, 1957; Ahrens et al, 1957; Wilkens, 1958; Avigan and Steinberg, 1958; Hegsted et al, 1965; Keys et al, 1965b; Spritz and Mishkel, 1969). Despite substantial differences in experimental design, there was general agreement that diets in which saturated fat is replaced in part or in whole by polyunsaturated fat, leads to a reduction in serum cholesterol levels. This response was noted in young normocholesterolaemic medical students, vitamin and protein deficient South African Bantus, and Americans with and without hypercholesterolaemia and IHD.

In most of these studies, the reduction in serum cholesterol concentration was less than 25%; the maximum response seems to have been achieved within 2 - 3 weeks in most cases (Ahrens et al, 1957).

The lower cholesterol level after unsaturated fat feeding is due to a reduction in low density lipoprotein cholesterol (Nickaman et al, 1967; Spritz and Mishkel, 1969), although Farquhar and Sokolow (1958) found a similar effect on high density lipoprotein cholesterol.

It is still not clear whether this effect is chiefly due to a reduction in the saturated fatty acid content of the diet or to an increase in the polyunsaturated fatty acid content. This difficulty arises because most studies have relied on isocaloric substitution of one type of fat by the other. Therefore, when the polyunsaturated fat content of the diet increases, it is inevitably accompanied by a decrease in saturated fat content. The effect of other dietary changes has also to be considered; cholesterol is frequently found in foodstuffs rich in saturated fats; the effect on serum lipids of reducing saturated fat intake should thus not be attributed to the modification of the glyceride content of the diet alone, but should take cholesterol content into consideration as well. These shortcomings may largely be overcome by the use of formula diets in which the exact contents can be carefully controlled (Ahrens et al, 1954). Such an approach, by eliminating cholesterol from the diet, has shown that the glyceride per se has the desired effect on serum cholesterol levels (Spritz et al, 1965; Connor et al, 1969). Using diets in which the total calorie content was kept constant, by testing as far as possible the effect of only one foodstuff at a time, Keys and his colleagues derived formulae for the relative effect of various fats on serum cholesterol concentrations (Keys et al, 1965a and b). They demonstrated the influence of a change in the saturated fat content of the diet on serum cholesterol to be about twice that of a quantitatively equal change in the opposite direction of unsaturated fatty acids. The unsaturated fatty acid used for the derivation of this formula was linoleic acid (18:2). They showed that isocaloric dietary substitution of C12 - 16 saturated fatty acids for carbohydrate, led to an increase of serum cholesterol of 2.4 mg./dl. for each 1% of total calorie intake. Conversely, isocaloric substitution of carbohydrate by polyunsaturated fat led to a 1.2 mg./dl. reduction in serum cholesterol. The effect of changing from one diet to another of different fatty acid composition is thus given by the difference between the expression 1.2 (2S' - P) for the two diets. (S = % of total calories as glycerides of saturated fatty acid of C12 - 16 length; P = % of total calories as glycerides of polyunsaturated fatty acids.) A diet in which the 2S - P is equal to zero, i.e. which contains twice as much unsaturated as saturated fat, would thus have no effect on serum cholesterol level when isocalorically substituted with carbohydrate. This has been confirmed experimentally

(Grande et al, 1972). This is not to say, however, that diets with a 2S' - P of 0 have no effect on serum cholesterol levels in an individual patient; if, for example, such a diet replaces one with a much higher saturated fat and a much lower unsaturated fat content, serum cholesterol will be expected to fall. Maximum cholesterol lowering is thus achieved by replacing saturated by polyunsaturated fat.

The saturated fatty acids chiefly responsible for a lipid raising effect are palmitic (16:0), myristic (14:0), and lauric (12:0) (Keys et al, 1965a and b; Hegsted et al, 1965). Stearic acid (18:0) and saturated fatty acids of shorter chain length than lauric little affect serum cholesterol levels in either direction; the monounsaturated oleic acid (18:1) also neither leads to an increase or a reduction in serum cholesterol.

Long chain fatty acids with a degree of unsaturation greater than that of linoleic acid, appear to have an even greater hypocholesterolaemic effect. Fish oils, for example, which contain low concentrations of linoleic, and relatively high concentrations of 20:5ω3 and 22:6ω3, lead to lower cholesterol levels than maize or safflower oil, both of which are rich in linoleic acid (18:2) in man, rat, chicken and mouse (Pfeiffer, 1967). Ahrens et al (1959) has claimed that the hypocholesterolaemic action of polyunsaturated fat is a reflection of their total degree of unsaturation. Hydrogenation of double bonds result in a loss of cholesterol lowering effect (Ahrens et al, 1957).

Not only the degree of unsaturation, but also the configuration about the double bond, is of importance. When compared with the oleic acid (18:1 Δ 9 cis), its trans isomer elaidic acid (18:1 Δ 9 trans), has a definite cholesterol raising effect (Vergroesen, 1972). This effect appears to depend on the presence of cholesterol in the diet. As the polyunsaturated fat used in the calculation of the 2S' - P formula of Keys et al (1965a and b) consisted largely of linoleic acid, it will be appreciated that their formula perhaps does not apply when the vegetable oil substituted is rich in fatty acids other than linoleic acid. The situation is further complicated by the finding that an extremely high intake of linoleic acid will not improve on the cholesterol lowering effect of a diet in which 35% of total calories are derived from safflower seed oil (Vergroesen, 1972). The same author has also suggested that saturated fats have little effect on serum cholesterol when present in the diet with much lower amounts of linoleic acids (Vergroesen, 1972).

Some studies have failed to show a lipid lowering effect of polyunsaturated fats (Connor et al, 1961; McOsker et al, 1962; Erickson et al, 1964). In all these, the quantity of polyunsaturated fat was low; it may be that a threshold exists, below which polyunsaturated fats fail to exert their cholesterol lowering effect.

Maximal cholesterol lowering thus appears to be achieved by replacing saturated fatty acids by polyunsaturated ones. Reduction of saturated fat intake has the further advantage that cholesterol intake is simultaneously reduced as most sources of saturated fat are also rich in cholesterol. Although most authorities agree that dietary cholesterol is an important determinant of serum cholesterol concentration, the degree of importance is still a matter of some dispute. Whereas Keys et al (1965c) believe it to play a relatively minor role, Connor et al (1968) assign to it far greater importance. According to Keys et al (1965c), the change in serum cholesterol equals 1.5 times the square root of the change in intake expressed as mg./1,000 K cals. More recently, Mattson et al (1972) have again attributed a greater importance to the contribution of dietary cholesterol, by showing a linear increase in serum cholesterol concentrations with increasing amounts of cholesterol in the diet. They fed formula diets of composition similar to that of the standard American diet (i.e. 40% of calories from saturated fat and 12% from unsaturated fat); the only variable was the cholesterol content. Each 100 mg. cholesterol/1,000 K cals of diet resulted in an increase in serum cholesterol of 12 mg./dl.; the highest level of cholesterol fed approximated that of the average American diet. A linear response was also found by Hegsted et al (1965). A further increase in the intake of cholesterol would probably have had little additional effect, as both Beveridge et al (1960) and Connor et al (1961) showed the effect to plateaux at levels of cholesterol intake just above the upper limit used in the study of Mattson et al (1972). This is probably due to the limited capacity for cholesterol absorption. As diets low in saturated fat also tend to be low in cholesterol, the relative contribution of these two factors is difficult to evaluate. Hegsted et al (1965) has suggested that reducing dietary saturated fat, increasing dietary polyunsaturated fat, and increasing cholesterol intake, each exerts an independent effect. Against this view is the data of Connor et al (1964) and Erickson et al (1964), which suggest the cholesterol to be the overriding factor. An interplay probably exists between dietary cholesterol and the composition of dietary fat; conflicting data, however, confuse the issue. Brown (1970) has pointed out that dietary fat must have a P/S ratio of 7 to significantly lower cholesterol levels when dietary cholesterol intake is that of the typical American diet (about 700 mg./day). On the other hand, when cholesterol free diets were consumed, changing the P/S ratio from 0.2 to 2.6 was shown to have no effect on serum cholesterol (Connor et al, 1964). If the exogenous cholesterol is below 400 mg. per day and the amount of saturated fat in the diet is controlled, the effect of the P/S ratio on cholesterol levels is lessened (Keys et al, 1957b; Stamler, 1960; Erickson et al, 1964; Reiser, 1966).

The exact interrelationship between dietary cholesterol and fat of different composition is thus not clearly understood. None the less, in long-term clinical trials, a significant cholesterol lowering effect has resulted from the feeding of diets low in cholesterol, with partial replacement of saturated by polyunsaturated fat. The United States National Diet Heart Study (National Diet Heart Study Research Group, 1968) attained a mean reduction in serum cholesterol of 26 mg./dl. from baseline values in men living at home, and of 36 mg./dl. in those living in institutions where dietary adherence was probably better. In the New York Anticoronary Club Trial (Christakis et al, 1966), serum cholesterol values fell by 11.6% in one year from a mean starting value of 260 mg./dl. The reduction was sustained, and in fact serum cholesterol fell by a further 5 mg./dl. over the ensuing four years. The Los Angeles Veterans Trial of Dayton et al (1969) showed that mean cholesterol values were 29.5 mg./dl. lower in the experimental group than in controls, and in the Helsinki Mental Hospital Trial (Miettinen et al, 1972), cholesterol concentrations changed significantly in the expected direction.

Mechanism of the reduction in serum cholesterol concentration

There are several ways in which polyunsaturated fat could lower serum cholesterol concentration. One possibility is that endogenous synthesis of cholesterol might be reduced; another is that absorption of dietary cholesterol might be diminished. Removal of cholesterol from the body could be enhanced either in the form of neutral steroids, bile acids or both. A further possibility is that the cholesterol lowering effect could be due to a redistribution of cholesterol from plasma to extravascular sites. A combination of any or all these factors could also be operative. Despite many years of careful research in this field, the exact mechanism is not known with certainty.

Little evidence exists to support the hypothesis of reduced endogenous cholesterol synthesis being the important mechanism. Avigan and Steinberg (1958), however, showed polyunsaturated fat to increase cholesterol synthesis in rat livers. This may not, however, relate to total cholesterol biosynthesis in the intact animal. The hypothesis of reduced cholesterol absorption has also received little support, although two studies have reported cholesterol absorption from the gut to be inhibited by polyunsaturated fat (Wood et al, 1966; Wood and Kinsell, 1967). Others have reported the opposite, i.e. enhanced cholesterol absorption during unsaturated fat feeding (Byers and Friedman, 1958; Bloomfield, 1964). More recently, McGovern and Quakenbush (1973) failed to explain the cholesterol lowering effect of polyunsaturated fats by reduced cholesterol absorption in rats fed labelled cholesterol.

The conflict with regard to the effect of polyunsaturated fatty acids on cholesterol excretion is somewhat more interesting. Gordon et al (1957) and Lewis (1958a) were among the first to demonstrate an increased excretion of bile acids on replacing saturated with unsaturated fat in the diet; they suggested that an enhanced conversion of cholesterol to bile acids might account for the lower cholesterol levels observed after unsaturated fat feeding. Since that time, steroid balance studies have confirmed that polyunsaturated induced reduction in serum cholesterol could be accounted for by an increased faecal steroid excretion. However, doubt has recently been cast on this mechanism and a shift of cholesterol from vascular to extravascular compartments has been claimed to be of major importance (Grundy and Ahrens, 1970).

After the initial studies of Gordon et al (1957) and Lewis (1958a), two groups of workers failed to find consistent changes in steroid excretion rates (Avigan and Steinberg, 1965; Spritz et al, 1965). Kinsell's group have, however, consistently found an increase in faecal steroid excretion on switching the dietary fat source from saturated to polyunsaturated (Sodhi et al, 1964; Wood and Kinsell, 1966; Wood et al, 1967; Sodhi et al, 1967; Kinsell et al, 1968). Using a chemical balance method, they observed greater steroid excretion, both neutral and acidic after polyunsaturated compared with saturated fat feeding, in every subject studied, regardless of the order in which the fats were fed. The fall in serum cholesterol on switching from saturated to polyunsaturated feeding was abrupt and maintained, and was associated with an immediate and maintained elevation of total faecal steroids which were considered to be due to increased excretion of bile acids, and neutral steroids, both of endogenous and exogenous origin (Kinsell et al, 1968). They suggest that the hypocholesterolaemic effect of dietary unsaturated fat is attributable, at least in part, to an increased excretion of cholesterol and its metabolites in the faeces; and remark on the probability that at least some of the increased excretion may be due to reduced absorption and re-absorption. They also showed the increased excretion rate to be prolonged (at least up to 80 days) and suggested that, as a new plateau of serum cholesterol is reached long before this time, an increased rate of addition of cholesterol to the plasma pool must occur. Although this could be due to an increased synthesis rate, little data is available in support of the hypothesis; increased mobilization of cholesterol from extravascular sites thus seems likely.

The studies of Moore et al (1962) and Moore et al (1968) are in agreement with those of the Kinsell group despite the use of different methods. Using an isotopic balance method following the intravenous injection of ^{14}C cholesterol, they showed a significant increase in total faecal steroids after isocaloric substitution of polyunsaturated for saturated fat. The increase was equally distributed between neutral steroid and bile acid fractions. The change in serum cholesterol concentration was found to be more than adequately balanced by a reciprocal change in faecal steroid excretion, sufficient to account for all the cholesterol lowering effects.

These findings are, however, not universally accepted. The main opposition comes from Ahrens and his co-workers (Spritz et al, 1963; Spritz et al, 1965; Grundy and Ahrens, 1970) who claim that the majority of patients they studied, failed to show an increase in faecal neutral steroid excretion, a reduced absorption of exogenous cholesterol or an increase in bile acid excretion. In view of this they thought it unlikely that the hypercholesterolaemic response to dietary polyunsaturated fat could be explained in terms of increased steroid excretion; they suggest the alternative possibility of redistribution of cholesterol between plasma and tissue pools to be the likely mechanism. It is, of course, tremendously important to exclude the possibility of cholesterol being redistributed to extravascular sites, which could include the arterial wall, in view of the widespread recommendation of such diets for the prevention and treatment of hyperlipidaemia and IHD. It is, therefore, especially important to know whether cholesterol is leaving the body or moving into the tissue.

Kinsell et al (1968) believe the differences between the two groups to be methodological. Samples have actually been exchanged; this, however, has failed to resolve the differences.

Further evidence against the proposals of Ahrens' group comes from Connor and his co-workers (Connor et al, 1969). Using identical methods to Ahrens, they showed enhanced neutral steroids and bile acid secretion sufficient to account for changes in serum cholesterol, to be present in all cases studied. Faecal bile acids accounted for the greater part of the change. In view of the similar methodology, these different results are of interest. As Grundy and Ahrens (1970) studied mainly subjects with hyperlipidaemia whereas Connor et al (1969) studied normal men, it is possible that subjects with metabolic abnormalities show a somewhat different response to unsaturated dietary fats. More likely, however, is that the long period known to exist before any change occurs in the plasma-liver cholesterol pool, is greater in subjects with expanded pool sizes (Connor et al, 1969).

Another possible mode of action of polyunsaturated fatty acids in reducing serum cholesterol concentrations, concerns structural changes in plasma lipoproteins. Qualitative changes could facilitate the catabolism and elimination of the contained cholesterol, or influence the handling of cholesterol at a cellular level.

Cholesterol esters were noted to become more unsaturated before serum cholesterol levels fell in human subjects fed sunflower seed oil (Lewis, 1958b). Arvidson and Malmros (1972) showed similar changes to also occur in triglycerides, free fatty acids and phospholipids. Further evidence that the composition of the cholesterol-bearing lipoproteins of plasma are important determinants of their own metabolism, is the observation that ^{14}C cholesterol-labelled lipoproteins containing unsaturated fatty acids derived from donor rats, induced an increased bile acid and steroid excretion when fed to recipients (Lewis et al, 1961). It is, therefore, of interest that Schrade et al (1961) reported the serum lipids of patients with hyperlipidaemia to contain relatively more saturated and monosaturated fatty acids and less polyunsaturated fatty acids than the serum lipids of healthy male controls.

More recently, Spritz and Mishkel (1969) showed the reduced cholesterol levels following polyunsaturated fat feeding to be due to a reduction in low density lipoprotein cholesterol without a significant change in the protein content of this lipoprotein. Compositional changes in the lipoproteins were also observed; linoleate increased at the β position of phospholipids during unsaturated feeding. It was postulated that in view of the non-linear configuration of unsaturated fatty acids, they occupy a greater space on the lipoprotein molecule than do saturated fatty acids. By altering the spatial configuration of the lipids into which they are incorporated, fewer lipid molecules could be accommodated by the apoprotein of low density lipoprotein, resulting in the lipid lowering effects of polyunsaturated fat. Although not proving this hypothesis, their data is consistent with it; a similar effect of polyunsaturated fat on VLDL might, therefore, account for a reduction in the lipid content of this lipoprotein.

POLYUNSATURATED FAT AND SERUM TRIGLYCERIDES

By contrast with the vast literature on the effect of polyunsaturated dietary fats on serum cholesterol, relatively little is known of their effects on serum triglycerides. The latter effect has received renewed attention in view of the now well established association between serum triglycerides and IHD. As with serum cholesterol, measures which lower serum triglycerides may prove of value in the prevention and treatment of atherosclerosis.

Effect on serum triglyceride concentration

In many of the earlier studies on the effect of polyunsaturated fats on serum cholesterol, triglyceride measurements were also performed. Little significance, however, was attributed to changes observed. By contrast, with the general agreement that polyunsaturated fat feeding reduces cholesterol, the effect on serum triglycerides is more variable. Some studies showed a triglyceride reduction in response to unsaturated fat feeding; others failed to show this effect. In many of these early studies, the dietary changes involved more than one dietary constituent. Not only was the fat content changed, but frequently carbohydrate content and composition were simultaneously altered. Not surprisingly, interpretation of much of this data is somewhat difficult.

A lowering of serum triglycerides in response to polyunsaturated fat feeding is well documented (Ahrens et al, 1957;

Kinsell et al, 1959; Beveridge et al, 1964; Anderson, 1967; Connor et al, 1969; Spritz and Mishkel, 1969; Bagdade et al, 1970; Grande et al, 1972; Macdonald, 1972; Whyte et al, 1973). This effect is rapid, Macdonald (1972) having found a 20% reduction in five days. That the effect is due to a change in the quality of the dietary fat is strongly suggested by the use of formula diets, in which the only switch was in the type of fat. For example, when formulas providing 40% of calories as fat were fed as either saturated or unsaturated fats, with other constituents being identical during both dietary periods, plasma triglycerides fell significantly in response to the unsaturated diet, (Spritz and Mishkel, 1969). The effect of certain saturated fatty acids on triglyceride levels differs from their effect on serum cholesterol. Whereas stearic acid has little effect on cholesterol levels (Keys et al, 1965a), it has a hypertriglyceridaemic effect relative to palmitate (Grande et al, 1970). Also, saturated fatty acids with less than 12 carbon atoms have little or no effect on serum cholesterol (Hashim et al, 1960; Grande et al, 1972); they do, however, cause an elevation of serum triglycerides when substituted for longer chain fatty acids such as palmitic and oleic (Uzawa et al, 1964). Saturated fats which contain large proportions of stearate, or fatty acids with less than 12 carbon atoms such as beef fat and butterfat respectively, have frequently been used in feeding experiments during saturated fat feeding periods. The triglyceride lowering effect on switching to polyunsaturated fat could thus result largely from a reduction in stearic and short chain fatty acids, rather than from some effect of polyunsaturated fat per se. Some feeding experiments, however, do suggest that polyunsaturated fats reduce triglycerides in their own right. When formula diets containing fats with only one long chain fatty acid were substituted, the triglyceride lowering effect of polyunsaturated fat feeding was still observed (Connor et al, 1969). The serum triglyceride concentration appears to be least affected in subjects with the lowest initial values (Spritz and Mishkel, 1969). Whereas in the same study serum cholesterol reduction was shown to be due to a change in the LDL cholesterol, it is not known which lipoprotein changed to account for the triglyceride effect, as measurements of VLDL were not made. Little information is available as to lipoprotein changes which account for this change.

A triglyceride lowering effect of polyunsaturated fat diets has not been universally found. Anderson et al (1973) using a diet specially designed to lower serum cholesterol, showed the desired effect to be accomplished without any significant change in serum triglyceride. Their cholesterol lowering diets, however, had much lower fat calories than the standard diet it replaced; an increased carbohydrate intake was used in order to keep the diets isocaloric. As isocaloric high carbohydrate diets are well known to be capable of inducing an elevation of serum triglycerides in normal subjects (Antonis and Bersohn, 1961; Glueck et al, 1969; Mancini et al, 1973) and in patients with endogenous hypertriglyceridaemia (Ahrens et al, 1961), this may explain the findings of Anderson et al (1973). What is perhaps more significant, is that when in the same study a cholesterol-raising diet was fed, triglyceride levels rose significantly (Anderson et al, 1973). The latter diet contained more egg and butterfat and less vegetable oil and miscellaneous vegetables, than did the standard diet with which it was compared. The fat and carbohydrate content of each were similar.

Arvidson and Malmros (1972) also failed to show any clear cut effect of polyunsaturated fat feeding on serum triglyceride. During one of two feeding periods, mean triglyceride concentrations were lower in the group of subjects ingesting unsaturated fat compared with those receiving saturated fat; the levels, however, failed to reverse when the diets were exchanged between the two groups. The reason for this observation is not clear.

Long-term trials have also shown a variable effect. The efficacy of dietary adherence is, however, frequently difficult to judge and changes in weight frequently confuse the issue. Using the Coronary Prevention Evaluation Programme Diet, Hall et al (1972) found the reduction in serum triglyceride to exceed the fall in serum cholesterol by 50% after one year's treatment in hypertriglyceridaemic men. In normolipaemic men, the reduced triglyceride level after one year was, however, not statistically significant. This diet is low in saturated fat and cholesterol, moderate in polyunsaturated fat, total fat and carbohydrate, and calorie controlled to lower weight where indicated. Of the hyperlipidaemic subjects, those who lost weight showed the most marked change in serum triglyceride; the group whose weight remained unchanged showed a smaller, but nonetheless significant reduction, suggesting that the quality of the diet as well as the calorie deficit, played a part in the fall in serum triglycerides. Further support for an independent effect of diet composition comes from Wilson et al (1971), who used the American Heart Association high polyunsaturated/saturated (P/S) diet. In this diet, total fat provided 40% of calories, saturated fat 10%, polyunsaturated fat 15% and carbohydrate 43%. They showed that after six months' dieting, serum cholesterol was reduced by 9.6% and triglycerides by 11.7% in a group of hyperlipidaemic subjects and normolipaemic non-obese men. Triglycerides fell rapidly during the first two weeks of the diet, then rose, but by the end of the six month period, were significantly lower than initially. The most marked reduction in triglyceride levels were seen in those subjects who had decreased their carbohydrate intake, and also in those who had increased their intake of polyunsaturated fat above the minimum recommended level.

In these long-term studies, not only was the type of dietary fat changed, but the intake of the fat was usually reduced as well. Either calorie intake is reduced, which may contribute to the fall in serum lipids, or a proportion of the saturated fat intake is isocalorically replaced by carbohydrate; the higher carbohydrate content might then cause an upward trend in triglyceride levels (Ahrens et al, 1961; Glueck et al, 1969; Mancini et al, 1973). For these reasons, it is somewhat difficult to compare these studies with carefully controlled dietary experiments involving an isocaloric substitution of one fat type for another while keeping the other constituent of the diet unchanged, and at a level comparable to that ingested by western societies.

Another way of examining possible long-term effects of high polyunsaturated fat diets is by studying lipid levels and coronary heart disease incidence in communities which habitually consume such diets. Not many such communities exist; despite incomplete evidence about their food consumption, the Eskimos appear to be the only people who fall into this category (Krogh and Krogh, 1914; Bang et al, 1971). It is, therefore, of interest that the Eskimos have significantly lower levels of cholesterol and especially triglycerides than many developed countries. Bang et al (1971) reported a mean triglyceride concentration of less than 60 mg./dl. and Ho et al (1972) a mean concentration of 85 mg./dl. Semi-quantitative lipoprotein electrophoresis has revealed that pre-beta lipoprotein levels are strikingly low (Bang et al, 1971; Ho et al, 1972); in fact, most electrophoretic strips showed a near-absence of pre-beta lipoprotein. Contrary to most western civilizations, triglyceride levels failed to increase with increasing age. That these effects are environmental rather than hereditary is strongly suggested by the fact that Eskimos living in Denmark, had serum lipids similar to the Danes. It is tempting to attribute the observations on the lipids and lipoproteins of the Eskimos to the high content of polyunsaturated fat in their diet and their low intake of saturated fat. Much fatty fish, whale and meat is eaten, but dairy products are seldom consumed. They do, however, also have an extremely low intake of carbohydrate (15 - 20% of total calories) which may partly account for the low triglyceride and pre-beta lipoprotein concentrations, and which makes conclusion regarding the effect of dietary polyunsaturated fat somewhat tenuous.

A further point of interest relating to the Eskimos, is their extremely low incidence of coronary heart disease (Bang et al, 1971).

Interrelationship between dietary fats and carbohydrates on serum triglyceride concentration

From the aforegoing, it is clear that the serum triglyceride concentration is to some extent determined by the intake of dietary saturated and polyunsaturated fat. It is well known that dietary carbohydrates can also influence serum triglyceride concentration. As mentioned earlier, high carbohydrate diets can result in an elevation of serum triglycerides in both normolipaemic (Antonis and Bersohn, 1961; Glueck et al, 1969; Mancini et al, 1973) and hypertriglyceridaemic individuals (Ahrens et al, 1961). In view of the possibility that the response of serum triglyceride to dietary fat may be modified by the type and amount of carbohydrate in the diet, the interrelationship between these two dietary components on plasma lipid metabolism is clearly of interest.

An acute increase in serum triglyceride levels in response to dietary sucrose is well described. This effect has, however, only been demonstrated when the level of carbohydrate intake has been very high, e.g. 7 grammes/Kg./day (Macdonald and Braithwaite, 1964; Macdonald, 1965; Antar and Ohlson, 1965; Szanto and Yudkin, 1969; Nestel et al, 1970). At lower, more usual levels of sucrose intake (e.g. 20% of total calorie intake), this hyperlipidaemic effect is not found (Dunnigan et al, 1970; Mann and Truswell, 1972). A further factor which influences the increase in serum triglyceride in response to dietary sucrose, is the fat content of the diet (Macdonald, 1967). More recently, Macdonald(1972) has shown that sunflower seed oil in the diet of normal men, reduces the serum triglyceride concentration regardless of the nature of the accompanying carbohydrate. This suggests that carbohydrate, especially sucrose, only influences serum triglycerides in situations where the polyunsaturated fat content of the diet is low. This has been confirmed in hypertriglyceridaemic subjects; sucrose exerted a hyperlipidaemic effect in the presence of dietary saturated fat and cholesterol (Antar et al, 1970), but not when the accompanying dietary fat was high in polyunsaturated fatty acids (Birchwood et al, 1970). Mann et al (1973) have recently shown the hyperlipidaemia induced by feeding high sucrose diets to be reversed when the accompanying fat was changed from saturated to polyunsaturated. These findings may have therapeutic implications; diets designed to lower serum triglyceride may not need to be low in carbohydrate content if sufficient polyunsaturated fat is ingested.

Mechanism of the reduction in serum triglyceride in response to feeding polyunsaturated fat

By contrast with the large number of studies on the mechanism of the cholesterol response to polyunsaturated fat feeding, little is known of the mode of action of unsaturated fats in reducing serum triglyceride. In order to exert its triglyceride lowering effect, dietary polyunsaturated fat must presumably either accelerate the removal of endogenous triglyceride from plasma, or decrease its rate of input into plasma.

As discussed in Chapter I, removal of triglyceride in plasma is largely controlled by lipoprotein lipase. Rats fed a polyunsaturated fat diet providing 20% of calories as corn oil, had significantly lower serum triglyceride concentrations than rats fed an isocaloric amount of saturated fat (Pawar and Tidwell, 1968). Adipose tissue lipoprotein lipase activity was significantly higher in the rats fed the polyunsaturated fat diet, suggesting enhanced removal of triglyceride to be responsible for the lower triglyceride levels seen. A similar effect has been reported in man; Bagdade et al (1970) showed PHLA to be greater after a 40% fat diet rich in polyunsaturated fat when compared with an isocaloric saturated fat diet. The increased PHLA was associated with a reduction in serum triglyceride suggesting that unsaturated fat might facilitate the removal of triglyceride from plasma. It is also conceivable that lipoproteins containing different fatty acids in their triglyceride components may be hydrolyzed at different rates by lipoprotein lipase, although Korn (1961) has shown lipoprotein lipase activity in vitro to be independent of the fatty acid nature of the substrate.

The alternative possibility is that polyunsaturated fat feeding lowers serum triglyceride concentration by reducing the secretion of triglyceride in its main transport form, VLDL. It is well established that during unsaturated fat feeding, the proportion of polyunsaturated free fatty acids in plasma increases in relation to saturated free fatty acid (Hallgren et al, 1960). Kohout et al (1971) perfused isolated rat livers with isomolar concentrations of different albumin-bound free fatty acids. They demonstrated triglyceride secretion to be dependent on the chemical nature of the free fatty acid perfused; when long chain fatty acids containing 18 carbons were perfused, the output of triglyceride decreased as the number of double bonds increased. If this pertains in man, the change in the ratio of linoleic to saturated long chain fatty acids which occurs during polyunsaturated fat feeding, could result in a diminished hepatic output of triglyceride. Nestel and Steinberg (1963) had earlier found less linoleic acid to be incorporated into hepatic triglyceride than palmitate, by the perfused rat liver and by rat liver slices. No significant difference in the triglyceride content of the perfusate was, however, detected between the two fatty acids. A possible explanation is that their perfusion lasted only 90 minutes whereas that of Kohout et al (1971) lasted four hours; it may be that esterification of FFA and secretion of triglyceride depends on a critical intracellular FFA concentration which had not been adequately achieved in the shorter perfusion time of Nestel and Steinberg (1963). Nestel and Barter (1971) also showed incorporation of labelled palmitate into serum triglyceride to exceed that of linoleate during constant infusion of these two free fatty acids in a heterogenous group of normolipaemic and hyperlipidaemic subjects. Data is, however, not available comparing hepatic triglyceride production from the same precursor free fatty acids in animals prefed saturated or polyunsaturated diets.

If, as suggested by these two groups of workers, palmitate is preferentially incorporated into serum triglycerides compared with linoleate, it is of interest to know of their relative roles in other metabolic pathways of hepatic FFA. Ketogenesis from palmitate, for instance, has been shown to be less than from isomolar concentrations of linoleate in the isolated perfused rat liver (Kohout et al, 1971). However, in fed rats, ketogenesis is only a minor pathway. When ketogenic rates were increased by fasting, the production of ketone bodies from a variety of fatty acids has been shown to be similar (Krebs and Hems, 1970). Whether hepatic palmitate and linoleic acid are oxidized at the same rate in vivo is not clear. Havel et al (1964) showed oxidation rates of palmitate and linoleate to be similar during exercise in man. However, in vitro studies with rat liver mitachondria showed the rate of CO_2 production to be greater from short chain than from long chain fatty acids (Bjöntorp, 1968). Furthermore, the rate of oxidation of 18C fatty acids appears to be directly related to the number of double bonds of the major free fatty acids occurring in plasma. Linoleic acid was shown to have a faster rate of oxidation than palmitic, stearic and oleic acids. It is, therefore, conceivable that, whereas palmitic acid reaching the liver tends to be incorporated into triglycerides, preferential oxidation of free linoleate may occur.

A further fate of hepatic FFA is incorporation into phospholipids. Besides being an essential component of membranes, phospholipids are important constituents of lipoproteins and of bile. Campbell et al (1972) showed the output of biliary phospholipid, cholesterol and bile salts to be enhanced by polyunsaturated feeding. Their data suggests the effect of corn oil on biliary bile salt output to be due to enhanced bile acid synthesis from cholesterol. The hypocholesterolaemic effect of polyunsaturated fat could thus be due to accelerated cholesterol catabolism to bile acids. Selective incorporation of polyunsaturated free fatty acids into phospholipid, rather than triglyceride, could thus be a factor in the triglyceride lowering effect of these fats.

A further possible explanation of the effect of dietary polyunsaturated fat serum is an extension of the hypothesis of Spritz and Mishkel (1969). They have suggested that unsaturated fatty acids occupy more space on the lipoprotein molecule than saturated fatty acids, thereby allowing fewer lipid molecules to be accommodated per unit of apoprotein. Their hypothesis is based on dietary induced changes found in LDL (see page 256); it could, however, equally apply to VLDL, and serum triglyceride could thus be reduced.

SCOPE OF THE PRESENT STUDY

Although the triglyceride lowering effect of high polyunsaturated fat diets is well documented, both in short term feeding experiments and in longer term trials, little is known as to the magnitude or mode of action.

The present studies have attempted to assess changes in serum lipid concentration during feeding experiments involving isocaloric substitution of saturated and polyunsaturated fat in normolipaemic and hyperlipidaemic men. Since little data is available as to changes in lipoproteins in response to polyunsaturated fat feeding, the lipid content of lipoprotein fractions was measured after ultracentrifugal separation.

From the aforegoing it can be seen that there is little information concerning the mode of action of diets rich in polyunsaturated fat in reducing serum lipid concentrations. The mechanism of the fall in triglyceride level has been little studied; increased removal has been suggested to be the dominant factor because increased adipose tissue lipoprotein activities have been reported in rats on such diets (Pawar and Tidwell, 1968). As unsaturated long chain fatty acids have been shown to be less well incorporated into triglycerides by the rat liver compared with saturated fatty acids (Kohout et al, 1971), a reduction in secretion of triglyceride in its main transport form, VLDL, could also be operative. These possibilities have been assessed in the present study.

The possible role of altered rate of uptake of triglyceride from plasma was studied using the intravenous fat tolerance test and by assaying post-heparin lipolytic activity.

Factors influencing the secretion of VLDL triglyceride were also investigated. Plasma FFA concentration and turnover were measured. The incorporation of free palmitic and free linoleic acids into VLDL-triglyceride was compared using a double isotope technique. These two fatty acids were chosen as representative of saturated and polyunsaturated fatty acids respectively.

As insulin secretion may influence the rates of triglyceride secretion into plasma (Topping and Mayes, 1970) and of its removal (Salaman and Robinson, 1966), serum insulin levels were also compared during the two dietary regimes.

CHAPTER V, SECTION B

Thirty-four men took part in a short-term feeding experiment to determine the effect of dietary polyunsaturated fat on fasting serum triglyceride and cholesterol, and lipoprotein concentration and composition. Each subject underwent two dietary periods; in one, the fat content was predominantly saturated, in the other, predominantly unsaturated. Measurements were made towards the end of each dietary period.

SUBJECTS

Eleven men (mean age 54 years, range from 30 - 67 years) with endogenous hypertriglyceride were studied. All had serum triglyceride levels exceeding 160 mg./dl. with serum lipoprotein electrophoresis showing increased pre-beta bonds. Subjects with known secondary causes of hypertriglyceridaemia were excluded from the study.

In addition, 23 normal men (mean age 30 years, range 20 - 52 years) being healthy members of the hospital and laboratory staff, were investigated. Their serum triglyceride levels were less than 160 mg./dl. and cholesterol concentrations less than 260 mg./dl.

DIETS

The feeding experiment consisted of a 3 - 5 day run in period followed by two 10 day dietary periods. Calorie intake was calculated for each individual from a detailed dietary history taken by a trained dietitian. In the normal men, initial body weight was 69.5 ± 1.9 Kg. (mean ± SEM); in the hypertriglyceridaemic patients, it was 78.4 ± 2.2 Kg. Body weight was recorded daily and average figures for each feeding period did not increase or decrease by more than 1 Kg. No alcohol was permitted throughout the study.

During one 10 day period, the dietary fat was predominantly saturated, with a P/S ratio of 0.20, whereas during the other period, most of the saturated fat content was isocalorically substituted by polyunsaturated fat; the P/S ratio during the unsaturated fat feeding period was 2.4. The diet consisted of normal foodstuff, being provided as three main meals and two snacks per day, in both dietary periods.

Fat provided 40% of total calories, carbohydrate 45% and protein 15%. In both periods, the major sources of protein were lean meat and poultry, and whole or skimmed milk. Throughout the study, the nature and content of dietary carbohydrate was held constant; starch provided 23%, sucrose 11% and fructose 5% of calories, galactose, maltose and other minor components making up the total carbohydrate content. In the saturated fat period, the fat was largely provided as milk, butter and cream. Mean cholesterol content was 420 mg./day. In the polyunsaturated fat period, the main source of fat was sunflower oil, which has a linoleic acid content of 61.5% (Press et al, 1974). Mean cholesterol content during this part of the diet was 160 mg./day.

In some experiments, the saturated fat period preceded the period of unsaturated fat feeding; in others, the sequence was reversed. Meals were provided by the Hammersmith Hospital Diet Kitchen. Physical activity was restricted throughout the periods.

Fasting serum lipids, lipoprotein ultracentrifugation and lipoprotein electrophoresis were determined as previously described, at the end of each period.

RESULTS

SERUM LIPIDS

A representative study in which the period of saturated fat feeding was followed by the high P/S diet is shown in Figure 22 . The reduction in serum triglycerides on switching to unsaturated can be seen to commence within one day.

The response of serum triglyceride and serum cholesterol to the saturated and polyunsaturated fat diets in the 34 men is seen in Figure 23.

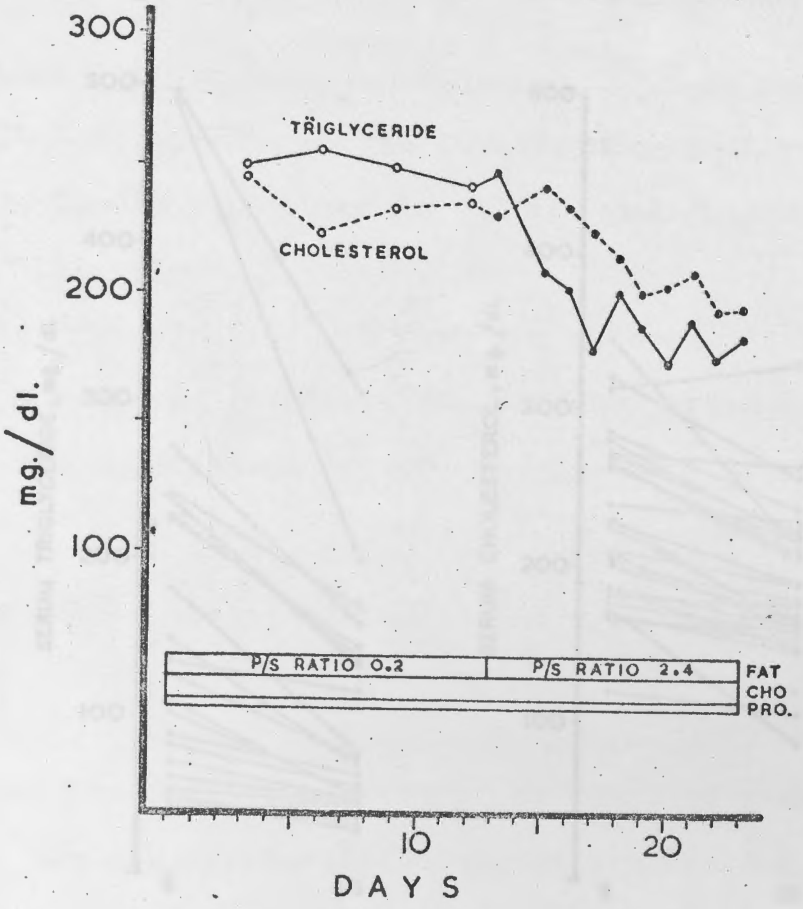

Fig. 22. Fasting serum triglyceride and cholesterol levels in a representative dietary study. Saturated fat feeding period preceded polyunsaturated fat.

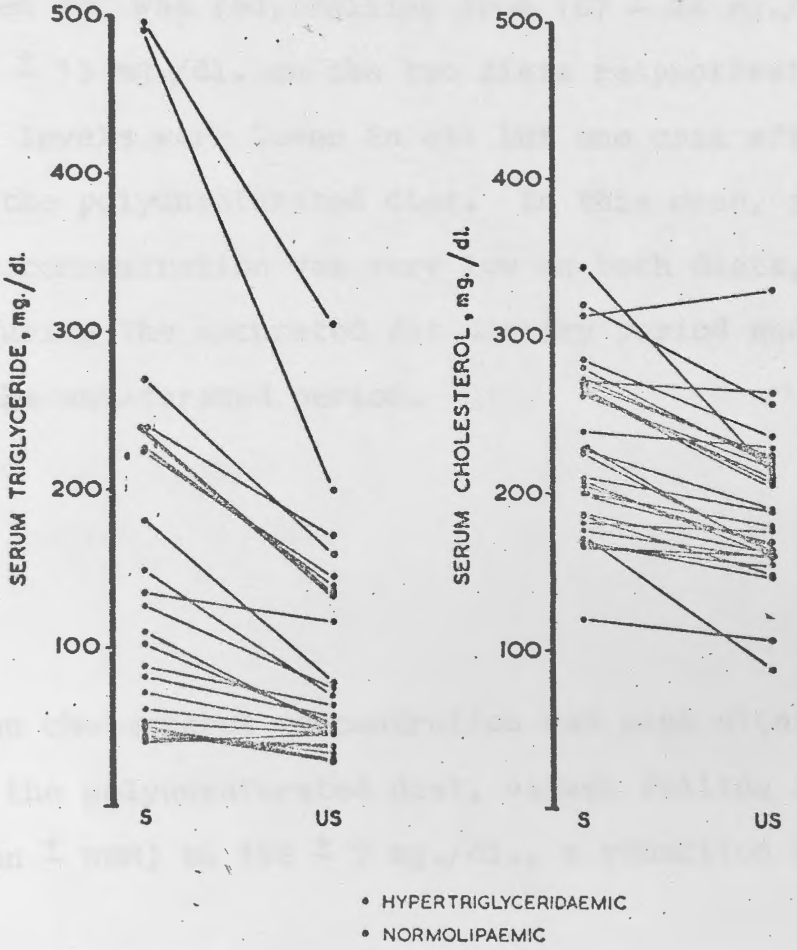

Fig. 23. Serum triglyceride and cholesterol levels during saturated fat diet (S) and polyunsaturated fat diet (US)

Triglyceride

The mean triglyceride concentration was 35% lower ($p < 0.001$ by the paired t-test) when unsaturated fat was fed than when saturated fat was fed, falling from 167 ± 24 mg./dl. (SEM) to 101 ± 13 mg./dl. on the two diets respectively. Triglyceride levels were lower in all but one case after consumption of the polyunsaturated diet. In this case, serum triglyceride concentration was very low on both diets, being 40 mg./dl. during the saturated fat dietary period and 46 mg./dl. during the unsaturated period.

Cholesterol

The mean cholesterol concentration was also significantly lower after the polyunsaturated diet, values falling from 222 ± 8 (mean ± SEM) to 188 ± 7 mg./dl., a reduction of 16% ($p < 0.001$)

Figure 23 also indicates that serum triglycerides and cholesterol concentrations respond to diet in a similar fashion both in hypertriglyceridaemic patients and normal subjects; those subjects with the highest lipid concentration during the saturated fat diet tended to show the more marked response to unsaturated fat feeding. Due to the similar response, results for normal and hyperlipidaemic patients have been pooled in the remainder of this Chapter.

Magnitude of response in relation to baseline values

The correlation between the triglyceride concentration during the saturated fat feeding period, and the difference between the triglyceride levels during this diet and during the high P/S diet, was exceedingly close, as shown in Figure 24 ($r = +0.94$, $p < 0.001$). A lower, but nonetheless significant correlation can also be seen to pertain for cholesterol levels ($r = +0.58$, $p < 0.001$ - see Figure 24). Subjects with the highest triglyceride cholesterol values while on the saturated fat diet, showed the most marked changes in response to feeding polyunsaturated fat.

LIPOPROTEINS

Quantitative

Changes in serum lipoprotein fractions during consumption of the two diets are shown in Table 8($n = 25$).

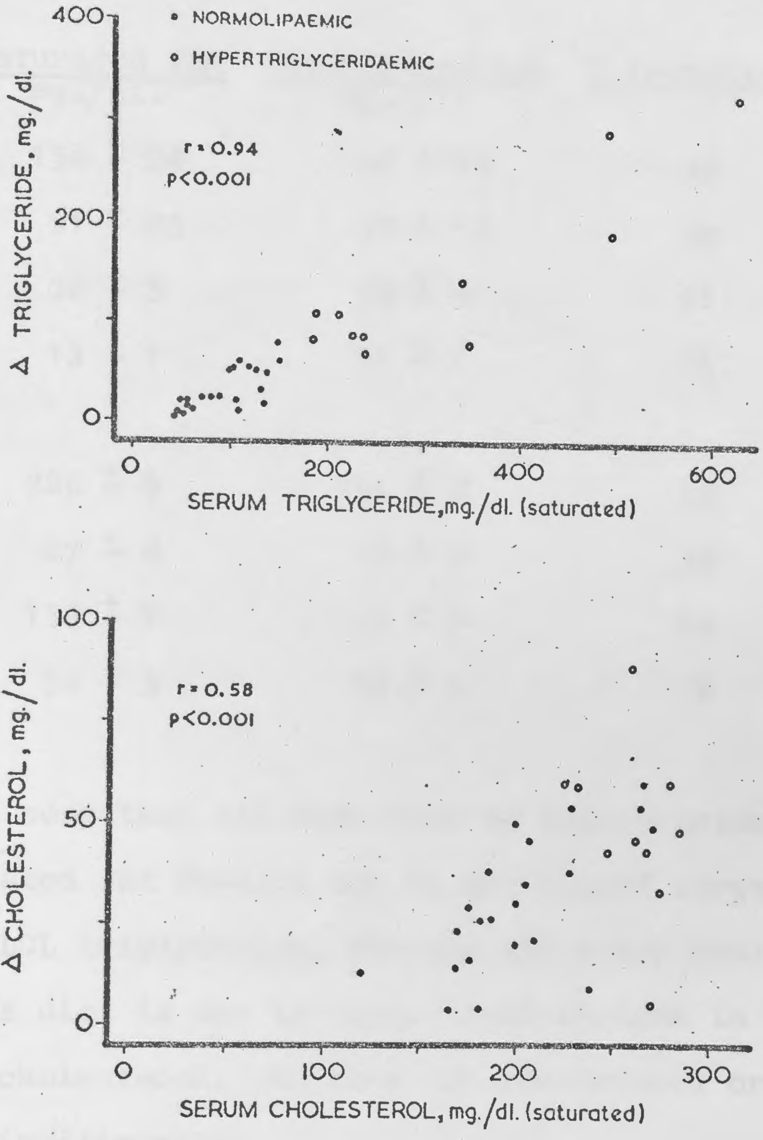

Fig. 24 Correlation between serum lipids during saturated fat feeding, and the difference between serum triglyceride levels during the two diets.

TABLE 8

EFFECT OF TYPE OF DIETARY FAT ON SERUM LIPOPROTEINS ON THE TWO DIETS

(mean ± S.E.M.)

Triglyceride	Saturated fat mg./dl.	Unsaturated fat mg./dl.	% Reduction	P
Total	154 ± 24	94 ± 13	39	< 0.001
VLDL	91 ± 23	53 ± 14	42	< 0.001
LDL	32 ± 5	28 ± 4	11	NS
HDL	13 ± 1	11 ± 1	15	NS
Cholesterol				
Total	226 ± 9	191 ± 8	16	< 0.001
VLDL	27 ± 4	18 ± 3	34	< 0.001
LDL	138 ± 7	125 ± 7	10	< 0.005
HDL	56 ± 3	56 ± 3	0	NS

It can be seen that the reduction in triglyceride level following saturated fat feeding can be attributed largely to reduction in VLDL triglyceride, whereas the lower cholesterol level after this diet is due to significant changes in both LDL and VLDL cholesterol. Neither HDL cholesterol or triglyceride changed significantly.

Qualitative

As VLDL triglyceride changed by 42% whereas VLDL cholesterol changed by only 34%, it appeared that VLDL composition must have differed during the two feeding periods. In Table 9 the ratio of triglyceride to cholesterol in VLDL is seen to decrease significantly when saturated fat was replaced by polyunsaturated fat in the diet ($p < 0.002$); there was, however, no significant change in the LDL triglyceride to cholesterol ratio.

TABLE 9

TRIGLYCERIDE/CHOLESTEROL RATIO (MEAN ± SEM)

	n	Saturated fat diet	Unsaturated fat diet	P
VLDL	25	2.70 ± 0.27	2.17 ± 0.18	< 0.002
LDL	24	0.21 ± 0.02	0.20 ± 0.02	NS

Because of the extremely low triglyceride content in HDL, calculations on it are inaccurate.

MECHANISM OF SERUM TRIGLYCERIDE LOWERING EFFECT OF A HIGH P/S DIET

In order to study the mode of action of the reduction in serum triglyceride concentration after unsaturated fat feeding, two experiments were performed. As Pawar and Tidwell (1968) have suggested that polyunsaturated fat may lower serum triglyceride by enhancing peripheral triglyceride removal, this process was assessed in 15 men, six hypertriglyceridaemic and nine normolipaemic. Removal of triglyceride from plasma was measured using the IVFTT (see Page 65); in addition, PHLA (see Page 76) was assayed as an indirect measure of lipoprotein lipase. These measurements were respectively performed after an overnight fast on the last two days of each dietary period as outlined on Page 272.

In the second experiment, six further men, aged 32 - 55 years (mean = 48 years), with normal serum lipids (mean cholesterol 193 mg./dl.; mean triglyceride 130 mg./dl.) received an infusion of labelled fatty acids to investigate the relative rates of incorporation of palmitate and linoleate into VLDL triglyceride. This was performed in the fasted state, the subjects having previously consumed a 'normal' western diet.

TRIGLYCERIDE REMOVAL FROM PLASMA

Figure 25 shows the fasting triglyceride levels of the 15 participants in this part of the experiment on the two diets; the value of 194 ± 38 mg./dl. (mean ± SEM) during saturated fat feeding was significantly higher than the mean level of 116 ± 19 mg./dl. during polyunsaturated fat feeding ($p < 0.005$). PHLA is also shown, together with the fractional removal rate of triglyceride (K_2) observed in the IVFTT after an injection of Intralipid at a dose of 0.1 gramme/Kg. body weight. By contrast with serum triglyceride concentration, neither PHLA nor the rate constant, K_2, of the IVFTT showed a significant change during the polyunsaturated fat dieting period.

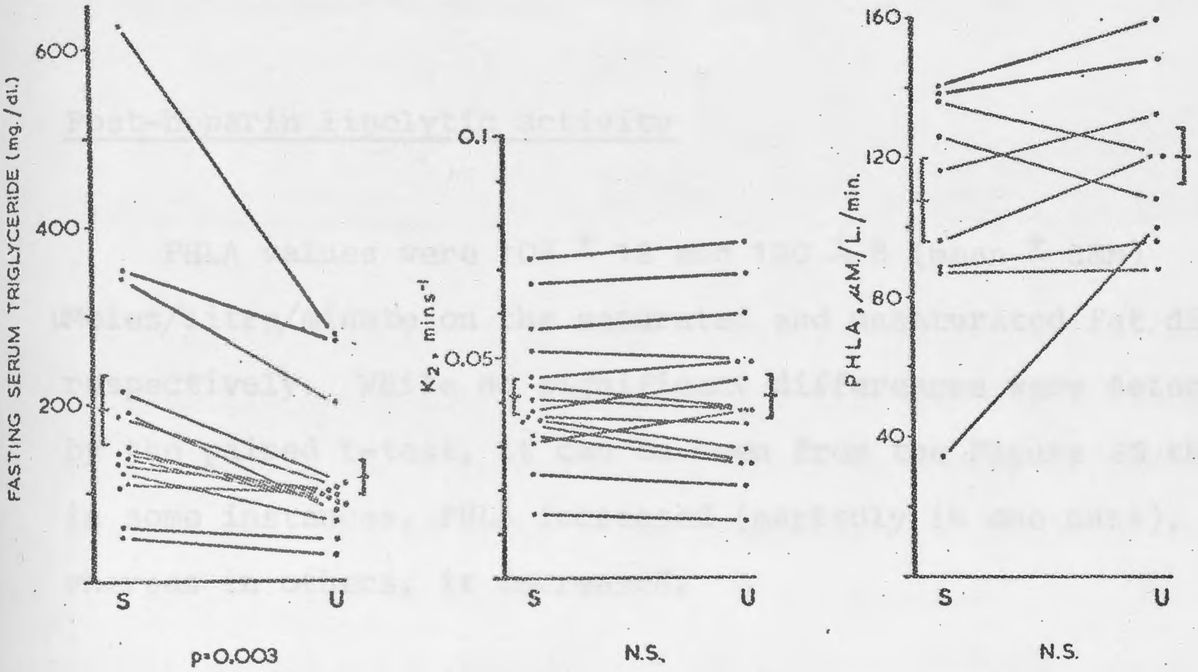

Fig. 25. Differences in serum triglyceride concentration, K_2 and PHLA during consumption of saturated fat (S) and polyunsaturated fat (US)

Intravenous fat tolerance test

The values for K_2 were 0.041 ± 0.004 min.$^{-1}$ (mean ± SEM) and 0.040 ± 0.005 min.$^{-1}$ on the saturated and unsaturated diets respectively. In almost all cases, the values for K_2 can be seen to be remarkably similar on the two diets.

Post-heparin lipolytic activity

PHLA values were 108 ± 12 and 120 ± 8 (mean ± SEM) µMoles/litre/minute on the saturated and unsaturated fat diets respectively. While no significant differences were detected by the paired t-test, it can be seen from the Figure 25 that in some instances, PHLA increased (markedly in one case), whereas in others, it decreased.

Thus no evidence was obtained in support of altered removal of triglyceride from plasma having accounted for the change in serum triglyceride concentration resulting from dietary substitution.

FREE FATTY ACID CONCENTRATION AND PLASMA INSULIN

Mean FFA levels and fasting plasma insulin concentrations shown in Table 10 did not differ significantly between the dietary periods.

TABLE 10

PLASMA FREE FATTY ACID AND BASAL INSULIN LEVELS ON THE TWO DIETS (mean ± SEM)

	Saturated	Unsaturated	Significance
FFA (μM/l.)	521 ± 35	550 ± 30	NS
Insulin (μU/ml.)	6.1 ± 0.6	5.0 ± 0.5	NS

INCORPORATION OF PALMITATE AND LINOLEATE INTO VLDL TRIGLYCERIDE

The incorporation of free palmitate and linoleate into VLDL-triglyceride was compared by a double isotope technique. These fatty acids were chosen to represent the major saturated and polyunsaturated fatty acids of plasma FFA respectively. The method used is described more fully on Page 78. The subjects were normolipaemic men on typical western diets.

1-^{14}C linoleic acid and (9,10-^{3}H) palmitic acid were complexed with fatty acid poor human albumin and infused simultaneously at a constant rate for a four-hour period following an overnight fast. Immediately before the experiment, they were added slowly, with mixing, to a solution containing 0.5 gramme human serum albumin and made up to volume with isotonic saline. Up to 10 μci ^{14}C linoleate and 25 μci ^{3}H palmitate were infused during each experiment.

Following an overnight fast, cannulation of both antecubital veins was performed; the subjects then lay quietly for 30 minutes prior to commencement of the infusion. The radioactive fatty acids were administered by a constant infusion through one venous cannula over a four-hour period. During the infusion, blood was sampled at hourly intervals from the cannula in the opposite arm for determination of the incorporation of ^{3}H and ^{14}C labels into VLDL triglyceride. After the infusion, blood was sampled for a further four hours to determine the isotope ratio in VLDL triglyceride during the disappearance phase.

VLDL was separated by ultracentrifugation (see Page 47) and extracted with chloroform-methanol. The lipids in VLDL and plasma were then separated on thin layer plates using free palmitic acid, triolein, lecithin and cholesteryl palmitate (Sigma 99% pure) which had previously been shown to yield a single spot on thin layer chromatography, as standards. The ^{14}C and ^{3}H in VLDL triglyceride, plasma free fatty acid, phospholipids and cholesterol ester fractions were counted in a liquid scintillation spectrometer.

In order to determine the specific activities of palmitate and linoleate in plasma, extracts of plasma were fractionated by thin layer chromatography (see Page 82). The free fatty acid spots were eluted, methylated and their composition analysed by gas liquid chromatography. Plasma FFA levels were also determined as previously described, and the concentrations of the individual FFAs determined from the fatty acid composition and concentration.

A representative study is shown in Figure 26(see Page 287); it is seen that incorporation of both isotopes into VLDL triglyceride was linear during the period of infusion. Extrapolation to the base-line suggests that labelled triglyceride started appearing in plasma after 30 - 40 minutes. After the infusion, VLDL radioactivity decreased exponentially, isotope ratios showing no significant change; mean $^{3}H/^{14}C$ ratios were 2.22 at one hour, 2.28 at two hours, 2.26 at three hours and 2.24 at four hours after the infusion.

Calculations

The absolute rate of incorporation cannot be reliably calculated from the incorporation curves partly because of simultaneous removal of triglyceride from serum and because of the possibility of recycling (Eaton et al, 1969). Having established, however, that there was no difference between the rates of disappearance of ^{3}H and ^{14}C, the slopes of the linear increase in radioactivity during the infusion was taken to be a measure of incorporation of the individual FFA into VLDL triglyceride, for the purpose of calculating the ratio between the incorporation of palmitate and linoleate as follows:

$$\text{Incorporation ratio} = \frac{\text{Rate of increase of } {}^{3}\text{H in VLDL-TG}}{\text{Specific activity of free palmitate}} \div \frac{\text{Rate of increase of } {}^{14}\text{C in VLDL}}{\text{Specific activity of free linoleate}}$$

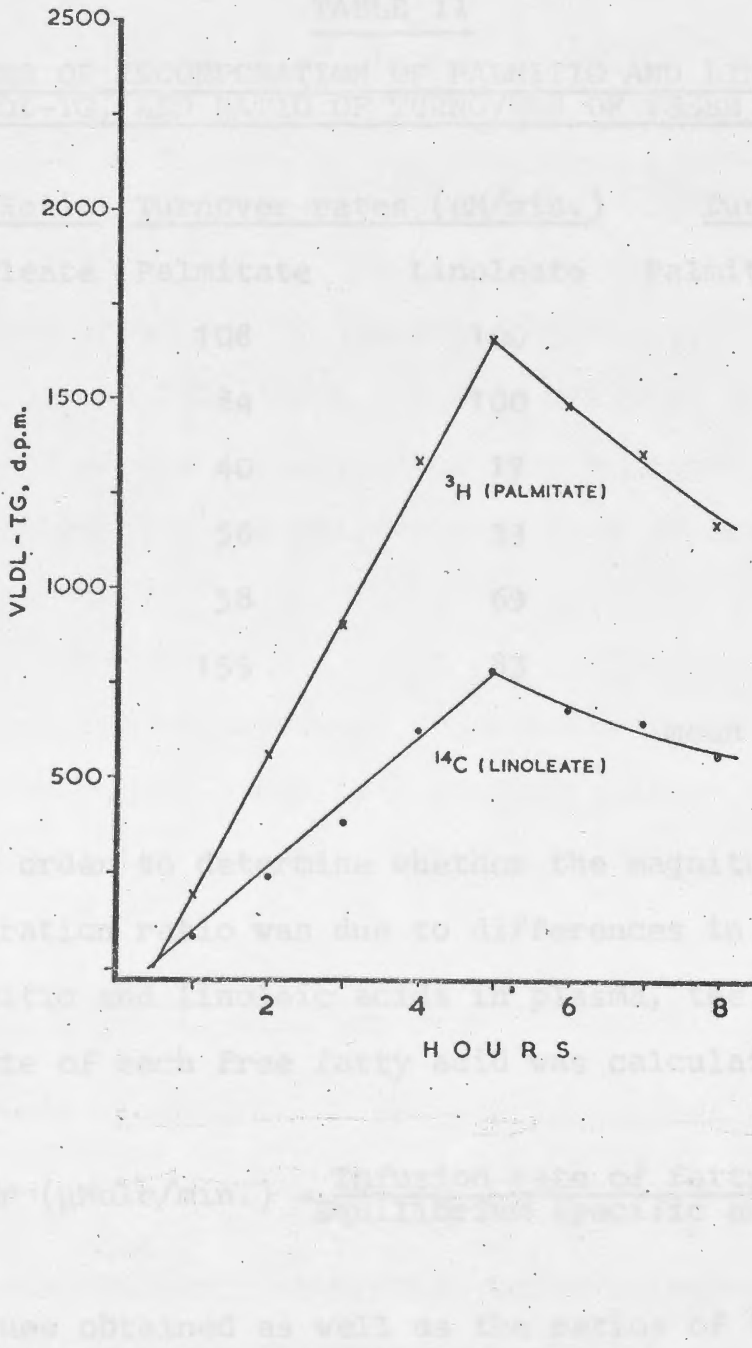

Fig. 26. A representative study of the incorporation of labelled palmitic and linoleic acids into VLDL-TG

This ratio exceeded unity in all studies, mean 3.19 (range = 2.72 - 4.13) as can be seen in Table 11.

TABLE 11

RATIO OF RATES OF INCORPORATION OF PALMITIC AND LINOLEIC ACIDS INTO VLDL-TG, AND RATIO OF TURNOVERS OF THESE ACIDS

Incorporation Ratio	Turnover rates (μM/min.)		Turnover Ratio
Palmitate/Linoleate	Palmitate	Linoleate	Palmitate/Linoleate
2.72	108	100	1.08
2.95	84	100	0.84
3.79	40	17	2.36
2.54	56	33	1.70
3.00	58	69	0.84
4.13	155	83	1.87
mean = 3.19			mean = 1.43

In order to determine whether the magnitude of this incorporation ratio was due to differences in the turnovers of palmitic and linoleic acids in plasma, the absolute turnover rate of each free fatty acid was calculated as follows:

$$\text{Turnover } (\mu\text{Mole/min.}) = \frac{\text{Infusion rate of fatty acid (DPM/minute)}}{\text{Equilibrium specific activity (DPM/}\mu\text{Mole)}}$$

The values obtained as well as the ratios of the turnover of palmitate to linoleate, are shown in Table 11. It can be seen that in four of the six cases, the absolute turnover of free palmitate exceeded that of free linoleate, the mean turnover of palmitate being 1.43 times greater than that of linoleate.

The proportions of the respective turnovers of palmitic and linoleic acids which appeared in VLDL triglyceride were compared thus:

$$\frac{\text{Rate of incorporation of }^{3}\text{H into VLDL-TG}}{\text{Turnover rate of free palmitic acid}} \div \frac{\text{Rate of incorporation of }^{14}\text{C into VLDL-TG}}{\text{Turnover rate of free linoleic acid}}$$

The values obtained for the six subjects were 2.52, 3.50, 1.62, 1.40, 3.58 and 2.21 (mean = 2.47). Thus in all subjects, the percentage of the free palmitic acid turnover incorporated into VLDL-triglyceride was greater than that of free linoleic acid turnover.

This data is expressed graphically in Figure 27; the incorporation ratio has been plotted against the ratio of the turnovers of the two free fatty acids concerned, i.e.

$$\frac{\text{Free palmitic acid turnover }(\mu\text{Moles/min.})}{\text{Free linoleic acid turnover }(\mu\text{Moles/min.})} \text{ for each subject.}$$

As is shown in Figure 27, the ratio of the rates of incorporation of palmitate and linoleate into VLDL-triglyceride, was greater in every study than could be accounted for by differences between the turnovers of plasma free palmitic and linoleic acids. These results suggest that free palmitic acid is preferentially incorporated into VLDL-triglyceride compared with free linoleic acid.

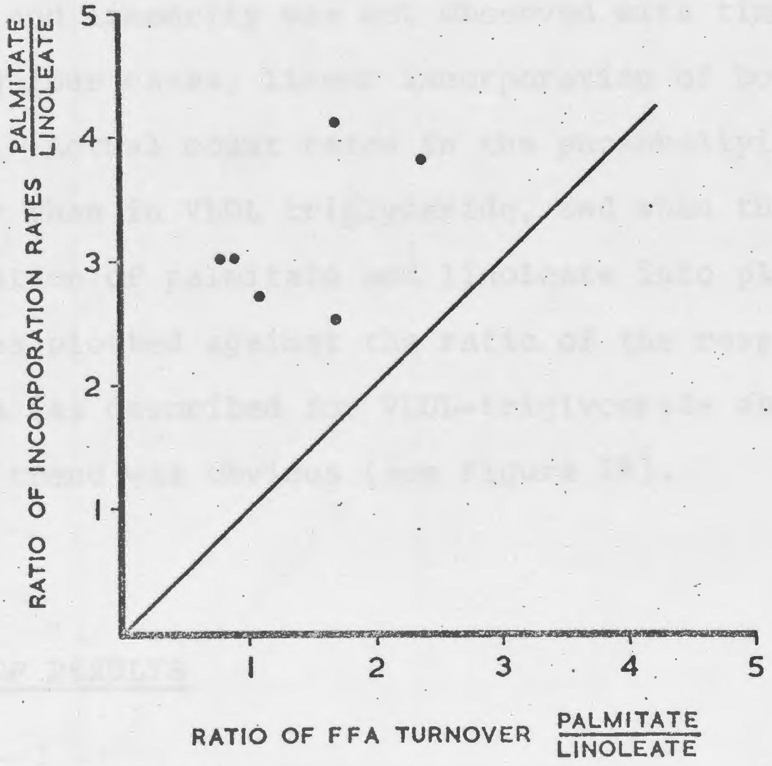

Fig. 27. Comparison of ratio of rates of incorporation of free palmitate and free linoleate into VLDL-TG, with the ratio of the turnovers of these acids as FFA

No measurable incorporation of counts into cholesterol esters was found during the duration of the experiment. Incorporation of isotope into phospholipid was also determined after elution of the phospholipid spot on TLC, with ethanol (see Page 84). In two cases, the counts in phospholipid were very low and linearity was not observed with time. In the remaining four cases, linear incorporation of both isotopes occurred. Actual count rates in the phospholipid fraction were far lower than in VLDL triglyceride, and when the ratio of incorporation of palmitate and linoleate into plasma phospholipids was plotted against the ratio of the respective FFA turnovers (as described for VLDL-triglyceride above), no definite trend was obvious (see Figure 28).

SUMMARY OF RESULTS

When isocaloric diets high in saturated fat or polyunsaturated fats were alternately fed to hyperlipidaemic and normal men, serum triglyceride was 35% lower and serum cholesterol 16% lower during the unsaturated fat feeding. A similar response occurred in normolipaemic subjects and in patients with primary endogenous hypertriglyceridaemia.

The magnitude of the reduction of both serum triglyceride and cholesterol due to the unsaturated fat was greatest in subjects with the highest base-line levels.

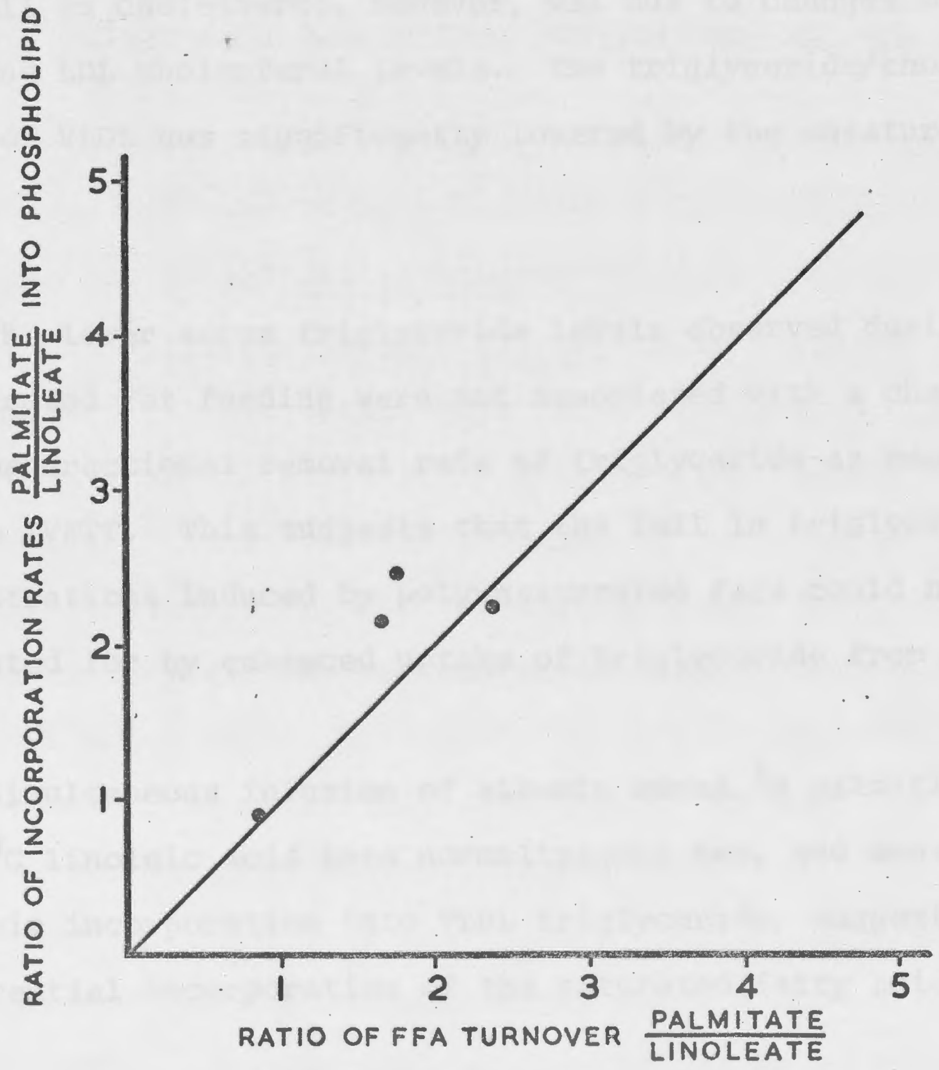

Fig. 28. Comparison of ratio of rates of incorporation of free palmitate and free linoleate into serum phospholipid, with the ratio of the turnovers of these acids as FFA

The reduction of triglyceride levels during the unsaturated fat period was due to a change in VLDL triglyceride. The fall in cholesterol, however, was due to changes both in VLDL and LDL cholesterol levels. The triglyceride/cholesterol ratio of VLDL was significantly lowered by the unsaturated fat diet.

The lower serum triglyceride levels observed during unsaturated fat feeding were not associated with a change in PHLA or fractional removal rate of triglyceride as measured by the IVFTT. This suggests that the fall in triglyceride concentrations induced by polyunsaturated fats could not be accounted for by enhanced uptake of triglyceride from plasma.

Simultaneous infusion of albumin bound ^{3}H palmitic acid and ^{14}C linoleic acid into normolipaemic men, and measurements of their incorporation into VLDL triglyceride, suggests preferential incorporation of the saturated fatty acid.

CHAPTER V, SECTION C

In choosing a diet to determine the effect of polyunsaturated fat feeding on serum lipids and lipoproteins, attention had to be paid to other constituents of the diet which could influence these. Of particular importance was the need to keep caloric intake constant as a negative calorie balance is known to lower serum triglyceride concentrations (Levy, 1972). Consideration had also to be given to the content and composition of dietary carbohydrate; the interaction between dietary fat and carbohydrate, discussed more fully on page 263, which may affect particularly the serum triglyceride concentration, was carefully considered. In many reported studies, for example, those of Wilson et al (1971) and Hall et al (1972) not only was the type of dietary fat changed, but the intake of fat was reduced during the polyunsaturated dietary period as well. Either calorie intake is reduced which may contribute to a fall in serum lipids, or a proportion of the saturated fat intake is isocalorically replaced by carbohydrate when the higher carbohydrate intake might cause an upward trend in glyceride levels (Mancini et al, 1969). To evaluate the effect on serum triglycerides of dietary saturated and unsaturated fats, carbohydrate and fat intakes were kept constant during the two dietary periods in the present study; the composition of the carbohydrate, i.e. the content of sucrose, fructose and starch, also remained unchanged. The provision of 40% of calories by fat, 45% by carbohydrate and 15% by protein is of a similar order to that of the typical western diet. Because formula diets were not employed, the saturated fat feeding period contained a higher cholesterol content that did the polyunsaturated fat feeding period; this is likely to have influenced the serum cholesterol concentration in its own right (Keys et al, 1965c; Connor et al, 1968; Mattson et al, 1972). No effect of cholesterol feeding on serum triglyceride levels has been observed when formula feeds in which the cholesterol content was the only change, were employed, (Connor et al, 1969). The effect noted in the present study on serum triglyceride is thus almost certainly due to the effect of exchanging dietary fat. It is, however, not possible to determine to what extent the effect observed was due to a reduction in saturated fat content, an increase in unsaturated fat content, or to a combination of both these effects.

SERUM LIPID CONCENTRATIONS

Using ten-day feeding periods, the mean serum triglyceride level was 35% lower during consumption of a diet rich in polyunsaturated fat than when saturated fat was fed. This response was greater in magnitude than the better known effect of such diets on serum cholesterol concentration, which was reduced by 16%.

Serum cholesterol

The reduction in serum cholesterol was of the order noted in previous studies (Ahrens et al, 1957; Connor et al, 1969), although lower than some (Spritz and Mishkel, 1969). Ahrens et al (1957) have shown the cholesterol change to have reached its maximum by two to three weeks; Spritz and Mishkel (1969) showed the effect of changing dietary fat on plasma cholesterol and triglyceride concentration to be complete within 10 - 14 days. Had the experiments in the present study been continued for a longer period, it is possible that even greater differences may have been observed. One study in which measurements were made throughout both dietary periods showed the onset of the change in both cholesterol and triglyceride levels to occur within a day of changing diets (see Figure 22page 274).

Serum triglyceride

The magnitude of reduction of serum triglyceride concentrations previously reported has been more variable. Some authors (Arvidson and Malmros, 1972; Anderson et al, 1973) have failed to find any changes, whereas many others have observed a reduction in serum triglycerides in response to feeding polyunsaturated fat (Ahrens et al, 1957; Kinsell et al, 1959; Beveridge et al, 1964; Connor et al, 1969; Spritz and Mishkel, 1969). Spritz and Mishkel found mean serum triglyceride to be about 25% lower after polyunsaturated feeding. Although this figure is somewhat lower than that observed in the present study, they noted that the least change in triglyceride concentration occurred in those with lowest triglyceride levels. A similar effect was observed in the present study (see Figures 23 and 24), the fall in triglyceride in response to polyunsaturated fat feeding showing a highly significant positive correlation with the triglyceride level after the saturated fat period. It is also noteworthy that the response of both cholesterol and triglyceride to polyunsaturated fat feeding was similar in normolipaemic and hypertriglyceridaemic individuals.

As triglyceride levels changed more in those with high initial levels and because the initial triglyceride levels were higher in the present study than in that of Spritz and Mishkel (1969), this may explain the difference in magnitude of triglyceride reduction between the two studies. The observation that individuals with a higher initial level experience a greater reduction in response to a stimulus than those with initially low concentrations, has previously been observed for serum cholesterol concentrations (Kingsbury et al, 1969; Ederer, 1972). The present findings are in conformity with these studies.

The experiments performed in these investigations were of short duration; extrapolations of observations to the situation in the long-term may not be entirely justified. Although serum cholesterol reductions have been shown to be sustained when high P/S diets have been maintained over long periods (Ahrens et al, 1957; Christakis et al, 1966; Dayton et al, 1969; Karvonen, 1972), the effect is less clear cut for serum triglycerides. Wilson et al (1971) showed serum triglyceride to fall rapidly during the first two weeks of a diet high in polyunsaturated fats. This was followed by a rise in triglyceride concentrations, although by the end of a six month period, triglyceride levels had fallen to levels significantly lower than at the commencement of the study. In such long-term studies, adherence to the prescribed diets is often not as strict as might be desired. Calorie control, in particular, seems to be a problem as weight changes are frequently observed in participants. As serum triglyceride levels are known to respond to changes in calorie content of the diet, it is conceivable that changes in calorie intake may override any effect produced by changes in the dietary fat content of the diet. This hypothesis could explain the lack of effect of unsaturated fat in lowering serum triglyceride levels noted by Bierenbaum et al (1973) despite the predicted change in fatty acid composition of adipose tissue being proof of dietary adherence.

LIPOPROTEINS

Whereas much has been reported of the effects of dietary fat on plasma lipids, little information is available that defines this effect in terms of circulating lipoproteins. Most of the triglyceride in fasted serum is present in the VLDL fraction, and the lower triglyceride levels during the unsaturated fat diet in the present study, was due to decreased VLDL triglyceride. The reduction in serum cholesterol concentration, however, was due to a significant reduction in both VLDL and LDL triglyceride content. Although Spritz and Mishkel (1969) showed that polyunsaturated fat diets reduce LDL cholesterol content without affecting HDL, they did not determine the effect on VLDL. Although the latter lipoprotein is the main carrier of endogenous triglyceride, about 15 - 20% of the molecule by weight is cholesterol (Levy et al, 1966). The present study shows that the significant fall in VLDL cholesterol in response to feeding polyunsaturated fat accounted for a substantial proportion of the overall reduction in serum cholesterol. In accordance with the findings of Spritz and Mishkel (1969), polyunsaturated fat was not found to affect HDL cholesterol.

The composition as well as the content of VLDL was influenced by the dietary exchange, the ratio of triglyceride to cholesterol falling significantly with the high P/S diet. The triglyceride to cholesterol ratio of LDL did not differ between the two diets. VLDL is well-known to be heterogenous in respect of particle size (Fredrickson et al, 1967; Levy, 1971). The findings in the present study of a change in its triglyceride to cholesterol ratio in response to diet, is further evidence of the heterogeneity of the VLDL molecule.

The reason for this compositional change is not clear. Spritz and Mishkel (1969) showed a lower ratio of cholesterol and phospholipid to protein in LDL, in subjects on a high P/S diet than in subjects on a saturated fat diet. They postulated that this effect could be accounted for by the greater space on the lipoprotein molecule occupied by polyunsaturated fatty acids; the lipid carrying capacity of LDL would thereby be diminished. Although VLDL protein measurements are not described in the present thesis, the ratio of triglyceride and cholesterol to VLDL apoprotein measured immunologically, has been shown not to change significantly on switching from a saturated to an unsaturated fat diet (Chait et al, 1974). In twelve subjects, the triglyceride/protein ratio in VLDL was 10.7:1, while on a saturated fat diet and 10.3:1 on an unsaturated fat diet. The hypothesis of Spritz and Mishkel (1969) would thus appear not to be applicable to VLDL.

MODE OF ACTION OF POLYUNSATURATED FAT ON SERUM TRIGLYCERIDE

Considerable uncertainty exists concerning the mode of action of polyunsaturated fat diets in reducing serum triglyceride concentrations. Increased uptake of triglyceride from plasma has been suggested by the findings of Pawar and Tidwell (1968) that lipoprotein lipase activity in rat adipose tissue was increased by feeding a polyunsaturated fat supplemented diet. In the present study, lipoprotein lipase activity was assayed indirectly after its release into plasma by heparin; no difference between the two dietary periods was found. This finding contrasted with that of Bagdade et al (1970) who reported increased PHLA in men fed 40% corn oil diets. In some cases in this study, an increased PHLA was accompanied by a rise in plasma triglycerides. Caloric balance was unfortunately not always maintained; many of these subjects actually gained weight during the experiments. In the present set of experiments, calorie intake was carefully controlled, as evidenced by the lack of weight change of greater than 1 Kg. in any subject throughout the study. A further difference between the two studies was that whereas Intralipid was used as substrate for estimation of PHLA in the present work, Bagdade et al (1970) used Ediol. This substrate, unlike Intralipid, contains substantial amounts of monoglyceride (Persson, 1972). As heparin releases not only lipoprotein lipase, but also monoglyceride hydrolase into plasma, the results are not directly comparable. As discussed in Chapter III, PHLA as estimated in the present study, is probably not a particularly good measure of adipose tissue lipoprotein lipase activity. The intravenous fat tolerance test was used as a further index of uptake of triglyceride from plasma. Reduction of plasma triglyceride concentration during the unsaturated fat diet was accompanied by a change in fractional removal of injected triglyceride from plasma. These findings did not support the possibility that the high P/S diet reduces plasma triglyceride concentration by increasing its clearance from circulation. Both PHLA determination and the IVFTT employ artificial emulsion as substrate for lipoprotein lipase. In view of the effect of high P/S diets in increasing the polyunsaturated fatty acid content of serum triglyceride (Spritz and Mishkel, 1969; Arvidson and Malmros, 1972), it is conceivable that such altered substrates could be differentially metabolized by lipoprotein lipase in vivo. This has been shown not to be the case in vitro (Korn, 1961), and in vivo support for this finding is obtained from the double isotope experiment. VLDL triglyceride labelled endogenously with two isotopes (^{14}C linoleate and ^{3}H palmitate), showed a constant $^{3}H/^{14}C$ ratio during its removal from plasma over a four-hour period. Recently, it has been shown in this laboratory, that the fractional removal rate of apo-VLDL did not change on switching from a saturated fat to a polyunsaturated fat diet (Lewis B., Sigurdsson G. and Nicoll A. - in preparation), providing further evidence against changes in the fat composition of the diet affecting triglyceride removal.

The role of insulin in promoting the uptake of triglyceride from plasma and in the hepatic secretion of VLDL has been discussed in Chapter III. In view of its role at many sites in the pathways of FFA and triglyceride metabolism, basal insulin levels were measured on the two diets; no significant difference was found, suggesting that the differences in triglyceride levels on the two diets are unlikely to be insulin induced.

In the light of the present study, it seems unlikely that the fall in serum triglycerides in response to diets rich in polyunsaturated fat and low in saturated fat could be explained by enhanced removal of triglyceride from plasma. The alternative possibility, namely, that of reduced input of triglyceride into plasma, was thus investigated.

It is well established that endogenous plasma VLDL-triglyceride fatty acids are largely derived from plasma FFA (Havel, 1965). The uptake of FFA from the liver is also known to be dependent on FFA concentration (Rose et al, 1964; Heimberg et al, 1969; Van Harken et al, 1969). Another possible mechanism for changes in plasma VLDL triglyceride concentration, therefore, is altered plasma FFA concentration. No difference in plasma FFA concentration was observed, however, when the diets were alternated in this study.

Differential incorporation of saturated and polyunsaturated fatty acids into triglycerides of lipoprotein needs also to be considered. Using an isolated rat liver preparation perfused with isomolar concentrations of different fatty acids, Kohout et al (1971) have shown triglyceride output into the medium to be decreased when unsaturated fatty acids replace long chain saturated fatty acids of equal length in the perfusate. Similar observations have been made using both rat liver slices and perfused rat livers (Nestel and Steinberg, 1963). If saturated FFA were selectively incorporated into VLDL-triglyceride by the liver in preference to unsaturated FFA, dietary induced changes in the concentration of plasma FFA might influence VLDL-triglyceride secretion. The possibility that saturated fatty acid might be preferentially incorporated into VLDL-TG compared with unsaturated ones has been assessed by simultaneously infusing labelled free palmitic and linoleic acid into normal men on typical western diets. These two fatty acids were chosen as representative of saturated and unsaturated fatty acids respectively. The incorporation of infused free ^{3}H palmitate into VLDL-triglyceride was compared with that of infused free ^{14}C linoleate.

It was appreciated that absolute secretion rates of VLDL-triglyceride cannot be reliably calculated from the slope of the specific activity/time curve during infusion of labelled FFA. It was, however, considered that the ratio of these slopes would provide a measure of the relative rates of incorporation of the two fatty acids into VLDL triglycerides. It was first necessary to exclude the possibility that any difference observed between the ^{3}H and ^{14}C slopes was due to differential removal rates of the two isotopes from the plasma VLDL-triglyceride pool; by following the radioactivity in VLDL for four hours after the end of the infusion it was established that the $^{3}H/^{14}C$ ratio did not change during the period. Any recycling that occurred during the short period of the experiment was assumed to be equal for the two fatty acids.

That the ratio of the incorporation slopes provide a measure of relative rates of incorporation, also depends on the assumption that plasma and hepatic pools of each constituent FFA reach rapid equilibrium, as plasma FFA has first to pass through a hepatic FFA pool before appearing as VLDL-triglyceride; direct measurement of this hepatic pool is, of course, impossible in man. That the two pools are in equilibrium is supported by the fact that the plasma FFA pool turns over exceedingly rapidly and is small (Eaton et al, 1969). The splanchnic area is known to take up a large proportion of FFA leaving plasma (Havel, 1965), and as hepatic FFA concentration in liver cells is small, equilibrium appears probable. Were this not so, the hepatic FFA pool would probably be rapidly expanding.

Further criticism of the use of the incorporation of a labelled FFA precursor into triglyceride as a measure of triglyceride turnover is that this method assumes the plasma FFA to be the only significant precursor of plasma triglyceride fatty acid; de novo synthesis of FFA in the liver is assumed to be negligible, while in rats, hepatic fatty acid synthesis may be relatively important (Windmueller and Spaeth, 1967; Baker and Scholtz, 1967). Fatty acid synthesis has, however, been shown to be strongly suppressed in the fasted state

(Gibson and Hubbard, 1960; Numa et al, 1961; Korchak and Masaro, 1962), and as the present experiment was performed in fasting men, the contribution of hepatic fatty acid synthesis was assumed to be negligible.

The incorporation ratio exceeded unity in every study (mean = 3.19) suggesting that at the prevailing composition of plasma FFA, more palmitate than linoleate was converted to VLDL triglyceride. Although there is more free palmitate in plasma than free linoleate, Nestel and Barter (1971) have shown that the fractional turnover rate of the latter exceeds that of the former. A greater proportion of the plasma free linoleate would thus pass through the liver per unit of time than palmitate. When absolute turnovers were calculated, it can, however, be seen that in four of the six cases studied, the turnover of palmitate exceeded that of linoleate (see Table 11). When the ratio of incorporation of the two fatty acids into VLDL-triglyceride was compared with the ratio of their absolute turnovers, the greater incorporation of palmitate into VLDL-triglyceride could not be accounted for by the differences in the turnovers of the free palmitate and linoleate components through plasma. Furthermore, a greater percentage of free palmitate turnover appeared in VLDL-triglyceride compared with linoleate. As mean palmitate turnover was 1.43 times greater than linoleate turnover (see Table 11), this suggests that free palmitic acid compared with free linoleic acid, is preferentially incorporated into VLDL-triglyceride. This conclusion was also reached by Nestel and Barter (1971) who used similar methodology to study FFA incorporation into total serum triglycerides in a heterogeneous group of normolipaemic and hyperlipidaemic subjects, assuming similarity of uptake of the two labels from plasma.

It is unclear why preferential incorporation of certain components of plasma FFA into VLDL-triglyceride should occur. Other metabolic fates of hepatic FFA include incorporation into phospholipids of plasma and bile. No consistent pattern was noted in relation to incorporation of the two fatty acids into serum phospholipids in the cases studied. Linear incorporation was, however, only observed in four cases, and count rates were low. More sensitive techniques could conceivably show a difference. Campbell et al (1972) have demonstrated an increased biliary output of phospholipid in rhesus monkeys fed a diet rich in linoleic acid. Preferential oxidation of linoleate may also occur (Bjöntorp, 1968). It is conceivable that palmitic acid reaching the liver tends to be esterified to triglyceride in preference to linoleate; the reverse trend may be operative with regard to phospholipid formation, and to oxidation.

The serum triglyceride lowering effect of diets rich in polyunsaturated fat might, therefore, be mediated, at least in part, by a change in the composition of plasma FFA, with an increased proportion of unsaturated fatty acids which are less readily incorporated into VLDL triglyceride. This change in fatty acid composition of plasma has been well documented (Ahrens et al, 1959; Hallgren et al, 1960, Spritz and Mishkel, 1969; Arvidson and Malmros, 1972). The latter authors have shown a rapid increase in the proportions of polyunsaturated fat in plasma free fatty acids, triglycerides, cholesterol esters and phospholipids in response to feeding a polyunsaturated fat rich diet. Although it is well established that adipose tissue triglycerides are the main source of plasma FFA in the fasted state (Havel et al, 1962; Havel, 1970), and that changes in the diet are reflected in the fatty acid composition of adipose tissue (Hirsch, 1965), the latter changes take some time to occur. Certainly, the time required for dietary changes to become fully manifest in adipose tissue fatty acid composition is greater than the three weeks required for almost complete reversal of fatty acid spectra of plasma noted by Arvidson and Malmros (1972). Eaton et al (1969) have described a kinetic model for triglyceride and free fatty acid metabolism; a considerable proportion of plasma FFA is believed to be derived from triglycerides by recycling through a small extravascular pool with a rapid turnover. It is conceivable that dietary fatty acids enter this pool either from chylomicron or VLDL triglyceride, resulting in their rapid appearance as plasma FFA.

It is also conceivable that a common mechanism mediates the effect of the high P/S diet upon levels of both lipids in plasma. Accumulating evidence for a precursor-product relationship between VLDL and LDL (Levy et al, 1971; Eisenberg et al, 1972) suggests a possible unitary concept for the response of cholesterol and triglyceride levels to this dietary manipulation. As the mean triglyceride reduction in response to the polyunsaturated fat feeding was greater than the reduction in cholesterol, this could lend some support to this hypothesis. Studies in which the reduction in cholesterol is not accompanied by a reduction in serum triglyceride (Anderson et al, 1973), however, point against it. A further apparent contradiction to this hypothesis is the observation that the lipid composition of VLDL was altered by dietary change, whereas that of LDL was not. The main role of VLDL is to transport triglyceride to adipose tissue and muscle (Robinson, 1973); following hydrolysis of its triglyceride lipoprotein lipase, further catabolism to LDL appears to occur (Levy et al, 1971; Eisenberg et al, 1972). It is conceivable that following catabolism, a fixed ratio of triglyceride to cholesterol is present in LDL irrespective of the amount of triglyceride in the VLDL precursor. Meaningful consideration of this line of reasoning must, however, await further knowledge of lipoprotein structure and function. Nonetheless, while it is possible that two independent mechanisms account for the responses of LDL and VLDL respectively, the possibility that a single effect underlines change in both lipoproteins, and hence both in cholesterol and triglyceride, merits further investigation. A primary effect of the high P/S diet in decreasing VLDL secretion by the liver could lead to a fall in the concentration of its product LDL, and, therefore, in the cholesterol content of serum.

One practical application of the finding that high P/S diets reduce plasma levels of both VLDL and LDL, may be the use of such diets in the treatment of hyperlipidaemia. In the dietary management of patients with hyperlipidaemia, it is common practice to treat hypercholesterolaemia (Type II) by the high P/S diet and hyperglyceridaemia (Type IV) by a carbohydrate restricted diet (Frederickson et al, 1967; Levy, 1972). In fact, six individual diets have been recommended for the six types of hyperlipoproteinaemias of the WHO classification

(Beaumont et al, 1970). The present data, and some long-term studies cited earlier (Wilson et al, 1971; Hall et al, 1972), suggest that this distinction may be unnecessary. Most cases of hyperlipoproteinaemia encountered in a general hospital, or even in a clinic dealing specifically with disorders of lipid metabolism, tend to have elevations of pre-beta lipoprotein, beta lipoprotein, or both of these. Most of these states are likely to respond to a single therapeutic diet, the high P/S diet. Even when the fat content of the diet is somewhat lower than that used in the present study, Hall et al (1972) have shown, as has Macdonald (1972) in controlled feeding experiments, that the effect of unsaturated fat is dominant, and leads to a fall in triglyceride concentration despite the increase in carbohydrate. Antar et al (1970) have suggested that increased carbohydrate intake raises triglyceride levels only in the presence of a substantial intake of saturated fat. It, however, does seem important to avoid weight gain, as this may lead to triglyceride elevation despite the use of polyunsaturated fats (Bagdade et al, 1970). This simplification of dietary treatment of hyperlipidaemias is currently being used at the Hammersmith Hospital Lipid Disorders Clinic and appears to reduce lipid levels in both hypercholesterolaemia and in endogenous hypertriglyceridaemia (Tabaqchali et al, 1974). Its use does, of course, not preclude the need for lipid lowering drugs in addition, where indicated.

IMPLICATION FOR FUTURE WORK

The present results suggest that enhanced removal of triglyceride from plasma does not account for the triglyceride lowering effect of polyunsaturated fat diets. They also suggest preferential incorporation of palmitic acid into serum triglyceride compared with linoleic acid, thereby providing a possible basis whereby such diets could result in a reduced hepatic triglyceride secretion.

No attempt has, however, been made to study whether pre-feeding different dietary fats results in a reduced hepatic output of triglyceride other than through an affect on plasma FFA concentration. It is for instance, conceivable that processes such as esterification of triglyceride, or apoprotein formation, could be affected.

Alternative fates of fatty acids have also not been fully investigated in the present study. There is some evidence to suggest that polyunsaturated fatty acids may be preferentially utilized for biliary phospholipid formation (Campbell et al, 1972). Measurements of this are difficult in man, but in vitro and in vivo studies in animals could yield useful information in this field.

Further understanding of mechanisms involved in the control of serum lipid and lipoprotein levels is likely to come from more sophisticated turnover studies. Most methods used over the past decade have relied on numerous assumptions, many of which have not been validated. Interpretation is, therefore, not without difficulty. Recently, methods for studying the turnover of the apoprotein moeity of the lipoproteins have become available. Interpretation of these too, is not without hazard, in view of the inter-conversion that occurs between various lipoprotein classes. However, their use over the next few years, may well lead to a better understanding of lipid metabolism and the mechanism whereby factors such as dietary fats exert their influence on serum lipid and lipoprotein metabolism.

CHAPTER V - REFERENCES

Adams, C.W.M., Bayliss, O.B., Abdulla, Y.H., Mahler, F.R. and Rott, M.A. (1969) J. Atheroscler. Res. 9, 87.

Adams, C.W.M., Morgan, R.S. and Bayliss, O.B. (1970) Atherosclerosis 11, 119.

Adams, C.W.M. (1971) Proc. Roy. Soc. Med. 64, 902.

Adams, C.W.M. and Bayliss, O.B. (1973) Atherosclerosis 18, 191.

Ahrens, E.H.,Jr., Blankenhorn, D.H. and Tsaltas, T.T. (1954) Proc. Soc. exp. Biol. (N.Y.) 86, 872.

Ahrens, E.H.,Jr., Tsaltas, T.T., Hirsch, J. and Insull, W.R.Jr. (1955) J. clin. Invest. 34, 918.

Ahrens, E.H.,Jr., Hirsch, J., Insull, W.,Jr., Tsaltas, T.T., Blomstrand, R. and Peterson, M.L. (1957) Lancet I, 943.

Ahrens, E.H.,Jr., Insull, W.,Jr., Hirsch, J., Stoffel, W., Peterson, M.L., Farquhar, J.W., Miller, T. and Thomasson, H.J. (1959) Lancet I, 115.

Ahrens, E.H.,Jr., Hirsch, J., Oette, K., Farquhar, J.W. and Stein, Y. (1961) Trans. Ass. Amer. Phycn. 74, 134.

Anderson, J.T. (1967) Amer. J. clin. Nutrit. 20, 168.

Anderson, J.T., Grande, F. and Keys, A. (1973) J. Amer. diet. Ass. 62, 133.

Antar, M.A. and Ohlson, M.A. (1965) J. Nutrit. 85, 329.

Antar, M.A., Little, J.A., Lucas, C., Buckley, G.C. and Csima, A. (1970) Atherosclerosis 11, 191.

Antonis, A. and Bersohn, I. (1961) Lancet I, 3.

Arvidson, G. and Malmros, H. (1972) Zeitschrift für Ernähningswissenschaft 11, 105.

Avigan, J. and Steinberg, D. (1958) Proc. Soc. exp. Biol. (N.Y.) 97, 814.

Avigan, J. and Steinberg, D. (1965) J. clin. Invest. 44, 1845.

Bagdade, J.D., Hazzard, W.R. and Carlin, J. (1970) Metabolism 19, 1020.

Baker, N. and Schotz, M.C. (1967) J. Lipid Res. 8, 646.

Bang, H.O., Dyerberg, J. and Brondum Nielsen, A. (1971) Lancet I, 1143.

Beaumont, J.L., Carlson, L.A., Cooper, G.R., Fejfar, Z., Fredrickson, D.S. and Strasser, T. (1970) Bull. W. Hlth. Org. 43, 891.

Beveridge, J.M.R., Connell, W.F. and Mayer, G.A. (1956) Canad. J. Biochem. Physiol. 34, 441.

Beveridge, J.M.R., Connell, W.F., Mayer, G.A. and Haust, H.L. (1960) J. Nutrit. 71, 61.

Beveridge, J.M.R., Jagannathan, S.N. and Connell, W.F. (1964) Canad. J. Biochem. Physiol. 42, 999.

Bierenbaum, M.L., Fleischman, A.I., Raichelson, R.I. Hayton, T. and Watson, P.B. (1973) Lancet I, 1404.

Birchwood, B.L., Little, J.A., Antar, M.A., Lucas, C., Buckley, G.C., Csima, A. and Kallos, A. (1970) Atherosclerosis 11, 183.

Biss, K., Ho. K-J., Mikkelson, H., Lewis, L. and Taylor, C.B. (1971) New Engl. J. Med. 284, 694.

Bjöntorp, P. (1968) J. biol. Chem. 243, 2130.

Blaton, V., Havard, A.N., Gresham, G.A., Vandamme, D. and Peeters, H. (1970) Atherosclerosis 11, 497.

Bloomfield, D. (1964) J. Lab. clin. Med. 64, 613.

Borgström, B., Lundh, G. and Hoffman, A.F. (1963) Gastroenterology 45, 229.

Bronte-Stewart, B., Antonis, A., Eales, L. and Brock, J.F. (1956) Lancet I, 521.

Brown, H.B. (1970) In Atherosclerosis: Proceedings of Second International Symposium (ed. R.J. Jones) Springer-Verlag, New York, Berlin.

Byers, S.O. and Friedman, M. (1958) Proc. Soc. exp. Biol. (N.Y.) 98, 523.

Campbell, C.B., Cowley, D.J. and Dowling, R.H. (1972) Europ. J. clin. Invest. 2, 332.

Carlson, L.A. and Böttiger, L.E. (1972) Lancet I, 865.

Chait, A.,Onitiri, A., Nicoll, A., Rabaya, E., Davies, J. and Lewis, B. (1974) Atherosclerosis (In press)

Christakis, G., Rinzler, S.H., Archer, M., Winslow, G., Jampel, S., Stephenson, J., Friedman, G., Fein, H., Kraus, A. and James, G. (1966) Amer. J. publ. Hlth. 56, 299.

Connor, W.E., Hodges, R.E. and Bleiler, R.E. (1961) J. clin. Invest. 40, 894.

Connor, W.E., Stone, D.B. and Hodges, R.E. (1964) J. clin. Invest. 43, 1691.

Connor, W.E. (1968) J. Amer. Dietetic Assoc. 52, 202.

Connor, W.E., Witiak, D.T., Stone, D.B. and Armstrong, M.L. (1969) J. clin. Invest. 48, 1363.

Dayton, S., Pearce, M.L., Hashimoto, S., Dixon, W.T. and Tomiyasu, U. (1969) Circulation 40, suppl.no.2.

Dayton, S., Chapman, J.M., Pearce, M.L. and Popjak, G.T. (1970) Ann. Int. Med. 72, 97.

Doyle, J.T. (1963) New York J. Med. 63, 1317.

Dunningen, M.G., Fife, T., McKiddie, M.J. and Crosbie, S.M. (1970) Clin. Sci. 38, 1.

Eaton, R.P., Berman, M. and Steinberg, D. (1969) J. clin. Invest. 48, 1560.

Ederer, F. (1972) J. chron. Dis. 25, 277.

Eisenberg, S., Bilheimer, D.W. and Levy, R.I. (1972) Biochim. Biophys. Acta 280, 94.

Erickson, V.A., Coots, R.H., Mattson, F.H. and Kligman, A.M. (1964) J. clin. Invest. 43, 2017.

Farquhar, J.W. and Sokolow, M. (1958) Circulation 17, 890.

Fredrickson, D.S., Levy, R.I. and Lees, R.S. (1967) New Engl. J. Med. 276, 34, 94, 148, 215, 273.

Gibson, D.M. and Hubbard, D.D. (1960) Biochem. Biophys. Res. Commun. 3, 531.

Glomset, J.A. (1968) J. Lipid Res. 9, 155.

Glueck, C.J., Levy, R.I. and Fredrickson, D.S. (1969) Diabetes 18, 739.

Gordon, H., Lewis, B., Eales, L. and Brock, J.F. (1957) Lancet II, 1299.

Gordon, H. (1959) Postgrad. Med. J. 35, 186.

Gordon, T. and Verter, J.I. (1969) In The Framingham Study: An epidemiological Investigation of Cardiovascular disease. Section 23. Bethesda Md, Natl. Inst. Hlth.

Grande, F., Anderson, J.T. and Keys, A.C. (1970) Amer. J. clin. Nutrit. 23, 1184.

Grande, F., Anderson, J.T. and Keys, A. (1972) Amer. J. clin. Nutrit. 25, 53.

Groen, J., Tijong, B.K., Kaminga, C.E. and Willibrands, A.F. (1952) Voeding 13, 556.

Grundy, S.M. and Ahrens, E.H.,Jr. (1970) J. clin. Invest. 49, 1135.

Hall, Y., Stamler, J., Cohen, D.B., Mojonnie,L., Epstein,M.B., Berkson, D.M., Whipple, I.T. and Catchings, S. (1972) Atherosclerosis 16, 389.

Hallgren, B., Stenhagen, S., Svanloorg, A. and Svennerholm, L. (1960) J. clin. Invest. 39, 1424.

Hashim, S.A., Arteaga, A. and Van Itallie, T.B. (1960) Lancet I, 1105.

Hashimoto, S. and Dayton, S. (1966) J. Atheroscler. Res. 6, 580.

Havel, R.J., Felts, J.M. and Van Duyne, C.M. (1962) J. Lipid Res. 3, 297.

Havel, R.J., Carlson, L.A., Elkelund, L.G. and Holmgren, A. (1964) J. Appl. Physiol. 19, 613.

Havel, R.J. (1965) In Handbook of Physiology: Section V: Adipose Tissue (eds. A.E. Renold and G.F. Cahill) Amer. Physiol. Soc. Washington D.C. p.499.

Havel, R.J. (1970) In Atherosclerosis: Proceedings of Second International Symposium (ed. R.J. Jones) Springer-Verlag, New York, Berlin, p.210.

Hegsted, D.M., McGrandy, R.B., Myers, M.L. and Stare, F.J. (1965) Amer. J. clin. Nutrit. 17, 281.

Heimberg, M., Weinstein, I. and Kohout, M. (1969) J. biol. Chem. 244, 5131.

Hirsch, J. (1965) In Handbook of Physiology: Section V: Adipose tissue (eds A.E. Renold and G.F. Cahill) Springer-Verlag, New York, Berlin, Amer. Physiol. Soc. Washington D.C. p.181.

Ho, K.J., Mikhelsohn, B., Lewis, L.A., Feldman, S.A. and Taylor, C.B. (1972) Amer. J. clin. Nutrit. 25, 737.

Howard, A.N., Blaton, V., Vandemnic, D., Van Landschoot, N. and Peeters, H. (1972) Atherosclerosis 16, 257.

Kannel, W.B. and Gordon, T. (eds) The Framingham Study: An epidemiological Investigation of Cardiovascular disease. Section 24. Bethesda Md, Natl. Inst. Hlth.

Kannel, W.B. (1971) Amer. J. clin. Nutrit. 24, 1074.

Karvonen, M.J. (1972) Proc. Nutr. Soc. 31, 355.

Keys, A. (1957) J. chron. Dis. 6, 552.

Keys, A., Anderson, J.T. and Grande, F. (1957a) Lancet I, 787.

Keys, A., Anderson, J.T. and Grande, F. (1957b) Lancet II, 959.

Keys, A. and Fidanza, F. (1960) Circulation 22, 1091.

Keys, A., Taylor, H.L., Blackburn, H.,Jr., Brozek, J., Anderson, J.T. and Simonson, E. (1963) Circulation 28, 381.

Keys, A., Anderson, J.T. and Grande, F. (1965a) Metabolism 14, 776.

Keys, A., Anderson, J.T. and Grande, F. (1965b) Metabolism 14, 747.

Keys, A., Anderson, J.T. and Grande, F. (1965c) Metabolism 14, 759.

Keys, A. (1970) Circulation 41, suppl.1.

Keys, A. (1971) Coronary Heart Disease (eds. H.I. Russek and B.L. Zolman) JB Lippincott, Philadelphia, p 59.

Kingsbury, K.J., Morgan, D.M., Aylott, C. and Emmerson, R. (1969) Lancet I, 739.

Kinsell, L.W., Partridge, J., Boling, L., Margen, S. and Michaels, G.D. (1952) J. clin. Endocr. 12, 909.

Kinsell, L.W., Michaels, G.D., Walker, G., Wheeler, P., Splitter, S. and Flynn, P. (1959) Diabetes 8, 179.

Kinsell, L.W., Wood, P.D.S., Shioda, R., Schlierf, G. and Lee, Y.L. (1968) Progr. Biochem. Pharm. 4, 59.

Kohout, M., Kohoutova, B. and Heimberg, M. (1971) J. biol. Chem. 246, 5067.

Korchak, H.M. and Masoro, E.J. (1962) Biochim. Biophys. Acta 58, 354.

Korn, E.D. (1961) J. biol. Chem. 236, 1638.

Krebs, H.A. and Hems, R. (1970) Biochem. J. 119, 525.

Krogh, A. and Krogh, M. (1914) Meddelelser om Gronland 51 1.

Lack, L. and Weiner, I.M. (1967) Clin. Chem. 18, 534.

Leren, P. (1970) Circulation 42, 935.

Levy, R.I., Lees, R.S. and Fredrickson, D.S. (1964) J. clin. Invest. 45, 63.

Levy, R.I., Bilheimer, D.W. and Eisenberg, S. (1971) In plasma lipoproteins (ed. R.M.S. Smellie) Academic Press, London, New York.p.3.

Levy, R.I. (1972) Ann. Int. Med. 77, 267.

Lewis, B. (1958a) Lancet I, 1090.

Lewis, B. (1958b) Lancet II, 71.

Lewis, B., Pilkington, T.R.E. and Hodd, K.A. (1961) Clin. Sci. 20, 249.

Lewis, B., Chait, A., Wootton, I.D.P., Oakley, C.M., Krikler, D.M., Sigurdsson, G., February, A., Maurer, B. and Birkhead, J. (1974) Lancet I, 141.

Macdonald, I. and Braithwaite, D.M. (1964) Clin. Sci. 27, 23.

Macdonald, I. (1965) Clin. Sci. 29, 193.

Macdonald, I. (1967) Amer. J. clin. Nutrit. 16, 458.

Macdonald, I. (1972) Clin. Sci. 43, 265.

Malmros, H. and Wigard, G. (1957) Lancet II, 1.

Mancini, M., Mattock, M., Rabaya, E., Chait, A. and Lewis, B. (1973) Atherosclerosis 17, 445.

Mann, J.I. and Truswell, A.S. (1972) Br. J. Nutrit. 27, 395.

Mann, J.I., Watermeyer, G.S., Manning, E.B., Randler, J. and Truswell, A.S. (1973) Clin. Sci. 44, 601.

Masironi, R. (1970) Bull. Wld. Hlth. Org. 42, 103.

Mattson, F.H., Erickson, B.A. and Kligman, A.M. (1972) Amer. J. clin. Nutrit. 25, 589.

Miettinen, M., Turpeinen, O., Karvonen, M.J., Elosuo, R. and Paavilainen, E. (1972) Lancet II, 835.

Moore, R.B., Anderson, J.T., Keys, A. and Frantz, I.D.,Jr. (1962) J. Lab. clin. Med. 60, 1000.

Moore, R.B., Anderson, J.T., Taylor, H.L., Keys, A. and Frantz, I.D.,Jr. (1968) J. clin. Invest. 47, 1517.

Morrison, L.M. (1964) J. Amer. med. Assoc. 173, 884.

Myant, N.B. (1971) In Plasma Lipoproteins (ed. R.M.S. Smellie) Academic Press, London, New York, p.99.

Myant, N.B. (1973) J. clin. Path. 26, suppl. 5,1.

McGovern, R.F. and Quackenbush, F.W. (1973) Lipids 8, 470.

McOsker, D.E., Mattson, F.H., Sweringen, B. and Kligman, A.M. (1962) J. Amer. med. Assoc. 180, 380.

National Diet-Heart Study Research Group (1968) Circulation 37, suppl.no.1.

Nestel, P.J. and Steinberg, D. (1963) J. Lipid Res. 4, 461.

Nestel, P.J., Carrol, K.F. and Havenstein, N. (1970) Metabolism 19, 1.

Nestel, P.J. and Barter, P. (1971) Clin. Sci. 40, 345.

Newman, H.A.I. and Zilversmit, D.B. (1966) Circulat. Res. 18, 293.

Nichaman, M.Z., Sweeley, C.C. and Olson, R.E. (1967) Amer. J. clin. Nutrit. 20, 1057.

Nilsson, I.M., Blomback, M. and Von Franken, I. (1957) Acta med. scand. 159, 35.

Numa, S., Matsuhashi, M. and Lynen, F. (1961) Biochem Zeitschr. 334, 203.

Pawar, S.S. and Tidwell, H.C. (1968) J. Lipid Res. 9, 334.

Persson, B. (1972) M.D. Thesis. Univ. Göteburg, Sweden.

Pfeiffer, J.J. (1967) In Fish Oils (ed. M.E. Stansby) Westport Connecticutt. p.322.

Press, M., Hartop, P.J. and Prottery, C. (1974) Lancet I, 597.

Reiser, P.J. (1966) Amer. Oil Chem.Soc. 43, 112A.

Research Committee to the Medical Research Council (1968) Lancet II, 693.

Robinson, D.S. (1973) J. clin. Path. 26, suppl. 5, 5.

Rose, H., Vaughan, M. and Steinberg, D. (1964) Amer. J. Physiol. 206, 345.

Salaman, M.R. and Robinson, D.S. (1966) Biochem. J. 99, 640.

Schrade, W., Biegler, R. and Böhle, E. (1961) J. Atheroscler. Res. 1, 47.

Shaper, A.G. (1972) Brit. med. J. IV, 32.

Siperstein, M.D. and Guest, M.J. (1960) J. clin. Invest. 39, 462.

Smith, E.B. and Slater, R.S. (1972) Atherosclerosis 11, 417.

Sodhi, H.S., Wood, P.D.S. and Kinsell, L.W. (1964) Clin. Res. 12, 108.

Sodhi, H.S., Wood, P.D.S., Schlierf, G. and Kinsell, L.W. (1967) Metabolism 16, 334.

Spritz, N., Grundy, S. and Ahren, E.H.,Jr. (1963) J. clin. Invest. 42, 981.

Spritz, N,,Ahrens, E.H.,Jr. and Grundy, S. (1965) J. clin. Invest. 44, 1482.

Spritz, N. and Mishkel, M.A. (1969) J. clin. Invest. 48, 78.

Stamler, J. (1960) Amer. J. publ. Hlth. 3, 14.

Stamler, J., Stamler, R. and Shekelle, R.B. (1970) In Ischaemic Heart Disease (eds. J.H. de Haas, H.C. Hemker, H.A. Sullen) Leiden University Press.

Stamler, J. (1971) Brit. Heart J. 33, suppl. p.145.

Szanto, S. and Yudkin, J. (1969) Postgrad. med. J. 45, 602.

Tabaqchali, S., Chait, A., Harrison, R. and Lewis, B. (1974) Brit. med. J. (In press)

Topping, D.L. and Mayes, P.A. (1972) Biochem. J. 126, 295.

Uzawa, H., Schlierf, G., Chirman, S., Michaels, G., Wood, P.D.S. and Kinsell, L.W. (1964) Amer. J. clin. Nutrit. 15, 365.

Van Harken, D.K., Dixon, C.W. and Heimberg, M. (1969) J. biol. Chem. 244, 2278.

Vergroesen, A.J. (1972) Proc. Nutr. Soc. 31, 323.

Walton, K.W. and Williamson, N. (1968) J. Atheroscler.Res. 8, 599.

Whyte, H.M., Nestel, P.J. and Pryke, E.S. (1973) J. Lab. clin. Med. 81, 818.

Wilkens, J.A. (1958) S. Afr. med. J. 32, 85.

Wilson, W.S., Hulley, S.B., Burrows, M.I. and Nichaman, M.Z. (1971) Amer. J. med. 51, 491.

Windmueller, H.G. and Spaeth, A.E. (1967) Arch. Biochem. Biophys. 122, 362.

Wood, P.D.S., Shimoda, R. and Kinsell, L.W. (1966) Lancet II, 604.

Wood, P.D.S. and Kinsell, L.W. (1967) Prog. biochem. Pharmacol. 2, 80.

Woolf, N. and Pilkington, T.R.E. (1965) J. Path. Bact. 90, 459.

Zilversmit, D.B. (1973) Circ. Res. 33, 633.

CHAPTER VI

DIETARY FAT TRANSPORT

CHAPTER VI, SECTION A

Current classifications of hyperlipidaemia are based on serum levels of the major lipoprotein classes; a sound classification of lipoproteins themselves is thus a prerequisite for the classification of hyperlipoproteinaemic patients. For this approach to be entirely valid, it would be necessary that the lipoprotein classes be chemically, or at least metabolically homogenous; this, however, is clearly not the case (Alaupovic, 1971), and the concept of a dynamic system of interconvertible lipoproteins is gaining wider acceptance (Lewis, 1973).

Classification of lipoproteins depend largely on operational definitions; physical properties such as charge, density and size, permit of classification into the four main classes of lipoproteins - high density lipoprotein (HDL), low density lipoproteins (LDL), very low density lipoprotein (VLDL) and chylomicrons. Such an approach may well lead to artificial division in that more sophisticated separation techniques permit of further sub-division into many more classes. Also, within the four classes mentioned, considerable heterogeneity exists both in terms of structure and function. The heterogeneity is perhaps most marked in the triglyceride bearing lipoproteins - VLDL and chylomicrons (Bierman et al, 1962; Zilversmit et al, 1966; Ockner and Jones, 1970).

The weak bonds linking the lipid and protein components of the lipoprotein molecule (Schumaker and Adams, 1969) result in a certain degree of lability of composition. Changes in the composition of LDL were described by Spritz and Mishkel (1969) as a result of dietary modification. Similarly in Chapter V, I have described diet induced changes in the lipid components of VLDL. Compositional changes in VLDL have also been shown to occur in response to carbohydrate feeding (Mancini et al, 1973) further demonstrating the extreme lability of this class of lipoprotein. High carbohydrate diets led to the appearance in plasma of lipoprotein particles with physicochemical properties more akin to those of chylomicrons. In abnormal circumstances, therefore, operational definitions are prone to certain anomalies.

Consideration needs also to be given to functional aspects of the lipoprotein classes. The demonstration that the protein moeity of LDL is derived largely as a catabolic product of VLDL (Eisenberg et al, 1972), suggests a precursor-product interrelationship between these two classes of lipoproteins.

The functional aspect that has been investigated in the present study, relates to the role of the chief triglyceride-bearing lipoproteins in the transport of dietary fat. It has long been assumed that dietary or exogenous fat enters plasma as chylomicrons via the thoracic duct; by contrast, the transport of plasma triglycerides synthesized endogenously by the liver is usually attributed to VLDL (Fredrickson et al, 1967). In the light of recent knowledge, this view point may require some revision. Ingestion of fat is followed by the appearance of both chylomicrons and VLDL in rat intestinal lymph (Ockner et al, 1969b), and the intestine can secrete VLDL even in the absence of dietary fat (Ockner et al, 1969a). The present series of experiments were designed to study the relative contributions of chylomicrons and VLDL to alimentary lipaemia in man. The results show a significant contribution of both lipoprotein classes to dietary fat transport. Future concepts of 'endogenous' triglycerides should, therefore, include an intestinal component, and 'exogenous' triglycerides should best be regarded as triglycerides of dietary origin, irrespective of the nature of the transporting lipoprotein.

I will begin briefly reviewing the structure and function of these two lipoprotein classes.

CURRENT CONCEPTS OF ENDOGENOUS AND EXOGENOUS LIPOPROTEINS

Triglycerides are transported through plasma mainly as chylomicrons and VLDL. Which of the two forms predominates, depends largely on whether the immediate source of the triglyceride is dietary or that synthesized within the body; these two forms are referred to as exogenous and endogenous respectively. Chylomicrons are frequently equated with exogenous particles, their appearance in plasma following the ingestion of a fat containing meal (Gage and Fish, 1924; Dole and Hamlin, 1962; Senior, 1964; Fredrickson et al, 1967). As they are not present to any significant extent in the normal post-absorptive state, they have been deemed to have a role limited to the absorption of dietary fat. VLDL on the other hand, is believed to transport endogenously synthesized triglyceride (Fredrickson et al, 1967), largely following studies on eviscerated animals which show the liver to be the major site of VLDL formation, although a small amount is also formed by the intestine (Roheim et al, 1966).

Nomenclature in the lipoprotein field is based mainly on operational terms. The two most frequently used methods of lipoprotein separation are electrophoresis and preparative ultracentrifugation; the former separates lipoproteins according to flotation characteristics, which depend on size and density, and the latter according to charge. Chylomicrons are, however, the most difficult lipoprotein species to isolate free of contamination by other proteins and lipoproteins.

Electrophoresis may be used to separate VLDL from chylomicrons. 'Primary particles' which appear to be identical with chylomicron particles in lymph, migrate in the alpha-2 globulin region in starch block electrophoresis. 'Secondary' particles appear in plasma later than do 'primary' particles following fat ingestion, and migrate in the beta globulin position. In this system, primary particles tend to be superimposed on endogenous particles (Fredrickson et al, 1967). With the simpler techniques of paper electrophoresis, especially when albumin is added to the buffer, 'chylomicrons' tend to remain at the origin, whereas endogenous triglyceride bearing lipoproteins move to the alpha-2 (the so-called pre-beta) position. Larger endogenous particles may trail between the pre-beta position and the origin (Fredrickson et al, 1967). 'Primary' particles are those which originate in the intestinal mucosal cell, appear in intestinal lymph, and have a fatty acid composition very similar to dietary fat (Bierman et al, 1962). 'Secondary' particles which are similar in particle size, distribution and lipid content (Bierman and Strandness, 1966), contain a mixture of fatty acids of both dietary and endogenous origin (Kayden et al, 1963) and are thought to derive largely from the 'primary' particle (Bierman et al, 1962). Preparative ultracentrifugation may be used to separate chylomicra from VLDL. Contrifugation for 0.66 x 10^6 g-min. against a background density of 1.006 produces flotation of 'chylomicrons', VLDL remaining in the infranatant. Centrifugation for a further 16 hours at 105,000 g at a background density of 1.006 yield the 'endogenous' or VLDL fraction(Havel et al, 1955; Hatch and Lees, 1968); these correspond respectively to $S_f > 400$ and S_f 20 - 400 classes as isolated by analytical ultracentrifugation. Contamination of the exogenous fraction by endogenous particles, however, occurs. As there is an overlap between chylomicra and VLDL in respect of particle size, density and flotation rate, the dividing line between these two classes is necessarily arbitrary. Endogenous VLDL may include particles with a diameter of up to 1,000 Å, which have an S_f value of about 800 (Ruderman et al, 1968); such lipoproteins would tend to separate as chylomicrons during preparative ultracentrifugation. More recently, a technique has been described whereby chylomicrons have been isolated uncontaminated in a single centrifugation step, using a stabilizing density gradient and a swing-out bucket rotor (Lossow et al, 1969).

Probably the best method of separating exogenous from endogenous particles relies on the use of polyvinylpyrollidone (PVP) gradients, which depend on a combination of gradient flotation and precipitation of lipoproteins by PVP. Gordis (1962) showed that using this technique, exogenous particles (chylomicrons) could be separated into 'primary' and 'secondary' particles. By comparison with fractions obtained by preparative ultracentrifugation, Boberg and Hallberg (1968) showed the top fraction to contain 'chylomicrons', the middle fraction to contain part of the VLDL class, and the bottom fraction to contain the rest of the VLDL together with LDL and HDL.

The terms derived from these methods of isolation tend to be used interchangeably. Pre-beta lipoprotein as separated by electrophoresis, has been shown to be identical to the VLDL fraction separated ultracentrifugally (Smith, 1957; Brown and Doyle, 1967). Similarly, Lees and Fredrickson (1965) showed the immobile chylomicron band on paper electrophoresis to be equatable with ultracentrifugally separated chylomicrons. A classification based on particle size, using microfiltration and nephelometry has also been demonstrated to show a high order correlation with results obtained by analytical ultracentrifugation (Stone et al, 1970). Most classification systems in current use tend to rely on plasma lipoprotein characterization based on separation by either preparative ultracentrifugation or electrophoresis. Membrane filtration and nephelometry has not gained general acceptance.

While these methods provide relatively clear cut distinctions between chylomicra and VLDL in physico-chemical terms, the correlation between structure and function has nevertheless to be considered. Authoritative definitions of chylomicrons include both a functional and an operational term (Fredrickson et al, 1967; Zilversmit, 1969). Zilversmit (1969) for example, is careful to refer to particles with both 'S_f greater than 400 and containing triglyceride derived primarily from dietary fat'. This definition is unexceptional; it, however, excludes particles of $S_f < 400$ which may contain dietary fat. Ockner et al (1969b) showed that dietary fat could be transported in intestinal lymph as VLDL in addition to chylomicrons; and a similar situation appears to pertain to man (Harlan and Bleisher, 1963; Edelin et al, 1968; Schlierf and Raetzer, 1972). Furthermore, under unphysiological conditions, endogenous particles with physico-chemical properties of chylomicrons may be present in plasma (Mancini et al, 1973). The PVP gradient column, the only method which appears to adequately separate endogenous from exogenous triglycerides, has the disadvantage of being cumbersome and very time-consuming; its widespread use is, therefore, impractical.

CHYLOMICRONS

Definition

From the foregoing, it can be seen that difficulties in definition might be anticipated. The term, chylomicron, was originally used to describe the visible particles that appeared in lymph and blood in response to fat feeding and which contained the dietary triglyceride (Gage and Fish, 1924); they were named chylomicrons because they appeared as 'microscopic bodies from chyle'. Quantification was initially performed by counting the number of particles in plasma visible under the microscope at varying intervals after a fatty meal (Frazer and Stewart, 1937).

The most widely accepted classification of lipoprotein disorders in current use is the WHO modification of the classification of Fredrickson et al, 1967 (Beaumont et al, 1970). For the purpose of classifying disorders of lipoprotein metabolism, they define 'chylomicrons' as particles with a 'density of about 0.9 (S_f value > 400). They collect at the top of plasma left standing for 16 - 24 hours at 4°C, they remain at the origin on paper or agarose electrophoresis on in the loading gel on polyacrilamide gel, and they behave in a certain way in PVP gradients, or on starch gel electrophoresis.' Whereas it is stated that most 'chylomicrons' are considered to be of exogenous origins, these authors are careful to point out that some dietary triglycerides also appears in particles having physico-chemical properties of VLDL. Attention is also drawn to observations that some VLDL have the immobility of chylomicrons on electrophoresis.

Size and composition

'Chylomicrons' appear as spherical particles that vary in size from 750 - 10,000 Å on light and electron microscopy

(Levy et al, 1971). A spectrum of particles varying widely in size has been shown to exist (Yokoyama and Zilversmit, 1965; Zilversmit et al, 1966); at the lower end of the size spectrum, they tend to merge with the larger molecules of VLDL. Although in the dog and rat, the median particle diameter is between 0.2 - 0.3 μ , few particles with a diameter greater than 0.5 μ were observed during feeding cream or corn oil (Pinter and Zilversmit, 1962). They consist mainly of triglyceride (80 - 95%) with lesser amounts of cholesterol and phospholipid, less than half the former being in the esterified form; chemical composition has also been shown to be variable (Yokoyama and Zilversmit, 1965). The larger the chylomicron, the greater its relative content of glyceride and smaller its relative content of phospholipid, cholesterol and protein. Protein constitutes between 0.5 and 2.5% of total weight; apoprotein content and composition differ between chylomicrons of lymph and blood (Robinson, 1955; Scanu and Page, 1959; Dole and Hamlin, 1962). Additional protein appears to be added to lymph chylomicra upon entering the blood stream (Lossow et al, 1967; Robinson, 1970) and electrophoretic mobility on starch gel changes (O'Hara et al, 1966). Changes occur in the lipid composition as well; a gradual loss of phospholipid is accompanied by a gain in free cholesterol during incubation of thoracic duct lymph chylomicrons with plasma in vitro (Zilversmit, 1967).

VERY LOW DENSITY LIPOPROTEIN (PRE-BETA LIPOPROTEIN)

Definition

Beaumont et al (1970) define VLDL as lipoproteins which are isolated in the ultracentrifuge in the fraction of density < 1.006, having S_f values of 20 - 400 and which have pre-beta mobility on paper electrophoresis. This definition fails to take into account the sinking pre-beta lipoprotein (Berg, 1963; Rider et al, 1970); this lipoprotein despite having pre-beta mobility on electrophoresis, sediments rather than floats at $d = 1.006$ whereas 'normal' VLDL would do the opposite. This definition would also include VLDL of dietary origin and for this reason should not be assumed to be equatable with endogenous triglyceride particles.

Size and composition

More than 85% of VLDL consists of lipid; protein providing 7 - 12% of total weight. The major lipid class is triglyceride which constitutes 50 - 70% of the molecule by weight (Levy et al, 1966). Considerable heterogeneity, however, exists, density covering a wide spectrum from 1.006 to 0.95; as the density decreases, the relative proportion of glyceride rises and that of protein falls (Fredrickson et al, 1967). At the lower end of their density range, they overlap with chylomicrons; at their highest end, they merge with LDL.

The need to differentiate exogenous from endogenous lipoproteins arises when considering the hyperlipoproteinaemias and their therapy. The work of Ahrens et al (1961) is a landmark in our understanding of hypertriglyceridaemia. They demonstrated two apparently distinct forms of hypertriglyceridaemia. One was sensitive to dietary fat, extremely high triglyceride levels being present in plasma during the consumption of a 40% corn oil diet. The serum triglyceride level fell slowly and fasting serum lost its turbid appearance on isocaloric substitution of carbohydrate for fat. An excess of chylomicrons were present in an ultracentrifugal density of $d < 1.006$ lipoprotein fraction even in the post-absorptive state. Their fatty acid composition was shown to be similar to that of the dietary fat. The defect was believed to be due to inefficient clearing of chylomicrons from plasma, as post-heparin lipolytic activity in plasma was low or absent, and a single fat meal cleared slowly. This so-called 'fat-induced lipaemia', is to-day classified as Type I in the WHO classification (Beaumont et al, 1970).

In the second type of lipaemia, termed 'carbohydrate-induced', serum triglycerides were lowest on high fat feeding and highest during consumption of a high carbohydrate diet. Post-heparin lipolytic activity was normal, and the fatty acid composition of the $d < 1.006$ fraction showed little similarity to either adipose tissue or dietary fat. Particle size in this ultracentrifugal fraction was shown to be smaller than chylomicrons. On the basis of the fatty acid composition of this fraction, Ahrens et al (1961) suggested the lipoprotein particles in the latter case to arise largely by endogenous synthesis of triglyceride. 'Carbohydrate-induced' or endogenous hypertriglyceridaemia is frequently referred to as Type IV hyperlipoproteinaemia (Beaumont et al, 1970).

Whereas exogenous hypertriglyceridaemia responds to dietary fat restriction, the dietary treatment of choice for endogenous hypertriglyceridaemia has been claimed to be carbohydrate restriction (Levy, 1972; La Rosa, 1972). As classification into these supposedly widely differing forms of hypertriglyceridaemia depends on a knowledge of the lipoprotein pattern of serum, a better understanding of the chief triglyceride-bearing lipoproteins, not only in terms of structure but also of function, would be desirable for the provision of optimal therapy. Clearly, there is also the need for a better understanding of the interrelationships between the various classes of lipoproteins, as well as of specific metabolic abnormalities underlying the various forms of hyperlipoproteinaemia.

FORMATION OF VLDL BY THE INTESTINE

Although the role of the intestine in chylomicron formation has been long known, it has only been more recently appreciated that this organ is also capable of producing VLDL. As this has been shown to occur in the post-absorptive period (Ockner et al, 1969a), concepts of endogenous triglycerides ought to include the intestinal as well as a hepatic component. VLDL also appears to play a role in the absorption of dietary fat (Ockner et al, 1969b; Schlierf and Raetzer, 1972), contrary to the early belief that exogenous triglyceride was transported wholly in the form of chylomicrons.

Post-absorptive state

The absence of chylomicrons from plasma in the fasted state led to the erroneous belief that the role of the intestine in lipid metabolism was limited to the absorption of fat; recent evidence, however, favours a wider role.

The intestine has, for example, been shown to be an important site of cholesterol biosynthesis (Dietschy and Siperstein, 1965; Wilson, 1968). Hepatectomized animals are capable of incorporating radioactive fatty acids into plasma triglycerides (Havel and Goldfein, 1961) and labelled amino-acids into the apoproteins of circulating VLDL (Roheim et al, 1966); the intestine would seem to be the likely site of these reactions. Ockner et al (1969a) have shown that even under fasting conditions, the intestinal lymph of rats contains triglyceride-rich particles similar to plasma VLDL. This fraction which has an S_f peak of 102 on the analytical ultracentrifuge, thus exhibited slower flotation that do chylomicrons; a different lipid composition and electrophoretic mobility was also demonstrated. This fraction also differed from secondary particles (Bierman et al, 1966) in that the latter particle, derived from the primary chylomicron, also had a flotation coefficient $>$ 400. Ockner et al (1969a) have shown that rat VLDL moves in the alpha-1 position on agarose gel electrophoresis. The 'VLDL' of intestinal lymph showed alpha-2 mobility, but when incubated with plasma VLDL before electrophoresis, moved to the alpha-1 position. They were similar to plasma VLDL in respect of lipid composition, flotation coefficient, and in the composition of their major apoproteins (Ockner et al, 1968), although total protein content was less than that of plasma VLDL. Fatty acid composition did, however, differ somewhat suggesting a different supply of precursor fatty acids. Confirmation of the presence of particles in intestinal lymph which closely resemble plasma VLDL has been obtained by electron microscopy (Ockner and Jones, 1970).

Some particles with characteristics of chylomicrons were demonstrable in intestinal lymph even in the fasted state; 'intestinal VLDL' was the major lipoprotein species present. VLDL of intestinal origin thus probably contributes to the plasma VLDL pool, although the extent of such a contribution is not known at the present time. Windmueller and Levy (1968) have estimated the intestinal contribution to be about 10% in rats, whereas Ockner et al (1969a) suggest that as much as 40% of plasma VLDL may derive from the intestine.

Post-prandial state

The role of 'intestinal VLDL' during fat absorption has also recently received attention. Ockner et al (1969b) showed that exogenous fat could be transported as VLDL as well as chylomicrons in intestinal lymph. They distinguished chylomicrons from VLDL by flotation in the analytical ultracentrifuge, agarose gel electrophoresis, and by lipid content. Boquillon et al (1972) arrived at similar conclusions using cellulose acetate electrophoresis and density gradient zonal centrifugation to separate the two particles. Following a radioactive fatty meal, rat intestinal lymph was found to contain two bands on cellulose acetate electrophoresis. Most of the radioactivity was found in the fast-moving band. Further separation by zonal centrifugation showed the moving particles to have a higher cholesterol and protein content than particles remaining at the origin; electron microscopy also showed the latter particles to be larger. These results are thus consistent with the view that dietary fat is transported in intestinal lymph both as chylomicrons and VLDL; in these experiments, the majority of the labelled lipids were carried by the smaller particles. Somewhat conflicting results have been obtained by Windmueller et al (1970); although demonstrating a continuous spectrum of lipoprotein particle size in rat lymph in both fasted and fed state, they showed most of the change on feeding fat, to be due to particles of $S_f > 3,200$.

Studies in man

Most studies on the roles of chylomicrons and VLDL during fat absorption in man have relied on measurement of these two particles in plasma during alimentary lipaemia. It appears from early studies on normal men that $S_f > 400$, pre-beta migrating particles of $< 1\mu$ diameter may contribute to dietary fat transport through plasma (Edelin et al, 1968; Stone et al, 1970). The former authors showed that in normal subjects and in those with Types IV and V hyperlipoproteinaemia (Beaumont et al, 1970), dietary fats were incorporated significantly into both 'endogenous' and 'exogenous' particles separated by paper electrophoresis of the $d < 1.006$ fraction of serum. Dietary fatty acids appeared in the chylomicron fraction earlier than they did in the 'VLDL' fraction; recycling of dietary fatty acids and their incorporation into endogenous triglycerides is thus a possibility. Schlierf and Raetzer (1972) also showed the $S_f < 400$ fraction of serum to carry a considerable portion of the alimentary lipaemia. Following a single large fat load of 120 g., Harlan and Beischer (1963) showed a considerable increase in S_f 20 - 100 and S_f 100 - 400 lipoproteins, whereas LDL (S_f 0 - 20) remained unchanged. In this latter study, lipoproteins of $S_f > 400$ were not separated from the S_f 100 - 400 fraction; 'chylomicrons' would thus be included in the latter fraction. At the high dose of fat fed the S_f 20 - 100 fraction nevertheless showed a significant increase. Fröberg and Hallberg (1968) used the PVP gradient column of Gordis (1962) to study alimentary lipaemia; they demonstrated a rise in the top fraction ('chylomicrons') and in the middle fraction ('some VLDL'), but not in lower fraction (VLDL, LDL and HDL) after the ingestion of fat. As this method is more specific in separating endogenous from exogenous particles, it suggests that some of the VLDL increment must have been of exogenous origin. Furthermore, Bergström et al (1972) have reported in abstract, that VLDL increases in response to fat feeding in human thoracic duct lymph. Additional studies on lymph lipoprotein are thus desirable, but in view of the scarcity of suitable experimental subjects, have their obvious difficulties and drawbacks.

Further confirmation of the role of the intestine in VLDL synthesis in man has been obtained by preparative ultracentrifugation of jejunal biopsies. Lipid particles with features of VLDL and of 'chylomicrons' of various sizes have been shown to be present (Tytgate et al, 1971).

Dietary fat composition

The type of fat ingested may also be of importance in determining the nature of the lipoprotein particle produced by the intestinal mucosa. Ockner et al (1969b) have shown that intraduodenal infusion of linoleic acid, a polyunsaturated fatty acid, led to the appearance of mainly 'chylomicrons' in intestinal lymph. Palmitic acid on the other hand led to an increase in both chylomicron and VLDL triglyceride. Electron microscopy showed lymph lipoprotein particle size to be considerably smaller after administration of palmitate than after linoleate (Ockner and Jones, 1970), confirming data obtained ultracentrifugally. Oleic acid occupied an intermediate position, but in this respect more closely resembled linoleic acid. A similar situation may pertain in the rabbit, where a greater percentage of thoracic duct cholesterol was transported as chylomicrons when corn oil was fed rather than a diet rich in palmitic acid (Zilversmit et al, 1967). Linoleate has been shown to be absorbed more proximally and over a shorter distance of intestine than palmitatic acid (Ockner et al, 1972).

This observation could be due to the lower solubility of palmitate in micellar solution (Hoffman and Borgström, 1962), but as fatty acids may be absorbed from emulsions as well as from micellar solutions (Simmonds et al, 1968), this possibility seems unlikely. Uptake of the two fatty acids by intestinal mucosa appears not to differ, but the rate of esterification of linoleate appears to significantly exceed that of palmitate (Ockner et al, 1972), especially at higher concentrations of the free fatty acid. The more proximal absorption of linoleate over a smaller length of intestine, coupled with rapid esterification, could result in the formation of a larger quantity of triglyceride per cell than during palmitate absorption. During absorption of unsaturated fat, lipid availability in relation to apoprotein, could be greater than during absorption of saturated fat. Such a hypothesis could explain the differences in particle size in intestinal lymph during absorption of these two fatty acids.

Other studies have, however, failed to detect differences in particle size after feeding saturated or unsaturated fat. Zilversmit et al (1966) could show no significant difference in the size of particles in thoracic acid lymph after feeding with cream or corn oil. In this study in which particle size was estimated by phase contrast microscopy, the median size was 0.5μ. The same group of workers (Zilversmit et al, 1967), however, showed more cholesterol to be transported in chylomicrons after unsaturated than saturated fat feeding. Boquillon et al (1972) failed to show a difference in distribution of 'chylomicrons' and 'VLDL' dependent on the nature of the fatty acids fed. Still other studies have shown saturated fats to result in larger particles than unsaturated fat. Courel and Clement (1964) reported 'cream chylomicrons' to be three times as large as 'corn oil chylomicrons'; their technique of lymph collection has been criticized by Zilversmit et al (1966) who suggest preferential disruption of the 'corn oil chylomicrons' as an explanation of their findings. Another reported study, however, supports the finding of larger 'cream chylomicrons' (Jones et al, 1962), and in one study of dietary fat transport in thoracic duct lymph in man, palmitic and oleic acids were carried in the VLDL fraction to a lesser extent than was erucic acid, a more unsaturated fatty acid (Bergström et al, 1972).

Fat load

Another factor which may be of importance in determining the type of lipoprotein formed, is the load of fat presented to the intestinal cell. Ockner et al (1969b) have shown that the more proximal absorption of linoleate, leads to a greater formation of triglyceride per cell than palmitate. They suggest that the greater availability of triglyceride relative to protein at the sites of lipoprotein synthesis in the enterocyte, leads to the formation of larger particles than would result with less triglyceride formed per cell. Certain evidence tends to support this hypothesis; Fraser et al (1968) showed the size of rabbit lymph chylomicrons to be smaller in animals fed low corn oil diets than in those fed high doses of fat. In rabbits fed 5% corn oil diets, the mean diameter of chylomicrons was 960 Å compared with 1,435 Å in rabbits fed a 30% corn oil diet.

Chylomicron size also varied at different phases of fat absorption. Early on, mean chylomicron size was smaller than during peak absorption, at which time it would seem likely that the triglyceride load per cell would be at a maximum.

Whereas it seems beyond a doubt that in both animals and man, 'chylomicrons' and 'VLDL', as defined operationally, play a role in the transport of dietary fat, we may as yet be adopting too rigid a view of the triglyceride-bearing lipoproteins. There appears to be considerable overlap between these two apparently distinct classes when viewed under the electron-microscope and in terms of flotation characteristics (Ockner and Jones, 1970); a continuous spectrum of triglyceride bearing lipoproteins may thus exist. Zilversmit et al (1966) showed a continuous range of particle size from 300 - 4,500 Å in lymph from fat fed rats. Lymph obtained from rats on fat free diets has also been shown to contain a continuous spectrum of lipoproteins between S_f 20 and S_f 3,200, most being in the S_f 60 - 1,100 range (Windmueller et al, 1970). Fat feeding resulted in a predominance of particles $S_f > 3,200$. There was also a substantial increase in S_f 400 - 3,200 particles, whereas those of S_f 20 - 400 remained relatively unchanged. A spectrum ranging from large triglyceride-laden particles with characteristics of 'chylomicrons' on the one hand, to smaller 'VLDL-like' particles, less rich in triglyceride on the other, may exist. Electron microscopic studies of human intestinal biopsies have also shown the presence of small 'VLDL-like' particles during the post-absorptive state. Following the ingestion of fat, a range of particle sizes from the small particles present in the fasted state to large chylomicrons was observed (Tytgat et al, 1971). These studies, as well as that of Windmueller et al (1970), which showed a continuous spectrum of triglyceride bearing lipoproteins which shifted in the direction of larger molecules on fat feeding, are consistent with the possibility of a dynamic system of triglyceride-bearing lipoproteins capable of being formed by the intestinal cell in response to a requirement. A 'chylomicron' may thus originate as a smaller lipoprotein particle whose size has become expanded in order to accommodate additional triglyceride available as the result of fat absorption.

ALIMENTARY LIPAEMIA

Chylomicronaemia has not been regarded as being of particular importance in the pathogenesis of IHD, largely because of lack of evidence of an increased incidence of atherosclerosis in patients with Type I hyperlipoproteinaemia (Fredrickson, 1966; Fredrickson et al, 1967); additional information on this rare disorder may, however, alter this belief. By contrast, patients with pre-beta hyperlipoproteinaemia have been shown to be at risk of developing IHD (Carlson and Böttiger, 1972). If pre-beta lipoprotein (VLDL) is in some way directly related to the development of atherosclerosis, the recent realization that dietary fat may also be transported through plasma in this form, assumes considerable importance in relation to the pathogenesis of, and protection from IHD.

Most studies of alimentary lipaemia have relied upon the measurement of total triglyceride levels or the increase in optical density in plasma following ingestion of a fatty meal, and have not attempted to distinguish between 'exogenous' and 'endogenous' components. In addition, few studies have measured triglyceride levels throughout the day; this may be of greater relevance to the development of atherosclerosis than fasting levels which only exist for a few hours in the 24-hour period. Indeed, alimentary lipaemia following the ingestion of a single fat containing meal is of a greater degree and longer lasting in patients with IHD than in age and sex matched normal controls (Barritt, 1956; Felch and Van Italie, 1960; Bouchier and Bronte-Stewart, 1961; Denborough, 1963).

The magnitude of alimentary lipaemia relates largely to the dose of fat fed. A measurable increment in serum triglyceride concentrations may be detected after a meal containing less than 10 g. of fat (Zweers et al, 1968). In this study, a 14 g. fat breakfast produced a statistically significant rise, compared with the initial level. Most studies of alimentary lipaemia have, however, used much larger doses of fat, 60 - 100 g. usually being employed. As this load is greater than that ingested during most normal meals, results obtained may not reflect the physiological situation.

Following the ingestion of a high fat meal, peak levels of triglyceride are apparent after 3 - 6 hours (Havel, 1957a; Sullivan, 1962; Denborough, 1963). The level reached appears to correlate with the non-fasting value; the higher the initial serum triglyceride concentration, the greater the degree of alimentary lipaemia (Denborough et al, 1963; Hollister et al, 1963; Zweers et al, 1968). The finding of a greater degree of alimentary lipaemia in subjects with IHD than in controls could thus possibly reflect the higher fasting triglyceride levels known to occur in IHD (Carlson, 1960; Hazzard et al, 1973). The data of Denborough (1963) support this view; fasting serum triglyceride levels correlate well with optical density and triglyceride concentration following a fatty meal. In addition, estimations repeated in the same subjects following lowering of their base-line serum triglyceride concentrations by diet or 'Atromid', were associated with a reduction in alimentary lipaemia. During the ingestion of a fat containing diet, serum triglyceride levels throughout the day also appear to correlate with fasting triglyceride levels; Maruhama et al (1967) showed fasting triglyceride concentration to be a good predictor of diurnal triglyceride fluctuation. Diabetic control, for instance, results in a lowering of both fasting and diurnal triglyceride levels (Sterky et al, 1966; Maruhama et al, 1967).

The positive correlation between the fasting and postprandial levels may be of importance from two points of view. Firstly, despite fasting levels existing for only a few hours of the day, it suggests that measurements of postprandial or diurnal triglyceride concentrations, may offer no particular advantage over fasting levels in individual patients. This possibility has been reassessed in the present study. From a point of view of standardization for purposes of comparison, fasting levels would seem far preferable. With non-fasting specimens, the dose and timing of fat ingestion, as well as other constituents of the meal, would need to be considered in assessing the individual case. Secondly, because of the difficulty in obtaining blood for triglyceride estimations under standardized fasting conditions in epidemiological studies, or in surveys for screening purposes, it suggests that non-fasting samples might suffice. Zweers et al (1968) have shown a non-fasting cut-off point of 224 mg./dl. to correlate well with a fasting cut-off level of 168 mg./dl. Subjects with non-fasting levels of 224 - 280 mg./dl. were regarded as 'borderline', and above 280 mg./dl., as suspect and definitely hypertriglyceridaemic. This study was performed on healthy volunteers; whereas such an approach may prove of practical value for screening, in as much as all individuals with non-fasting levels above a predetermined cut-off point could be called back for examination under fasting conditions, its routine application in epidemiological studies may lead to erroneous conclusions. A normolipaemic subject at the time of peak lipaemia after a large fat meal, may for instance have a higher serum triglyceride concentration than a patient with mild elevation of triglyceride levels, who thereby may be at risk of developing IHD (Carlson and Böttiger, 1972).

CLASSIFICATION OF DISORDERS OF THE CHIEF TRIGLYCERIDE BEARING LIPOPROTEINS IN PLASMA

As can be seen from the foregoing, division of the chief triglyceride bearing lipoproteins into distinct classes based on physico-chemical properties is at best an approximation. Classifications of hyperlipoproteinaemic states based on serum lipoprotein patterns do thus not rest on a secure foundation. As Carlson (1970) has said, we are classifying the plasma and not the patient.

In modern medicine the aim of classification systems should be definition of disease in terms of specific pathogenetic mechanisms such as in terms of defective proteins or enzymes. At present only one form of genetic hyperlipoproteinaemia satisfies this criterion. Familial hyperchylomicronaemia (Type I) is believed to be due to an inherited deficiency of the enzyme lipoprotein lipase (Havel and Gordon, 1960). Such enzyme as is present, is qualitatively different from normal lipoprotein lipase; it is less inhibited in vitro by known inhibitors of lipoprotein lipase and has different substrate affinities (Bradford and Furman, 1968). No circulating inhibitors of PHLA have been found. Even in this condition, however, some questions remain unanswered. VLDL as well as chylomicron triglyceride is known to be hydrolyzed by lipoprotein lipase (Havel, 1965); a deficiency of this enzyme might, therefore, be expected to be associated with an elevation of VLDL as well. Analytical ultracentrifugation has shown some elevation of this lipoprotein in fact to exist in this disorder (Fredrickson et al, 1968), but the story is as yet incomplete.

The situation is somewhat more complicated in respect of Types IV and V of the WHO classification. A Type IV pattern is considered to exist when there is an elevation of pre-beta lipoprotein alone; the co-existence of increased pre-beta lipoprotein and fasting chylomicronaemia is classified as Type V. The latter type is often regarded as indicating the presence of both endogenous and exogenous triglyceride in plasma. Such a situation could potentially occur as a result of impaired triglyceride removal from plasma. In contradistinction to the Type I disorder, these patients frequently have normal or mildly reduced PHLA's (Fredrick et al, 1963). The pattern may vary with time in an individual patient (Lewis, 1973). Although a Type V pattern may be evident in some samples, other samples from the same patient may show the presence of increased pre-beta lipoprotein only. Furthermore, affected families may show either the Type V pattern or pre-beta hyperlipoproteinaemia alone (Nixon et al, 1969; Fredrickson, 1969). These considerations, taken in conjunction with the functional overlap between chylomicra and VLDL, and the evidence that the origin of the triglyceride in these lipoproteins cannot reliably or easily be predicted from their physico-chemical nature, raises the possibility that Type IV and V patients may in fact be suffering from the same disorder, the difference merely being one of degree. As previously discussed, if the load of triglyceride presented to the lipoprotein synthesizing mechanisms of the gut is large, bigger 'chylomicron-like' particles may result. A similar situation could pertain to the liver; in favour of this hypothesis is the demonstration of particles with features of chylomicrons in plasma of carbohydrate induced individuals at a time when no exogenous triglyceride is present

(Mancini et al, 1973; Schonfeld and Kudzma, 1973). Type V hyperlipoproteinaemia could thus merely be a more severe stage of pre-beta hyperlipoproteinaemia. As chylomicrons and VLDL are believed to be removed by a common removal mechanism (Havel, 1965; Brunzell et al, 1973), saturation of such a mechanism by VLDL could lead to impaired disposal of chylomicrons.

Schonfeld and Kudzma (1973) were unable to distinguish patients who developed chylomicronaemia from non-chylomicronaemic ones by their plasma lipoprotein lipase activity, although chylomicronaemia occurred more commonly during high fat than during a high carbohydrate feeding. Saturation of removal mechanisms by VLDL could explain delayed clearing of 'chylomicrons' either exogenous or endogenous. On the basis of their observations, they suggest that mixed hypertriglyceridaemia, i.e. endogenous and exogenous (Type V), may not be a genetically separate phenotype, but simply a variant of Type IV. Further support for this concept comes from the work of Brunzell et al (1973). When fat calories were kept constant and carbohydrate calories were varied in hypertriglyceridaemic subjects, Types IV and V appeared interchangeable. Restricting carbohydrate intake led to the disappearance of chylomicrons from the plasma of patients with the Type V lipoprotein pattern, whereas chylomicronaemia appeared in response to an increased carbohydrate intake in patients who initially had the Type IV pattern. Because fat intake remained unchanged throughout, they postulated alterations in endogenous triglyceride concentrations in response to changes in carbohydrate intake. Competition between chylomicrons and VLDL for a common saturable removal mechanism could explain their findings. Using PVP floculation these authors showed the larger particles observed during the period of carbohydrate excess to be of exogenous origin. Further evidence of saturability was provided by studying the relationship of endogenous triglyceride concentration to plasma lipolytic rate during prolonged heparin infusion; classical Michaelis-Menton kinetics were observed.

It has been suggested that whereas patients with the Type V pattern should receive fat restricted diets; those with the Type IV pattern should be treated by carbohydrate restriction (Levy, 1972; La Rosa, 1972). From the above considerations, it will be appreciated that such considerations may be unnecessary; a more rational approach to dietary management will come from a better understanding of the pathogenesis of these disorders.

SCOPE OF THE PRESENT WORK

Chylomicrons and VLDL have been shown both to play a role in the transport of dietary fat in experimental animals and in man. Most studies of alimentary lipaemia in man have relied upon the use of large unphysiological doses of ingested fat. Studies of fat transport in rabbit lymph have suggested that the load of fat fed may be of importance in determining the nature of the lipoprotein formed by the intestine (Fraser et al, 1968). This possibility has been tested in man by the feeding of breakfast of varying fat content; the contribution of the chief triglyceride-bearing lipoproteins to alimentary lipaemia has been measured. At the lower end of the spectrum, a fat free meal was fed, ranging through more physiological fat loads to the 100 g. load frequently used in oral fat tolerance tests.

Diets rich in polyunsaturated fats are gaining widespread acceptance both in the treatment of hyperlipoproteinaemia and in the prevention of IHD, as described in Chapter V. The effect of such diets on alimentary lipaemia are thus of interest; reports on the nature of the lipoprotein carrier following the ingestion of unsaturated compared with saturated fats are conflicting. Most studies have relied on separation of lipoprotein fractions from lymph of animals in some instances fed by intraduodenal tube. In order to determine the relative effects of saturated and unsaturated fat on dietary fat transport under physiological conditions in man, healthy male volunteers received butterfat and sunflower oil containing breakfasts of varying dosage. The lipoprotein contribution to alimentary lipaemia was measured.

As discussed earlier, the triglyceride concentration in a blood sample obtained after 12 - 14 hours fast, is not necessarily representative of the fluctuating levels throughout the day, although there appears to be a reasonably good correlation. As polyunsaturated fat feeding lowers fasting serum triglyceride levels (see Chapter V), it was of particular interest to investigate the effect of such a diet on diurnal changes as well, especially as polyunsaturated fats have been claimed to be more rapidly absorbed by the intestine (Ockner et al, 1972). It is, therefore, important to exclude the possibility that diurnal triglyceride levels could be higher after unsaturated fat feeding despite lower base-line levels, before recommending widespread use of high P/S diets.

Two patients with Type V hyperlipoproteinaemia received dietary modifications designed to alter their lipoprotein patterns. The relevance of the findings in these cases is discussed in relation to classification of the hyperlipoproteinaemias.

CHAPTER VI, SECTION B

1. THE ROLE OF THE TRIGLYCERIDE-BEARING LIPOPROTEINS IN DIETARY FAT TRANSPORT

In view of the demonstration that VLDL participates in the transport of dietary fat in rats (Ockner et al, 1969b), the present studies were performed in order to determine whether a similar situation pertains to man. Experiments were designed to demonstrate the variability of the distribution of triglycerides among VLDL and chylomicrons in plasma during the absorption of a fat-containing meal. Fat loads of 0, 20, 50 and 100 g. were fed with an experimental breakfast, samples of blood being obtained for eight hours thereafter, for the determination of the increment in chylomicron and VLDL triglyceride.

Subjects

Nine male members of the hospital and laboratory staff aged 22 - 43 years were studied. All were clinically normal, consumed a typical western diet and had fasting serum cholesterol and triglyceride levels below 260 mg./dl. and 160 mg./dl. respectively.

Composition of the experimental meals

Each subject consumed four experimental breakfasts separated by periods of 3 - 4 days. Each meal contained 1,000 calories; the fat and carbohydrate content were adjusted to provide 1, 20, 50 and 100 g. of saturated fat respectively. The carbohydrate was derived from bread, cereal and sucrose. The fat was provided as butter, milk and double cream. Their composition is shown in Appendix 1. The meals were well tolerated in all subjects, but a tenth, who experienced discomfort and nausea following the 100 g. fat meal, was excluded from the study.

Experimental design

Base-line measurements were made in duplicate after an overnight fast of at least 12 - 14 hours duration. The experimental breakfast was then consumed over a period of less than 20 minutes. In the first experiment, a single blood sample was taken four hours after each breakfast to correspond roughly to the time of peak alimentary lipaemia. In the second experiment, the time course of the alimentary lipaemia was studied; blood samples were obtained 2, 4, 6 and 8 hours after the experimental meal for determination of the triglyceride rich lipoprotein fractions. After the clot retracted, the serum was separated, EDTA added to a final concentration of 0.05%, and preparative ultracentrifugation commenced as described in Chapter II. The following triglyceride rich fractions were separated at a background density of 1.006:

$S_f > 400$, S_f 20 - 400, and in the second experiment also S_f 100 - 400 and S_f 20 - 100. The triglyceride content of whole serum, of these ultracentrifugally separated fractions, and of the residue after ultracentrifugation, were measured. Mean recovery was 95%.

RESULTS

Increments over fasting levels following different oral fat loads

The results of the first study are shown in Figure 29. During absorption of the 100 g. fat meal, there was a pronounced chylomicronaemia, the $S_f > 400$ fraction rising by a mean of 133 mg./dl.; at the same time, VLDL (S_f 20 - 400) triglyceride concentration increased by 44 mg./dl. above the fasting level. The contribution of VLDL at a fat load of 100 g. was thus substantial, accounting for about a quarter of the total increment in serum triglyceride. The relative contributions of VLDL and chylomicron triglyceride changed when smaller fat loads were fed to the same subjects. The 50 g. fat meal resulted in mean chylomicron and VLDL borne increments in serum triglyceride of 45 mg./dl. and 39 mg./dl. respectively and when 20 g. fat was consumed, most of the increment was contributed not by chylomicrons (8.5 mg./dl.), but by VLDL (22 mg./dl.). The control meal containing $<$ 1 g. fat was not followed by a significant change in triglyceride content of either fraction.

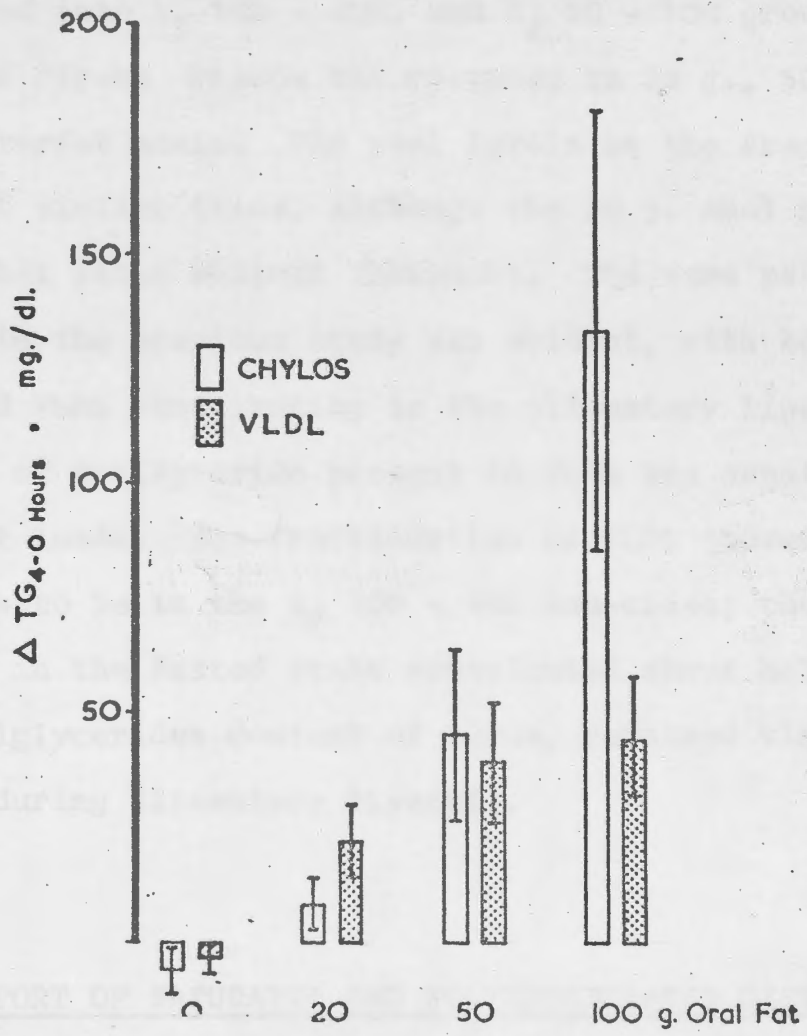

Fig. 29. Increments in serum triglyceride content over fasting levels (mean ± SEM) in chylomicrons and VLDL after different oral fat loads

Time course study

In the second study, the time course of the change in triglyceride content was studied. VLDL was further subfractionated into S_f 100 - 400, and S_f 20 - 100 groups. Table 12 and Figure 30 show the response to 20 g., 50 g., and 100 g. butterfat meals. The peak levels in the fractions occurred at similar times, although the 20 g. meal resulted in a somewhat later maximum increment. The same pattern as described in the previous study was evident, with both chylomicrons and VLDL contributing to the alimentary lipaemia; the proportion of triglyceride present in VLDL was greater with smaller fat loads. Sub-fractionation of VLDL showed most of the changes to be in the S_f 100 - 400 sub-class; the S_f 20 - 100, which in the fasted state contributed about half the $S_f > 20$ triglycerides content of serum, remained virtually unchanged during alimentary lipaemia.

2. TRANSPORT OF SATURATED AND POLYUNSATURATED DIETARY FATS

Conflicting views on the lipoprotein carrier in intestinal lymph following the ingestion of saturated and polyunsaturated fat, are discussed on page 338. The following experiments were performed in order to determine the distribution of triglyceride among chylomicrons and VLDL during the absorption of meals containing saturated and polyunsaturated fat.

TABLE 12

PLASMA TRIGLYCERIDE CONCENTRATION IN $S_f > 400$ ("CHYLOMICRON") FRACTION AND S_f 20 - 400 ("VLDL") FRACTION, AS INCREMENT OVER FASTING LEVELS (mg/dl ± S.E.M.), FOLLOWING 20, 50 AND 100 g ORAL FAT LOADS

	20 g		50 g		100 g	
	$S_f > 400$	S_f 20 - 400	$S_f > 400$	S_f 20 - 400	$S_f > 400$	S_f 20 - 400
2 h	4.8 ± 2.2	12.0 ± 4.2	30.4 ± 12.7	24.2 ± 7.3	118 ± 50.0	24.0 ± 7.5
4 h	18.8 ± 8.0	28.2 ± 13.4	50.7 ± 14.5	33.0 ± 13.9	159 ± 67.5	48.6 ± 21.1
6 h	30.0 ± 11.9	33.7 ± 17.0	51.9 ± 21.0	29.5 ± 12.8	99.2 ± 41.6	54.5 ± 22.2
8 h	6.6 ± 3.4	29.6 ± 16.5	14.4 ± 8.1	10.8 ± 5.7	23.3 ± 10.4	25.7 ± 14.1

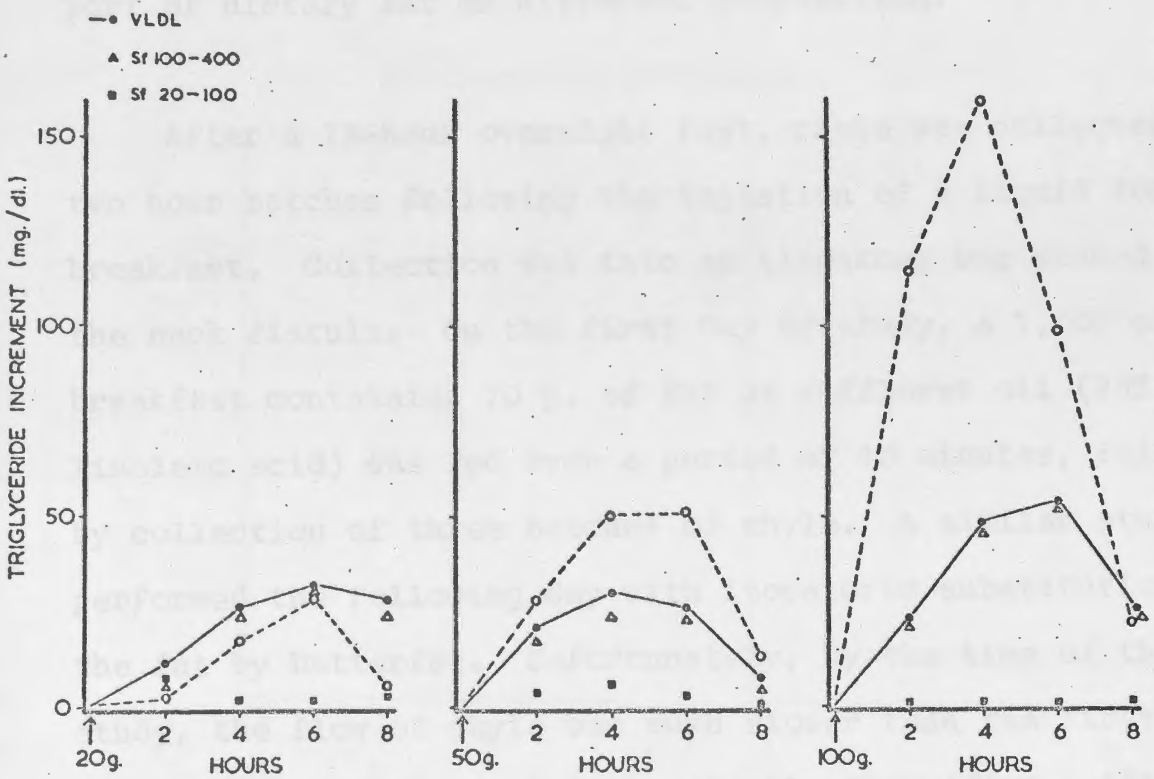

Fig. 30. Time course of serum triglyceride after meals containing 20, 50 and 100 g. fat in S_f 400 ('chylomicron') fraction, S_f 20–400 ('VLDL') fraction, S_f 100–400 fraction and S_f 20–100 fraction; results are expressed as increments over fasting levels.

(a) Thoracic lymph lipoproteins

One patient, a sixty year old female with a traumatic thoracic duct fistula, was studied in order to determine the relative contributions of chylomicrons and VLDL to the transport of dietary fat of different composition.

After a 12-hour overnight fast, chyle was collected in two hour batches following the ingestion of a liquid formula breakfast. Collection was into an ileostomy bag placed over the neck fistula. On the first day of study, a 1,000 calorie breakfast containing 70 g. of fat as safflower oil (76% linoleic acid) was fed over a period of 10 minutes, followed by collection of three batches of chyle. A similar study was performed the following day with isocaloric substitution of the fat by butterfat. Unfortunately, by the time of the second study, the flow of chyle was much slower than the first day, and in fact ceased completely, shortly thereafter. After storage at 4°C until collection for each day was completed, the chyle samples were fractionated into $S_f > 400$, and S_f 20-400 by preparative ultracentrifugation, and the triglyceride content of the fractions and of the unfractionated specimens measured. Figure 31 shows the results obtained in this study. As the volume of lymph draining from the fistula was much less on the day of the butterfat experiment than on the previous occasion, it was not considered valid to compare the results obtained on the two days. However, it does illustrate that following both meals, there was an increased triglyceride content of both $S_f > 400$ and S_f 20 - 400 fractions of chyle.

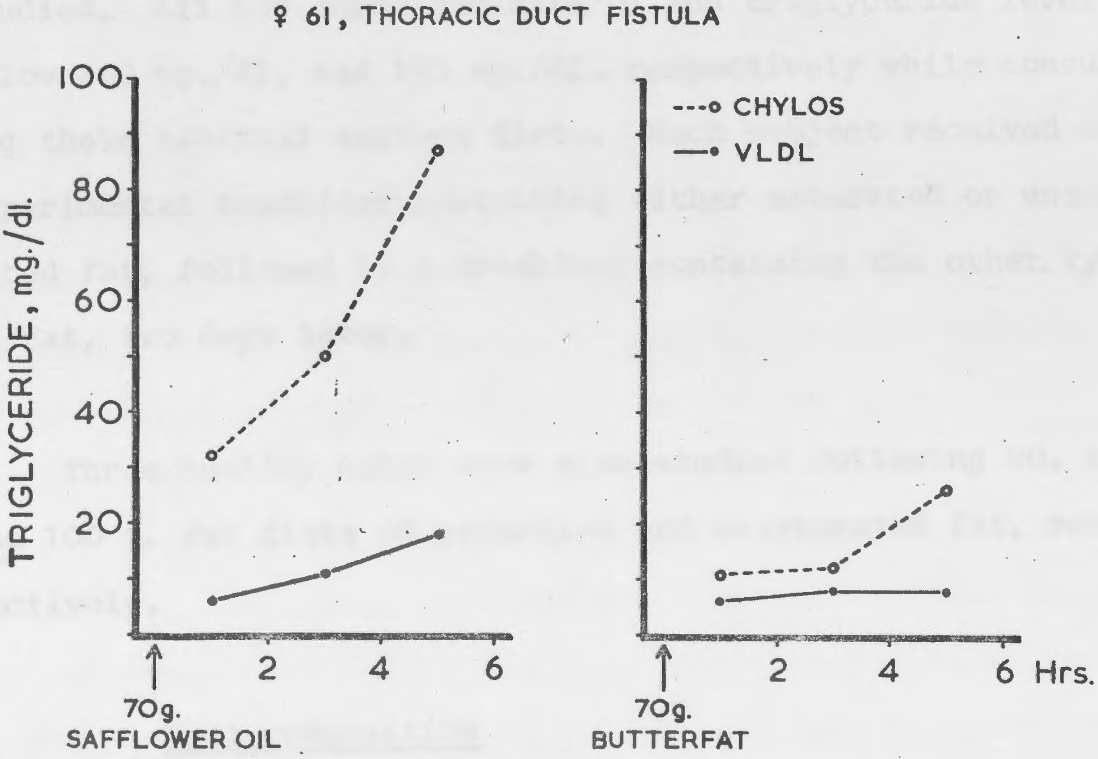

Fig. 31. Lymph triglyceride after 70 g. oral fat loads; results expressed as increments over fasting levels.

(b) Plasma lipoprotein

Subjects

Ten healthy, working members of the laboratory and hospital staff, being five males and five females aged 17 - 32, were studied. All had serum cholesterol and triglyceride levels below 260 mg./dl. and 160 mg./dl. respectively while consuming their habitual western diets. Each subject received an experimental breakfast containing either saturated or unsaturated fat, followed by a breakfast containing the other type of fat, two days later.

Three healthy males were also studied following 20, 50 and 100 g. fat diets of saturated and unsaturated fat, respectively.

Meal composition

In the first set of experiments, the two meals each comprised 750 calories and 50 g. fat, the only difference being the type of fat used. The saturated fat meal was fed in the form of a flavoured milkshake. The unsaturated meal was made to appear and taste similar to the saturated meal. The composition of the meals is shown in Appendix 2.

In the second study, six meals were fed to each subject after a 12 - 14 hour fast. The meals contained 20, 50 and 100 g. saturated fat and 20, 50 and 100 g. of polyunsaturated fat. Intervals of one day separated each experiment. Each meal consisted of 1,000 calories, the fat content of the saturated meals being derived from butterfat, and that of the unsaturated meal, from pure sunflower oil. The composition of these meals is shown in Appendix 3.

Experimental design

In the first experiment, base-line measurements were again made in duplicate after an overnight fast of 12 - 14 hours. The experimental breakfast was consumed over a period of less than 20 minutes after which the subjects undertook minimal activity. A further blood sample was obtained at 4 hours and the serum was separated and ultracentrifuged into $S_f > 400$ and S_f 20 - 400 fractions as described on page 47; ultracentrifugation started on the day of the experiment. In some studies, the saturated meal preceded the unsaturated; in others, the order was reversed. Triglyceride concentration was measured in whole serum and in the two fractions. Mean recovery of triglyceride was 95%.

In the second study, base-line measurements were made after which the experimental meal was consumed over a period of less than 20 minutes. Physical activity was restricted for the following 8 hours, and no further food or drink was permitted for the same period. Blood was sampled through an indwelling venous cannula at 2, 4, 6 and 8 hours after the start of each meal. The serum was separated into $S_f > 400$, and S_f 20 - 400 fractions by ultracentrifugation, and the triglyceride content of these fractions and of whole serum determined.

RESULTS

Figure 32 shows that during absorption of a 50 g. saturated fat meal, serum triglyceride rose by a mean of 72 mg./dl. above base-line levels. As in the previous set of experiments, the increment was due both to a chylomicronaemia and an elevation of VLDL triglyceride, these fractions rising by 50 mg./dl. and 21 mg./dl. respectively. At this fat load, VLDL accounted for 29% of the increase in total triglyceride.

When the same subjects ingested the unsaturated fat meal, a similar result was obtained. Although at 4 hours, the mean increment in total triglyceride was only 62 mg./dl., 20 mg./dl. (32%) was due to VLDL triglyceride. These results suggest that the relative contribution of chylomicrons and VLDL to alimentary lipaemia is independent of the nature of the fat ingested.

The results of the second experiment are shown in Figure 33. The proportionately greater contribution of VLDL to the alimentary lipaemia of a low fat load (20 g.), is observed both after saturated and unsaturated fat feeding. After the 100 g. fat meal, both types of fat led to a greater increment in chylomicra than in VLDL triglyceride. A difference in the response to the type of fat was, however, observed following the 50 g. fat meal; the chylomicron contribution to the alimentary lipaemia was proportionately greater after the unsaturated meal than after the saturated one. The time of peak lipaemia also differed according to the type of fat ingested;

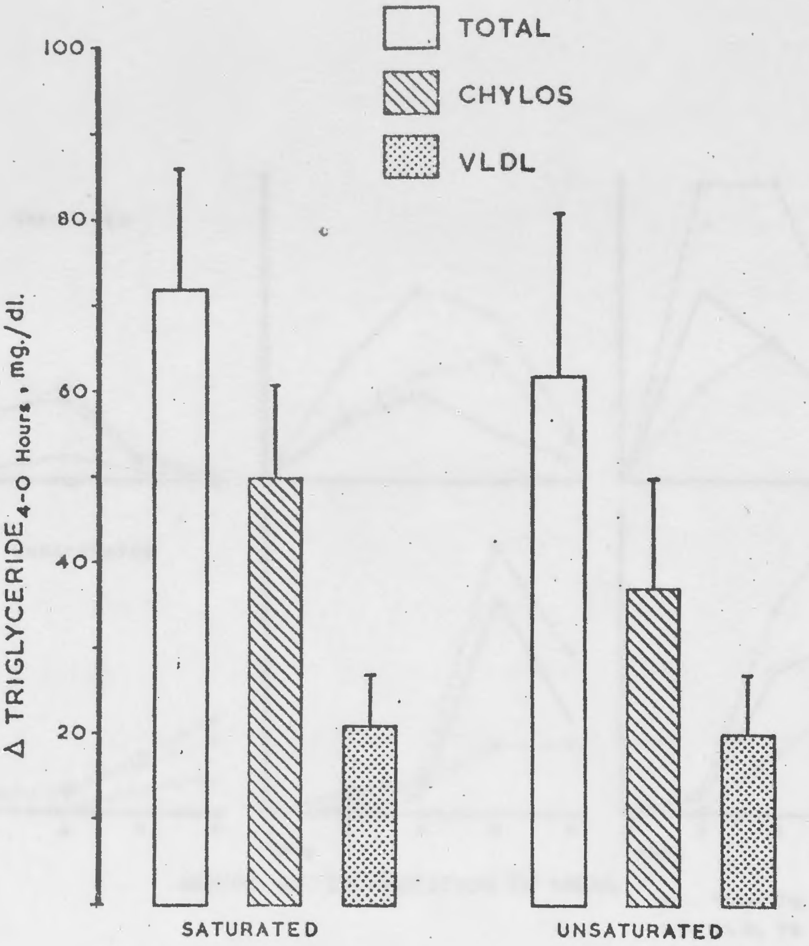

Fig. 32. Increments in serum triglyceride over fasting levels (mean ± SEM) in S_f 400 ('chylomicrons') and S_f 20-400 ('VLDL') during consumption of saturated and polyunsaturated meals.

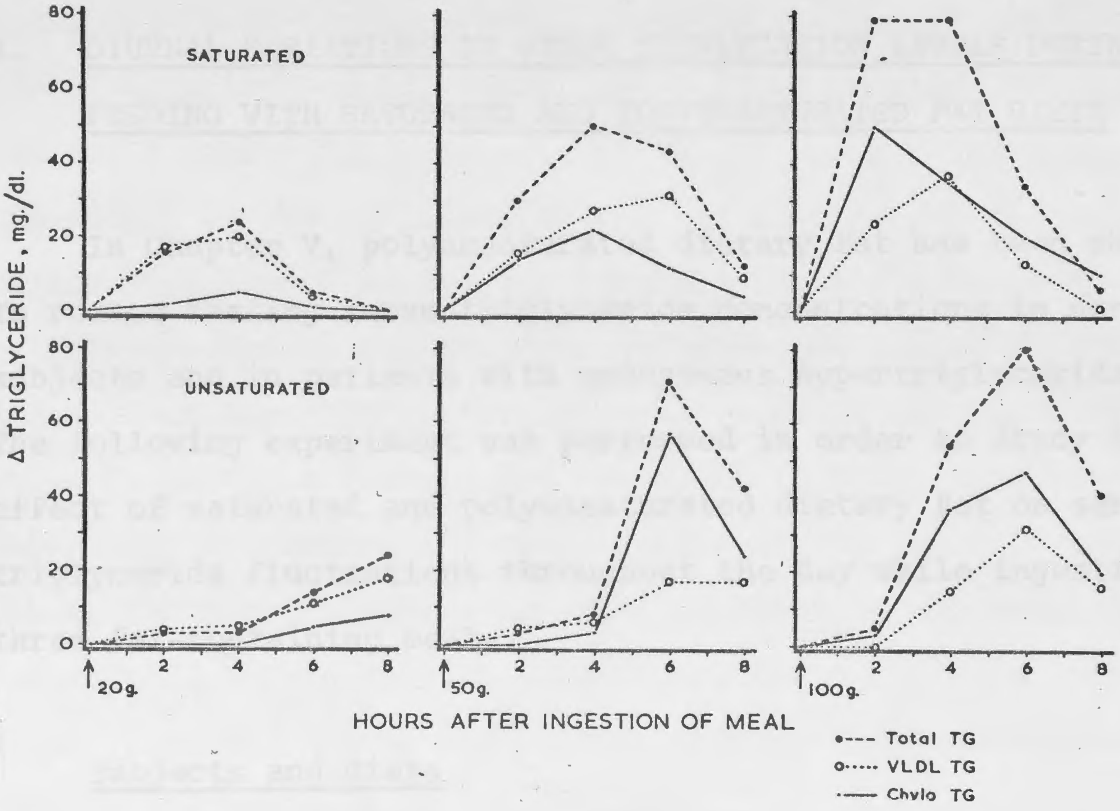

Fig. 33. Time course of mean serum triglyceride concentration after different oral loads of saturated and polyunsaturated fat; results are expressed as increments over fasting levels.

the polyunsaturated fat meal resulted in a slower appearance of triglyceride in plasma than did the saturated fat meal, at each fat load tested.

3. DIURNAL VARIATIONS IN SERUM TRIGLYCERIDE LEVELS DURING FEEDING WITH SATURATED AND POLYUNSATURATED FAT DIETS

In Chapter V, polyunsaturated dietary fat has been shown to reduce fasting serum triglyceride concentrations in normal subjects and in patients with endogenous hypertriglyceridaemia. The following experiment was performed in order to study the effect of saturated and polyunsaturated dietary fat on serum triglyceride fluctuations throughout the day while ingesting three fat-containing meals.

Subjects and diets

Sixteen men took part in this experiment. Ten were normolipaemic (mean age = 36 years; mean serum triglyceride concentration = 94 mg./dl.); six had endogenous hypertriglyceridaemia (mean age = 55 years; mean serum triglyceride concentration = 323 mg./dl.). Measurements were made on the last day of the two 10-day sequential periods of either saturated or polyunsaturated fat feeding. The compositions of the diets were identical to those described on page 272. In some cases, the saturated fat period preceded the unsaturated one; in others, the order was reversed.

Experimental design

After a 12 - 14 hour overnight fast, an indwelling polyethylene cannula was inserted into the antecubital vein and duplicate blood samples taken for base-line measurements. Three meals (at 9 a.m., 1 p.m. and 6 p.m.) provided 20%, 45% and 35% of total calories for the 24 hours respectively. The nature of the fat ingested was the same as for the antecedent nine days. Meals were consumed in less than 20 minutes; subjects performed minimal activities on the day of the study and no food was allowed between meals. Blood samples were taken at hourly intervals for the first 12 hours, and at longer intervals thereafter, for triglyceride estimation. In some cases, ultracentrifugal separation of the $S_f > 400$ fraction was carried out on samples taken every three hours for the first 12 hours, and on the 24 hour sample; triglyceride concentration was measured in this fraction.

Not only were fasting levels lower during unsaturated fat feeding, but post-prandial levels were also decreased. This is illustrated in Figure 34 by two representative studies, one in a hypertriglyceridaemic, the other in a normolipaemic subject. As in the previous experiment (see page 363), the peak lipaemia after feeding unsaturated fat occurred later than after saturated fat.

In order to ascertain whether this difference reflected the lower fasting level during the unsaturated fat feeding period, or whether there was also a difference between the two diets in the rise of serum triglyceride levels during fat

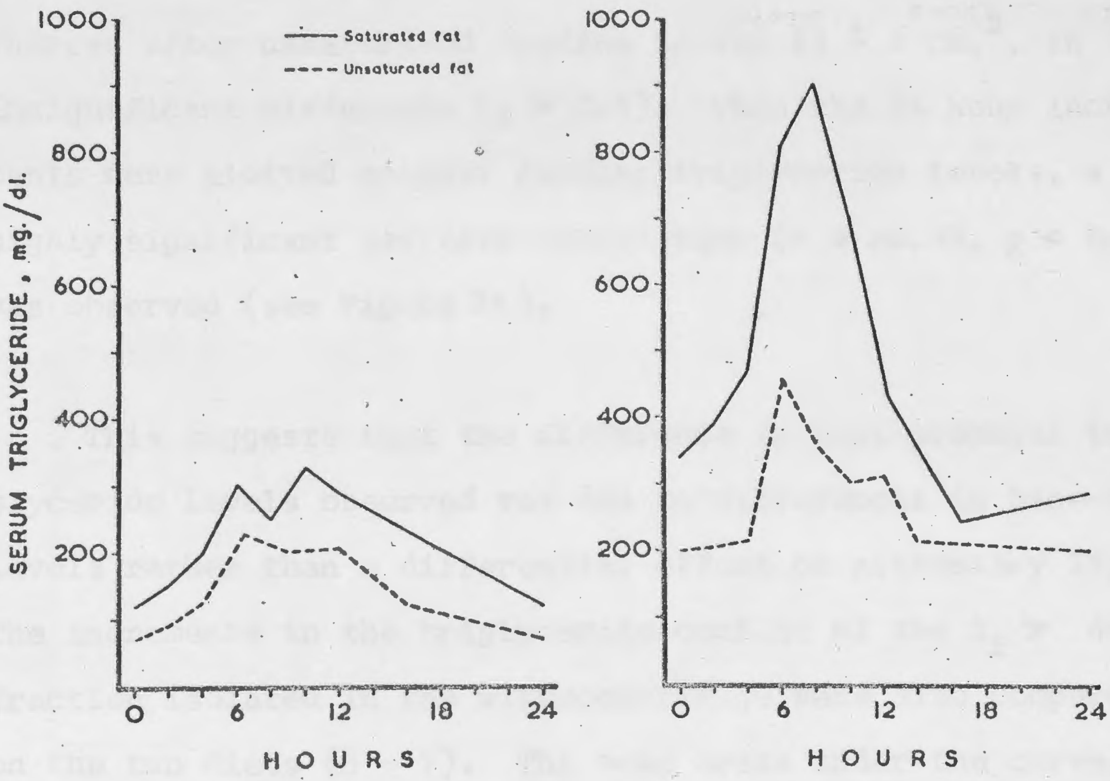

Fig. 34. Diurnal changes in serum triglyceride during consumption of the experimental diets

Left : Normolipaemic subject

Right: Hypertriglyceridaemic patient

Meals were fed at 0, 4 and 9 hours

absorption, the increments in serum triglyceride concentrations were plotted and the areas measured under the curves. During saturated fat feeding, the area was 81 ± 13 $cm.^2$ (mean ± SEM), whereas after unsaturated feeding it was 64 ± 8 $cm.^2$, an insignificant difference ($p > 0.1$). When the 24 hour increments were plotted against fasting triglyceride levels, a highly significant positive correlation ($r = +0.49$, $p < 0.01$) was observed (see Figure 35).

This suggests that the difference in post-prandial triglyceride levels observed was due to differences in base-line levels rather than a differential effect on alimentary lipaemia. The increments in the triglyceride content of the $S_f > 400$ fraction isolated in the ultracentrifuge were also compared on the two diets ($n = 7$). The mean areas under the curves were 56 ± 12 and 45 ± 10 $cm.^2$ during saturated and polyunsaturated fat feeding respectively. This difference was also not significant ($p > 0.1$).

4. DIETARY FAT TRANSPORT IN HYPERLIPOPROTEINAEMIA

As discussed on page 346, Type V hyperlipoproteinaemia is regarded by some authorities as severe endogenous hypertriglyceridaemia, whereas others believe both endogenous and exogenous triglycerides to occur in excess in plasma obtained in the fasted state. Two patients with Type V hyperlipoproteinaemia were studied in order to ascertain the effect of dietary modification on their serum triglycerides and lipoprotein patterns.

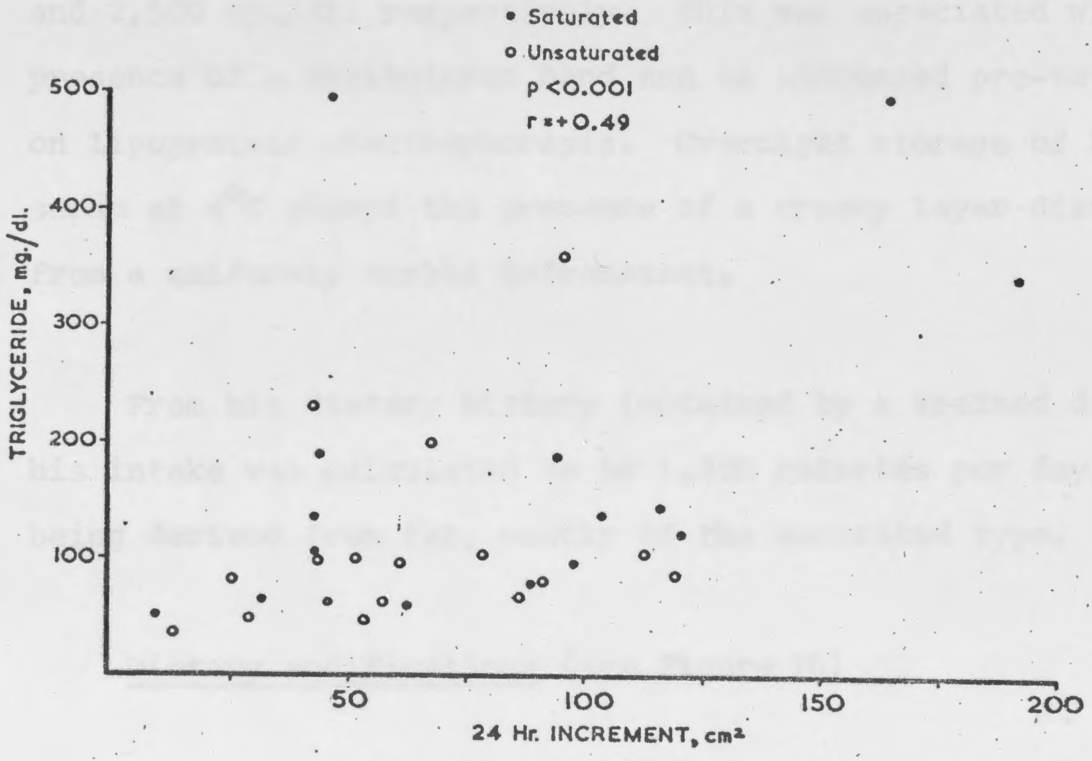

Fig. 35. Correlation between serum triglyceride and 24 hour increments

Case 1

Mr. W. E. aged 54, while on a 'normal' western diet had elevated cholesterol and triglyceride levels of 522 mg./dl. and 2,500 mg./dl. respectively. This was associated with the presence of a chylomicron band and an increased pre-beta band on lipoprotein electrophoresis. Overnight storage of his serum at 4°C showed the presence of a creamy layer distinct from a uniformly turbid infranatant.

From his dietary history (obtained by a trained dietitian), his intake was calculated to be 1,800 calories per day, 40% being derived from fat, mostly of the saturated type.

Dietary modifications (see Figure 36)

(a) Calorie, carbohydrate and fat restriction was commenced using an 800 calorie 40 g. fat diet.

(b) After a period of 12 days during which time the patient's weight had decreased by 1.4 Kg., the diet was altered. Fat content was kept unchanged at 40 g. daily, while calorie intake was increased chiefly by increasing carbohydrate intake. During this period his weight increased by 1.8 Kg.

(c) After 14 days, a further dietary alteration was made; the calorie content was kept at 1,800 per day, but the fat intake was more than doubled, being increased to 100 g. per day, again mainly as butterfat.

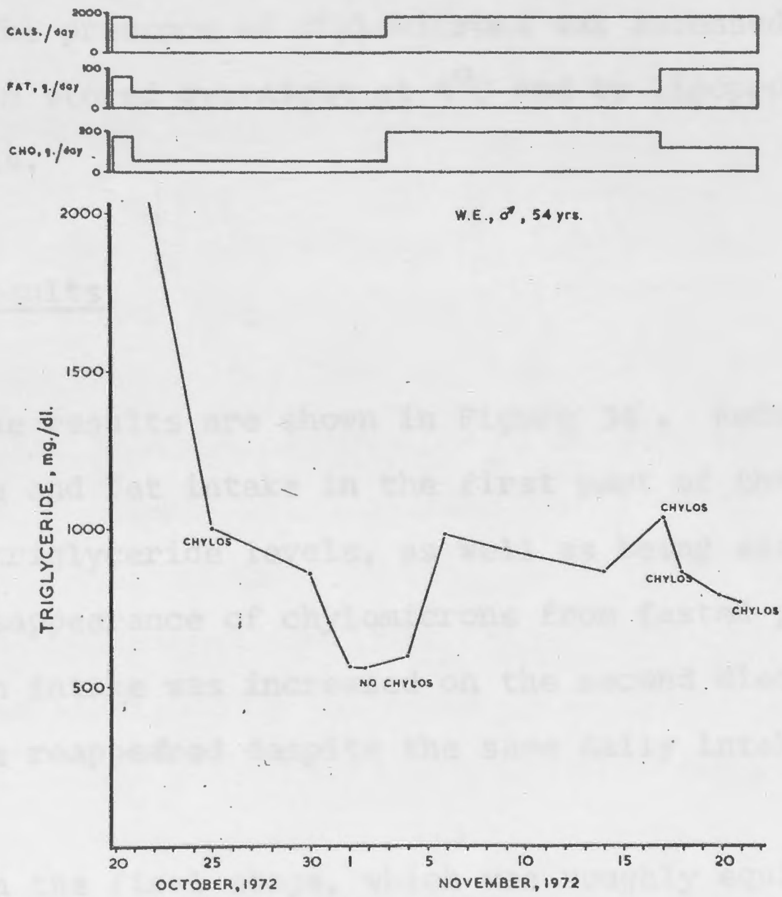

Fig. 36. Serum triglycerides during dietary modification in a patient with Type V hyperlipoproteinaemia

Fasting blood samples were obtained at intervals on the three diets; cholesterol and triglyceride content was determined and lipoprotein electrophoresis of the serum was carried out. The presence of chylomicrons was assessed by inspection of serum stored overnight at 4°C and by lipoprotein electrophoresis.

Results

The results are shown in Figure 36 . Reduction of both calorie and fat intake in the first part of the study decreased serum triglyceride levels, as well as being associated with the disappearance of chylomicrons from fasted plasma. When calorie intake was increased on the second diet, the chylomicrons reappeared despite the same daily intake of fat.

In the final stage, which was roughly equivalent to his usual diet, no further elevation of triglyceride level was achieved by raising the fat content of the diet, while keeping calorie intake constant. Chylomicronaemia persisted.

Case 2

Mr. H. R. aged 48, presented with planar xanthomata and a Type V lipoprotein pattern with cholesterol and triglyceride levels of 370 mg./dl. and 1,590 mg./dl. respectively. Dense pre-beta and chylomicron bands were present on lipoprotein electrophoresis of fasting serum, and chylomicron and VLDL triglyceride levels of 1,165 mg./dl. and 490 mg./dl. respectively, were detected after separation in the preparative ultracentrifuge.

Ingestion of labelled fat

In order to determine whether dietary fat appeared in both chylomicron and VLDL fractions in plasma, 25 μCi^{3}H tripalmitin (Radiochemical Centre, Amersham) in a gelatin capsule, was ingested with a 500 calorie breakfast containing 30 g. of butterfat. Venous blood samples were obtained at 2, 4, 6 and 8 hours after the test meal, and chylomicrons and VLDL separated ultracentrifugally. After extraction of the lipid in these fractions by the Folch technique (Folch et al, 1957), the chloroform phases were evaporated to dryness under a nitrogen stream and the radioactivity counted in a Tricarb Liquid Scintillation Spectrometer (Packard Co., U.S.A.) using Toluene/POPOP/PPO as scintillant. Counts were present in both fractions as seen in Figure 37, the peak in chylomicrons being observed six hours following the meal. A substantial number of counts was also observed in the VLDL fraction, although by eight hours after the meal, no reduction in count rate was observed in this fraction.

Dietary modification

A series of dietary manoeuvres were then performed which differed from those carried out in subject W. E. The following diets were fed after a run-in period of one week on his usual 1,800 calorie 100 g. fat diet:

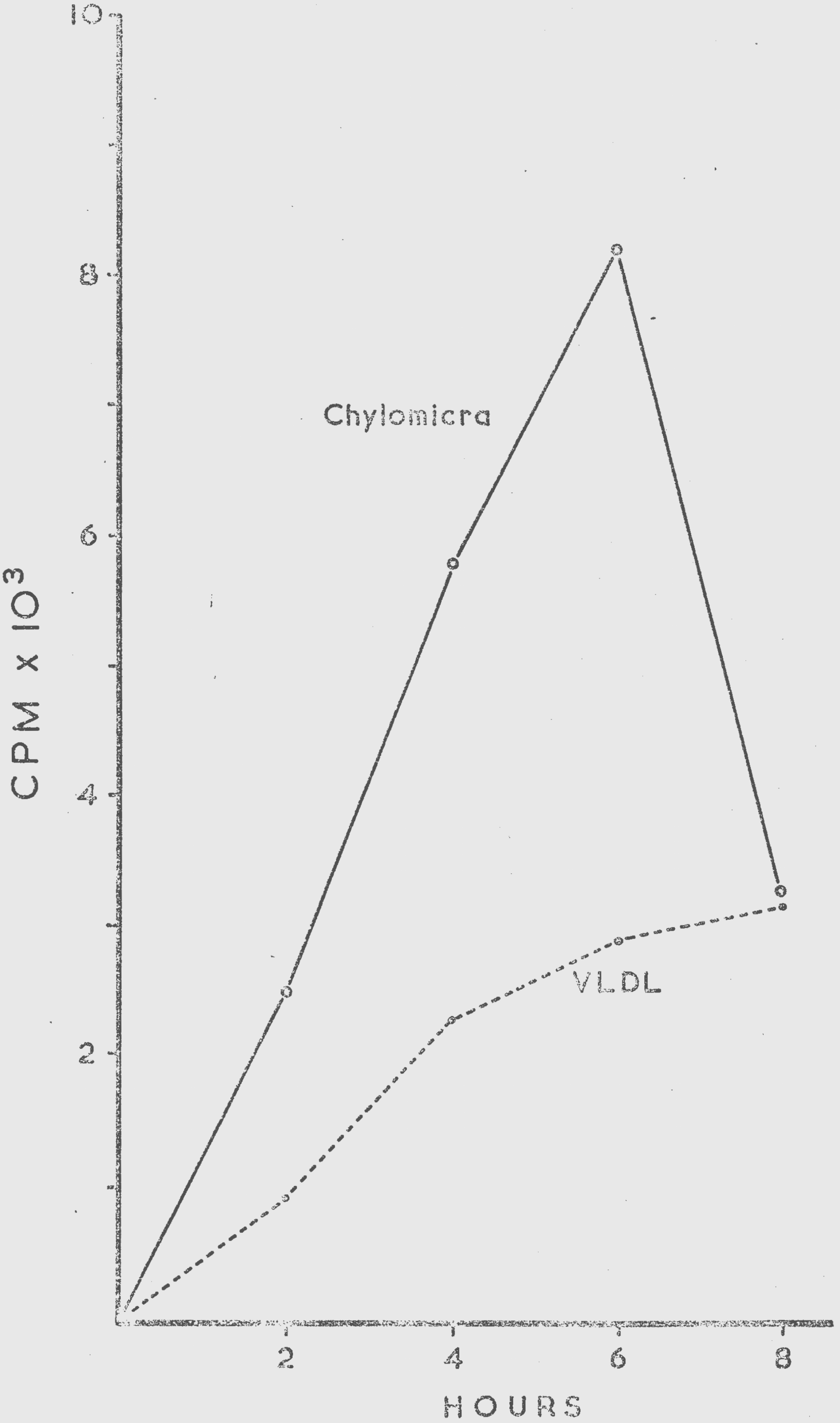

Fig. 37. Time course of appearance of counts in chylomicrons and VLDL

(a) An isocaloric diet containing less than 5 g. fat.

(b) An 800 calorie 40 g. fat diet.

Blood was sampled daily for estimation of triglyceride content of the chylomicron and VLDL fraction separated ultracentrifugally as previously described. The results are shown in Figure 38. At the time of institution of the diet containing less than 5 g. of fat, triglyceride concentration in both chylomicrons and VLDL was increasing. It can be seen that during the isocaloric dietary period (1,800 calories $<$ 5 g. fat), triglycerides in both VLDL and chylomicron increased further despite a virtual absence of fat from the diet. These 'chylomicrons' must thus have been of 'endogenous' origin. During the next dietary period, triglyceride concentration in both fractions fell during calorie restriction despite increasing the fat content of the diet to 40 g. per day.

SUMMARY OF RESULTS

During alimentary lipaemia in man, chylomicronaemia was accompanied by a simultaneous rise in VLDL triglyceride in plasma. After large oral fat loads, chylomicronaemia predominated but after smaller, more physiological meals, the increase in VLDL triglyceride was similar to or even exceeded that in chylomicron triglyceride. In one study in which lymph was collected from a thoracic duct fistula, the triglyceride concentration of both chylomicrons and VLDL increased following ingestion of a fatty meal.

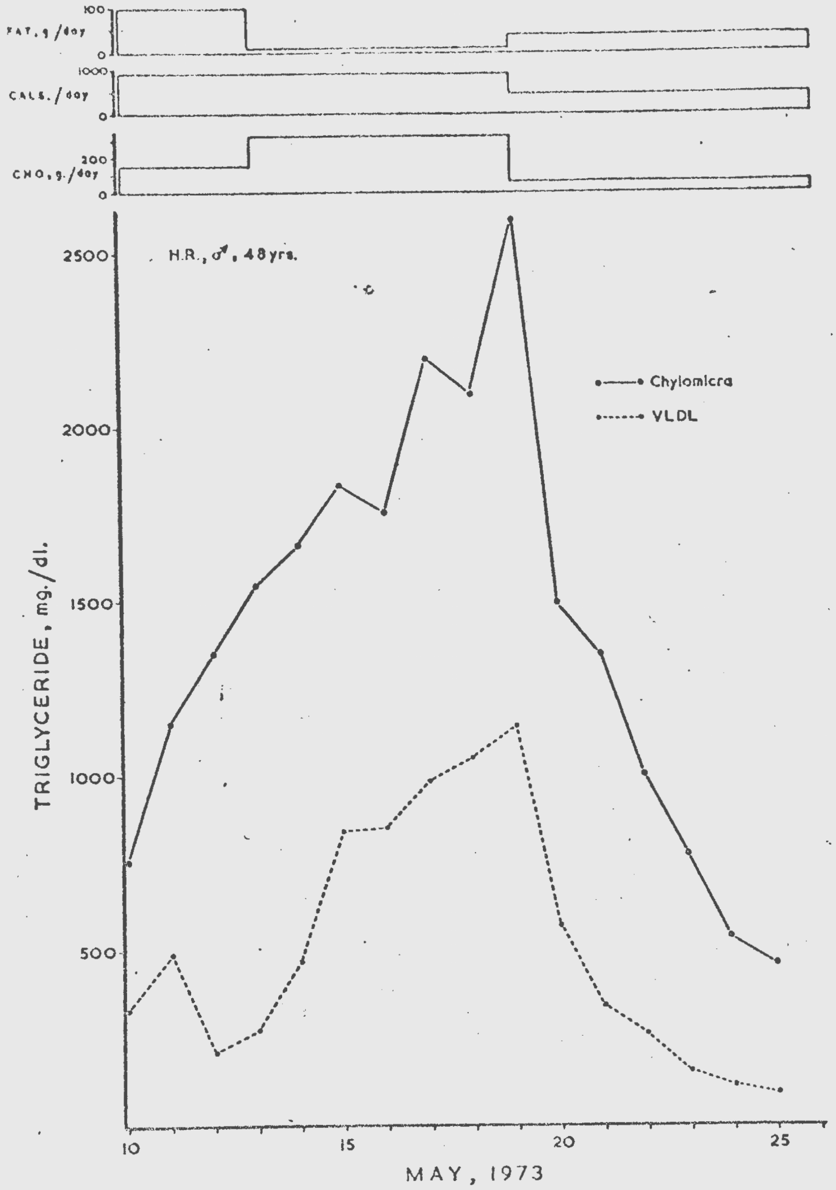

Fig. 38. Serum triglycerides in S_f 400 ('chylomicron') and S_f 20-400 ('VLDL') fractions during dietary modification in a patient with Type V hyperlipoproteinaemia

No conclusive evidence was found for either of these lipoprotein classes showing a differential contribution to alimentary lipaemia when saturated and polyunsaturated fat meals were fed.

The diurnal fluctuation of serum triglyceride concentration was greater after a period of saturated fat feeding than after a polyunsaturated fat feeding period. This difference could largely be attributable to differences in base-line (endogenous) triglyceride levels.

Two subjects with Type V serum lipoprotein patterns underwent dietary manipulations which resulted in changes in their serum triglyceride concentrations. In one, fat and calorie restriction led to the disappearance of chylomicrons from plasma. They returned when calories were increased despite fat intake being kept constant. In the other subject, triglyceride concentrations in VLDL and chylomicrons actually increased while on an isocaloric fat free, high carbohydrate diet; they fell again once calories and carbohydrate were restricted, despite an increase in dietary fat content.

CHAPTER VI, SECTION C

THE ROLE OF VLDL IN DIETARY FAT TRANSPORT

The finding that VLDL triglyceride rises during alimentary lipaemia could be attributed to a primary role of this lipoprotein class in dietary fat transport. This is in accord with findings in the rat where VLDL has been shown to contribute substantially to fat absorption in the rat (Ockner et al, 1969b; Boquillon et al, 1972). Studies of the relative roles of chylomicrons and VLDL in dietary fat transport in humans have been few; because of the difficulty in obtaining samples of lymph, most studies in humans have measured the appearance of lipid and lipoprotein in peripheral blood following the ingestion of a fat containing meal. VLDL as well as chylomicra have been shown to appear in human lymph in response to fat feeding (Bergström et al, 1972) as well as in plasma (Sdolin et al, 1968; Schlierf and Raetzer, 1972). The present study is in accord with these, and points in favour of dietary fat transport being a primary function of VLDL as well as its better known role in the transport of endogenous triglyceride (Fredrickson et al, 1967).

The post-prandial rise in VLDL could alternatively be due to endogenous triglyceride derived from the recycling of dietary fatty acids. The latter concept gains some support from the observation that plasma only assumes the fatty acid pattern of dietary fat some hours after peak lipaemia, suggesting an endogenous component to the lipaemia (Bragdon and Karmen, 1960). Chylomicrons have also been shown to reflect the dietary fatty acid composition more rapidly than do VLDL, following the ingestion of fat with a different fatty acid composition to that of plasma and adipose tissue (Edelin et al, 1968). However, the similarity in time course of the chylomicrons and VLDL curves in the present study favours the former explanation. Furthermore, chylomicron and VLDL peaks in the diurnal variation study of Schlierf and Raetzer (1972) show a similar time relationship; a three-meal a day feeding pattern led to distinct peaks being detectable in both fractions, which coincided temporally with each other and with peaks of total serum triglyceride concentration. In view of current knowledge of triglyceride and FFA kinetics (Eaton et al, 1969), recycling of dietary fat acids is likely to be superimposed; the extent of the VLDL peak due to recycling, however, remains speculative.

The presence of chylomicrons as well as VLDL in human thoracic duct lymph both in the present study, and in that of Bergström et al (1972), as well as in numerous studies in animals (Zilversmit et al, 1966; Ockner et al, 1969b; Ockner and Jones, 1970; Ockner et al, 1972; Boquillon et al, 1972) also favours a primary role of VLDL in dietary fat transport in humans. Because the amount of triglyceride recovered in the thoracic duct lymph of the patient studied, only represents a small fraction of the amount fed, and because the lymph volumes were small, it is likely that the bulk of lymph was still reaching the blood stream. Simultaneous blood sampling was unfortunately not performed. The only conclusion that can thus be drawn from this single case, is that during the absorption of both saturated and polyunsaturated dietary fat, lymph contains both chylomicrons and VLDL, suggesting the importance of both these lipoprotein classes in dietary fat transport.

While in the present study, the triglyceride concentrations in the S_f 20 - 100 and S_f 100 - 400 fractions were similar in the fasted state, the increase in the S_f 100 - 400 component accounted largely for the rise in VLDL during fat absorption; it is possible, therefore, that the S_f 20 - 100 component contains triglycerides of mainly endogenous origin. Harlan and Bleischer (1963) did, however, observe an increase in the S_f 20 - 100 component of VLDL in addition to a rise in S_f 100 - 400 during fat absorption; it is possible that the larger dose of fat fed (120 g.) may account for the discrepancy between their findings and those of the present study.

Most fat tolerance tests rely on the ingestion of fat loads larger than are usually eaten at a single meal and may be the equivalent of a whole day's intake. The high doses used are thus clearly unphysiological. They did, however, provide suitably high plasma triglyceride levels for the purpose of studying fat tolerance before a reliable intravenous fat tolerance test became available, but are not ideal for the study of intestinal fat absorption. For the purposes of the present study, it seemed more pertinent to test a range of fat loads some of which would be approximately equivalent to a normal sized meal. Studies in rats have suggested that the fat load may be of importance in determining the nature of the lipoprotein molecule produced; Fraser et al (1968) showed 'chylomicron' size to increase in rabbits with increasing fat load. Even during alimentary lipaemia, thoracic duct particle size rises and falls, being largest at the time of peak lipaemia (Fraser et al, 1968; Forte and Nichols, 1972). In the present study, when 100 g. of fat were fed, about one-quarter of the increment in plasma triglyceride at four hours was due to VLDL. However, the relative contribution of chylomicrons and VLDL to alimentary lipaemia was altered by the size of the fat load; the rise in VLDL triglyceride at 4 hours was about one-half of the rise in total plasma triglyceride during the absorption of 50 g. of fat. After the ingestion of the 20 g. fat meal, almost three-quarters of the increase in plasma triglyceride concentration occurred in the VLDL fraction. Particles lowest in density, and presumably highest in triglyceride, thus seem to predominate when very large loads of triglyceride are presented to the intestinal mucosa. When smaller, more physiological quantities of fat are ingested, the spectrum of triglyceride rich lipoproteins of plasma tends to drift towards VLDL. When Schlierf and Raetzer (1972) fed physiological amounts of fat divided into three equal meals, similar quantities of chylomicrons and VLDL accounted for the increments above base-line triglyceride levels over the 24-hour period. By doubling the number of meals and hence reducing the fat load per meal, the contribution of VLDL to alimentary lipaemia rose to about two-thirds of the total. The finding in the present study of a greater chylomicron contribution at high fat loads, is also consistent with that of Fraser et al (1968) who showed an increased fat load to result in the appearance of larger chylomicrons in rabbit thoracic duct lymph.

They also lend support to the hypothesis of intestinal lipoproteins being a dynamic system capable of responding to different fat loads by producing lipoproteins of various sizes and triglyceride content. A continuous spectrum of triglyceride bearing lipoproteins has been shown to be present in rat intestinal lymph (Ockner and Jones, 1970; Windmueller et al, 1970), which shifts towards the larger non-triglyceride rich molecule on ingestion of fat.

FAT COMPOSITION AND LOAD

Ockner et al (1972) have proposed a mechanism whereby the differences they observed between the absorption of saturated and unsaturated fats in rats, could fit in with this concept. They showed linoleic acid administered by duodenal intubation, to result in the appearance of mainly chylomicrons in intestinal lymph whereas palmitic acid resulted in VLDL as well (Ockner et al, 1969b). They suggested that as the unsaturated fatty acid is absorbed more proximally and by a shorter length of intestine than was required for the absorption of palmitic acid, more of the former would then be available per intestinal cell for lipoprotein formation; larger particles might then be anticipated. These findings have not been borne out in the present study in humans after oral fat administration. After 50 g. fat formula feeds which were identical in every respect other than in fat composition, no difference was detected in the relative proportions of chylomicrons and VLDL in plasma between butterfat and sunflower oil. Roughly 30% of the increase at 4 hours was contributed to by VLDL after both test meals. This is a somewhat lower value than in the previous experiment when about one-half of the 50 g. fat meal appeared as VLDL. Other constituents of the meal were, however, not equal and could possibly account for the difference.. The latter experimental meals contained only three-quarters of the calorie content of the earlier experiment; protein content was also higher and carbohydrate lower. In the three subjects who had saturated and unsaturated fat meals of varying load, sampling for 8 hours after the meal again showed no consistent difference between saturated and unsaturated fat. With the 20 g. meal, VLDL contributed the greater proportion of fat load, the opposite holding for the 100 g. meal. Only after the 50 g. meal were differences detected; whereas the alimentary lipaemia during saturated fat absorption could be accounted for by a proportionately larger contribution by VLDL, the chylomicron increment was greater during unsaturated fat feeding. Closer examination of the graphs show the four hour values not to be in disagreement with the earlier experiment; the peak lipaemia after the unsaturated meal occurred somewhat later by which time the relationship of chylomicrons to VLDL had altered. Further studies should perhaps attempt to compare chylomicron with VLDL triglyceride increments at the peak of lipaemia, and also compare areas under the peaks following single meals of saturated and unsaturated fat. The results available at present, however, fail to demonstrate a definite differential effect of the saturated and unsaturated meals on the relative production of chylomicrons and VLDL in man. This conforms with the findings of Zilversmit et al (1966), who showed no differences in particle size in thoracic duct lymph in rats fed cream or corn oil.

Another point of interest is the observation that peak lipaemia following the ingestion of sunflower oil, occurs later than after ingestion of the same amount of butterfat. The reason for this is not clear and would tend to be in conflict with the observation of Ockner et al (1972). They demonstrated more rapid and more proximal absorption of unsaturated fatty acids, which might be expected to result in the reverse of what was found in the present study. They, however, administered their fat by interduodenal infusion, thereby eliminating potential effects of these triglycerides on gastric motility and emptying. Fat ingestion is known to delay gastric emptying, unsaturated fats being more efficient inhibitors than saturated (Roberts, 1931; Tidwell and Cameron, 1942). This may explain the present findings of a later peak lipaemia occurring after feeding unsaturated fat. It may also explain the lack of difference in distribution of chylomicrons and VLDL found following the ingestion of the two types of fat; delayed gastric emptying after polyunsaturated fat feeding might tend to counteract the greater fat load per cell which has been postulated by Ockner et al (1972) to result in chylomicron formation during its absorption.

DIURNAL VARIATION

Studies of levels throughout the day on a three meal feeding pattern confirmed the findings of Schlierf and Raetzer (1972) that VLDL triglyceride contributes substantially to diurnal fluctuations in serum triglyceride concentration. Not only base-line triglyceride levels, but levels throughout the day were lower after the feeding of unsaturated fat. Kuo and Carson (1959) had previously shown a corn oil diet to result in lower and more short-lived elevations of serum triglyceride throughout the day than did a typical American diet. These observations could either reflect differential absorption of the two fats or simply be due to differences in endogenous triglyceride levels. Although the area between the peaks and the base-line levels did not differ significantly on the two diets, thus suggesting differential absorption not to occur, values were higher after the saturated feeding period. The number of subjects studied was small, and by studying larger numbers, levels of significance may have been achieved. Fasting serum triglyceride concentration did, however, correlate significantly with the diurnal change in triglyceride (i.e. the area between the peaks and the fasting level) as seen in Figure 35 . This suggests that the chief determinant of diurnal changes may be the fasting level, high triglyceride levels tending to be associated with larger lipaemic peaks during fat feeding. It is of interest that Schlierf and Raetzer (1972) also noted alimentary lipaemia to be more marked in hypertriglyceridaemic subjects than in normals and Maruhama et al (1967) showed the fasting triglyceride level to be a good predictor of the degree of diurnal fluctuations. Denborough (1963) also showed a positive correlation between fasting triglyceride levels and maximal values obtained during an oral fat tolerance test. These observations could possibly be explained by the slower triglyceride removal rates seen in association with high triglyceride levels (Boberg, 1971). As K_2 does not change when triglyceride concentration falls during polyunsaturated fat feeding (see page 283), this line of reasoning could, however, not explain the findings illustrated in Figure 35. The present study, nevertheless, lends support to the concept that triglyceride levels throughout the day are dependent on endogenous triglyceride levels; modification of the diet by increasing the polyunsaturated fat content could then reduce not only endogenous levels, but diurnal fluctuations as well. It also lends further credence to the lack of advantage in measuring diurnal triglyceride fluctuations, over simple measurements of fasting levels in the assessment and management of the hyperlipidaemic individual.

CLINICAL STUDIES IN RELATION TO CLASSIFICATION OF HYPERLIPOPROTEINAEMIA

The studies of dietary modification in two patients with Type V hyperlipoproteinaemia patterns in their serum, raises some interesting points. The serum of the first patient was transformed into a type IV pattern by restriction of calories, carbohydrate and fat (see Figure 36). When fat content was kept constant and caloric intake elevated by increasing the amount of carbohydrate fed, the serum triglyceride concentration again increased and chylomicrons reappeared in serum obtained after an overnight fast. As the fat content of the diet remained unchanged while carbohydrate content increased, these chylomicrons were likely to have been of endogenous origin due to carbohydrate induction (Ahrens et al, 1961; Glueck et al, 1969; Mancini et al, 1973). It is, however, possible that the increased carbohydrate load, by slowing triglyceride removal (Mancini et al, 1973), resulted in a delayed clearance of some dietary fat. An increased input of VLDL into plasma consequent on carbohydrate induction could thus conceivably have resulted in saturation of chylomicron removal mechanisms, thereby inhibiting the removal of chylomicrons formed in response to the 40 g. of fat fed. This suggestion is rendered less likely by the results of the study in the second patient. In this case, chylomicron (and VLDL) triglyceride increased substantially, despite virtual absence of fat from the diet. Carbohydrate induction of both VLDL and chylomicrons thus seem likely; the chylomicrons in this situation could thus be considered as being of endogenous origin. Both chylomicron and VLDL triglyceride concentrations subsequently fell on an 800 calorie diet, despite the intake of fat being increased to 40 g. per day.

Both cases could thus be made to alternate between the Type V and Type IV pattern by modifying the diet. In the second case, the Type V pattern, which is claimed to be due to excess endogenous and exogenous triglyceride, was present even during the consumption of a virtually fat free diet. Brunzell et al (1973) also showed Type IV and V patterns to be interchangeable, by varying the calorie and carbohydrate content of the diet while keeping fat calories constant. Therapeutic implications may be of importance. Whereas many authorities treat endogenous hypertriglyceridaemia by calorie and carbohydrate restriction, and exogenous hypertriglyceridaemia by fat restriction (e.g. Levy, 1972; La Rosa, 1972), this distinction may not be necessary in Type V hyperlipoproteinaemia.

A further point of interest in the present study, is that radioactivity appears later in VLDL than in chylomicrons following the ingestion of labelled tripalmitin together with a fat containing meal. One possible explanation would be recycled dietary fatty acids appearing in VLDL (Eaton et al, 1969). However, as the removal rate of chylomicron triglyceride exceeds that of VLDL triglyceride (Havel, 1965), competition at the site of removal could also contribute to the later VLDL peak observed in this case. Chylomicron and VLDL triglyceride peaks measured chemically were, however, found to occur at equal times in the experiments described on page and also in the studies of Schlierf and Raetzer (1972).

A functional overlap between particles classified as either 'chylomicrons' or 'VLDL' on physiochemical grounds thus appears to exist. Both chylomicrons and VLDL appear to contribute to dietary fat transport, in agreement with similar studies in man (Schlierf and Raetzer, 1972) and with studies of dietary fat transport through intestinal lymph in animals (Zilversmit et al, 1966; Ockner et al, 1969b; Ockner and Jones, 1970; Boquillon et al, 1972). These observations, taken in conjunction with the related trend of a shift of endogenous particles towards lower density triglyceride rich particles during carbohydrate induction in the two cases studied, as well as in those reported by Ruderman et al (1971) and Mancini et al (1973), emphasize the flexibility of the triglyceride-bearing lipoproteins. Classification systems of disorders of the hyperlipoproteinaemic state based on such flexible foundations must, therefore, clearly be regarded as provisional, Although in practice, the currently used classifications of disorders of the lipid metabolism seem useful and appear to work fairly well, they tend to result in too rigid an approach to the problem. They have, however, resulted in this group of diseases being thought of in lipoprotein rather than lipid terms, with obvious advantages. Furthermore, a more manageable vocabulary has been provided, and terms such as familial type IIa pattern have obvious advantages over the more tongue-twisting alternatives. The shortcomings should, however, not be forgotten and such classifications should be regarded as provisional, pending a more rational classification based on a pathogenetic and mechanistic approach.

CHAPTER VI - REFERENCES

Ahrens, E.H.,Jr., Hirsch, J., Oette, K., Farquhar, J.W. and Stein, Y. (1961) Trans. Ass. Amer. Phycns. 74, 134.

Alaupovic, P. (1971) Atherosclerosis 13, 141.

Barritt, D.W. (1956) Brit. med. J. 2, 640.

Beaumont, J.L., Carlson, L.A., Cooper, G.R., Fejfar, Z., Fredrickson, D.S. and Strasser, T. (1970) Bull. Wld. Hlth. Org. 43, 891.

Berg, K. (1963) Acta path. microbiol. scand. 59, 369.

Bergström, K., Blomstrand, R. and Devi, C.D. (1972) Europ. J. clin. Invest. 2, 272.

Bierman, E.L., Gordis, E. and Hamlin, J.III, (1962) J. clin. Invest. 41, 2254.

Biermen, E.L., Strandness, D.E.,Jr. (1966) Amer. J. Physiol. 210, 13.

Boberg, J. and Hallberg, D. (1968) J. Atheroscler. Res. 8, 351.

Boberg, J. (1971) M.D. Thesis. Acta Univ. Upsal. 105.

Boquillon, M., Paris, R. and Clement, J. (1972) Lipids, 7, 409.

Bouchier, I.A.D. and Bronte-Stewart, B. (1961) Lancet I, 363.

Bradford, R.N. and Furman, R.H. (1968) Biochim. Biophys. Acta 164, 172.

Bragdon, J.H. and Karmen, A. (1960) J. Lipid Res. 1, 167.

Brown, D.F. and Doyle, J.T. (1967) Amer. J. clin. Nutrit. 20, 324.

Brunzell, J.D., Hazzard, W.R., Porte, D.,Jr. and Bierman, E.L. (1973) J. clin. Invest. 52, 1578.

Carlson, L.A. (1960) Acta med. scand. 167, 399.

Carlson, L.A. (1970) Atherosclerosis 12, 181.

Carlson, L.A. and Böttiger, L.E. (1972) Lancet I, 865.

Courel, E. and Clement, J. (1964) Compt. Rend. Soc. Biol. 158, 715.

Denborough, M.A. (1963) Clin. Sci. 25, 115.

Dietschy, J.M. and Siperstein, M.D. (1965) J. clin. Invest. 44, 1311.

Dole, V.P. and Hamlin, J.T.III (1962) Phys. Rev. 42, 674.

Eaton, R.P., Berman, M. and Steinberg, D. (1969) J. clin. Invest. 48, 1560.

Edelin, Y.H., Kinsell, L.W., Michaels, G.D. and Splitter, S.D. (1968) Metabolism 17, 544.

Eisenberg, S., Bilheimer, D.W. and Levy, R.I. (1972) Biochim. Biophys. Acta 280, 94.

Felch, W.C. and Van Italie, T.B. (1960) New Engl. J. Med. 263, 1125.

Folch, J., Lees, M. and Stanley, G.H.S. (1957) J. biol. Chem. 226, 497.

Forte, T. and Nichols, A.V. (1972) Advan. Lipid Res. 10, 1.

Frazer, A.C. and Stewart, H.C. (1937) J. Physiol. (Lond.) 90, 18.

Fraser, R., Cliff, W.I. and Courtice, F.C. (1968) Quart. J. exp. Physiol. 53, 390.

Fredrickson, D.S., Ono, K. and Davis, L.L. (1963) J. Lipid Res. 4, 24.

Fredrickson, D.S. (1972) In The Metabolic Basis of Inherited Disease (eds.J.B.Stanbury, J.B. Wyngaarden and D.S.Fredrickson) McGraw-Hill, New York. p.430.

Fredrickson, D.S., Levy, R.I. and Lees, R.S. (1967) New Engl. J. Med. 276, 34, 94, 148, 215, 273.

Fredrickson, D.S., Levy, R.I. and Lindgren, F.T. (1968) J. clin. Invest. 47, 2446.

Fredrickson, D.S. (1969) Trans. Ass. Amer. Phycns. 82, 68.

Fröberg, S. and Hallberg, D. (1968) J. Atheroscler. Res. 8, 991.

Gage, S.H. and Fish, P.H. (1924) Amer. J. Anat. 34, 1.

Glueck, C.T., Levy, R.I. and Fredrickson, D.S. (1969) Diabetes 18, 739.

Gordis, E. (1962) Proc. Soc. exp. Biol. (N.Y.) 110, 657.

Harlan, W.R. and Bleischer, D.E. (1963) Amer. Heart J. 66, 61.

Hatch, F.T. and Lees, R.S. (1968) Advan. Lipid Res. 6, 1.

Havel, R.J., Eder, H.A. and Bragdon, J.H. (1955) J. clin. Invest. 34, 1345.

Havel, R.J. (1957a) J. clin. Invest. 36, 848.

Havel, R.J. (1957b) J. clin. Invest. 36, 855.

Havel, R.J. and Gordon, R.S.,Jr. (1960) J. clin. Invest. 39, 1777.

Havel, R.J. and Goldfein, A. (1961) J. Lipid Res. 2, 389.

Havel, R.J. (1965) In Handbook of Physiology: Section B: Adipose Tissue (eds. A.E. Renold and G.F. Cahill) Amer. Physiol. Soc. Washington D.C. p.499.

Hazzard, W.R., Goldstein, J.L., Schrott, H.G., Motulsky, A.G. and Bierman, E.L. (1973) J. clin. Invest. 52, 1569.

Hoffman, A.F. and Borgström, B. (1962) Fed. Proc. 21, 43.

Hollister, L.E. (1963) Amer. J. clin. Nutrit. 13, 214.

Jones, R., Thomas, W.A. and Scott, R.F. (1962) Exptl. Mol. Pathol. 1, 1965.

Kayden, H.J., Karman, A. and Dumont, A. (1963) J. clin, Invest. 42, 1373.

Kuo, P.T. and Carson, J.C. (1959) J. clin. Invest. 38, 1384.

La Rosa, J.C. (1972) Postgrad. Med. 52, 75.

Lees, R.S. and Fredrickson, D.S. (1965) J. clin. Invest. 44, 1968.

Levy, R.I., Lees, R.S. and Fredrickson, D.S. (1966) J. clin. Invest. 45, 63.

Levy, R.I., Bilheimer, D.W. and Eisenberg, S. (1971) In Plasma Lipoproteins (eds. R.M.S. Smellie) Academic Press, London. p.3.

Levy, R.I. (1972) Ann. intern. Med. 77, 267.

Lewis, B. (1973) J. clin. Path. 26, suppl. 5, 26.

Lossow, W.J., Lindgren, F.T. and Jensen, L.C. (1967) Biochim. Biophys. Acta 144, 670.

Lossow, W.J., Lindgren, F.T., Murchio, J.C., Stevens, G.R. and Jensen, L.C. (1969) J. Lipid Res. 10, 68.

Mancini, M., Mattock, M., Rabaya, E., Chait, A. and Lewis, B. (1973) Atherosclerosis 17, 445.

Maruhama, Y., Goto, Y. and Yamagata, S. (1967) Metabolism 16, 985.

Nixon, J.C., Martin, W.G., Kalab, M. and Monahan, G.J. (1969) Clin. Biochem. 2, 389.

O'Hara, D.D., Porte, D. and Williams, R.H. (1966) J. Lipid Res. 7, 264.

Ockner, R.K., Bloch, K.J. and Isselbacher, K.J. (1968) Science 162, 1285.

Ockner, R.K., Hughes, F.B. and Isselbacher, K.J. (1969a) J. clin. Invest. 48, 2079.

Ockner, R.K., Hughes, F.B. and Isselbacher, K.J. (1969b) J. clin. Invest. 48, 2367.

Ockner, R.K. and Jones, A.L. (1970) J. Lipid Res. 11, 284.

Ockner, R.K., Pittman, J.P. and Yager, J.L. (1972) Gastroenterology 62, 981.

Pinter, G.G. and Zilversmit, D.B. (1962) Biochim. Biophys. Acta 59, 116.

Rider, A.K., Levy, R.I. and Fredrickson, D.S. (1970) Circulation 42, suppl. 3, 10.

Roberts, W.M. (1931) Quart. J. Med. 24, 133.

Robinson, D.S. (1955) Quart. J. exp. Physiol. 40, 112.

Robinson, D.S. (1970) In Comprehensive Biochemistry (eds. M. Florkin and E.H. Stortz) Elsevier, Amsterdam. p.51.

Roheim, P.S., Gidez, L.I. and Eder, H.F. (1966) J. clin. Invest. 45, 297.

Ruderman, N.B., Richards, K.C. Valles de Bourges, V. and Jones, A.L. (1968) J. Lipid Res. 9, 613.

Ruderman, N.B., Jones, A.L., Krauss, R.M. and Shafrir, E. (1971) J. clin. Invest. 50, 1355.

Scanu, H. and Page, I.H. (1959) J. Exptl. Med. 109, 239.

Schlierf, G. and Raetzer, H. (1972) Nutrit. Metab. 14, 113.

Schonfeld, G. and Kudzma, D.J. (1973) Arch. intern. Med. 132, 55.

Schumaker, V.N. and Adams, G.H. (1969) Ann. Rev. Biochem. 38, 113.

Senior, J.R. (1964) J. Lipid Res. 5, 495.

Simmonds, W.H., Redgrave, T.G. and Willex, R.L.S. (1968) J. clin. Invest. 47, 1015.

Smith, E.B. (1957) Lancet 273, 910.

Spritz, N. and Mishkel, M.A. (1969) J. clin. Invest. 48, 78.

Sterky, G.C.G., Persson, B.E.H. and Larsson, Y.A.A. (1966) Diabetologia 2, 14.

Stone, M.C., Thirpe, J.M., Mills, G.L. and Dick, T.B.C. (1970) Clin. chim. Acta 30, 809.

Sullivan, J.F. (1962) Amer. J. clin. Nutrit. 11, 317.

Tidwell, H.C. and Cameron, E.S. (1942) Bull. John Hopkins Hosp. 70, 362.

Tytgat, G.N., Ruben, C.E. and Saunders, D.R. (1971) J. clin. Invest. 50, 2065.

Wilson, J.D. (1968) J. clin. Invest. 47, 175.

Windmueller, H.G. and Levy, R.I. (1968) J. biol. Chem. 243, 4878.

Windmueller, H.G., Lindgren, F.T., Lossow, W.J. and Levy, R.I. (1970) Biochim. Biophys. Acta 202, 507.

Yokoyama, A. and Zilversmit, D.B. (1965) J. Lipid Res. 6, 241.

Zilversmit, D.B., Sisco, P.H.,Jr., and Yokoyama, A. (1966) Biochim. Biophys. Acta 125, 129.

Zilversmit, D.B., Courtice, F.C. and Fraser, R. (1967) J. Atheroscler. Res. 7, 319.

Zilversmit, D.B. (1969) In Structural and Functional Aspects of lipoproteins in living systems (eds. A. Scanu and E. Tris) Academic Press, London. p.329.

Zweets, A., Yaron, E. and Groen, J.J. (1968) Clin. chim. Acta 19, 267.

SUMMARY

Elevated plasma triglyceride levels are associated with an increased incidence of ischaemic heart disease. The processes by which plasma triglyceride concentration is controlled in health and disease are incompletely understood.

This thesis describes studies on some of the major determinants of plasma triglyceride metabolism, viz. dietary fat, alcohol and diabetes mellitus, and the mechanisms whereby they affect plasma triglyceride concentration.

A new intravenous fat tolerance test, which relies on measurement of the removal rate of a triglyceride emulsion from plasma, has been applied.

In uncontrolled diabetes mellitus, more than a half of the patients studied had hypertriglyceridaemia, whether the diabetes was diet responsive or insulin dependent. The raised triglycerides were associated with elevated VLDL concentrations. Plasma free fatty acid turnover was elevated in nearly all the untreated diabetics; this was especially evident in insulin-dependent cases; turnover was also somewhat elevated in non-diabetic obese subjects.

The rate constant for the removal of exogenous triglyceride, K_2, correlated inversely with serum triglycerides, and in many cases was towards the lower end of the normal

range. Diabetic control, whether by diet alone, or by diet and insulin, resulted in a reduction of serum triglyceride concentration towards normal. At the same time, K_2 values increased significantly. The changes in K_2 were paralleled by changes in the rate constant for the removal of exogenous triglyceride operating under conditions of saturation kinetics (K_1). Plasma FFA turnover also fell during diabetic control.

Weight reduction in obese non-diabetics resulted in a reduction of serum triglycerides; associated with this, FFA turnover increased, in marked contrast to the obese diabetic patients.

It was concluded that diabetic hypertriglyceridaemia may be associated with impaired removal of endogenous triglyceride from plasma, although simultaneous increased input of triglyceride into plasma was not excluded.

Alcoholic hyperlipidaemia was found to occur commonly amongst patients attending a clinic for disorders of lipid metabolism. K_2 values tended to be low and did not correlate with serum triglyceride levels.

Alcohol withdrawal resulted in a significant lowering of serum triglyceride concentration; no associated change in fractional removal rate of triglyceride was found, in sharp contrast to the significant increase associated with correction of diabetic hypertriglyceridaemia.

It is concluded that correction of alcoholic hypertriglyceridaemia is not due to an enhanced fractional rate of removal of triglyceride from plasma. The findings are consistent with the view that alcohol results in an increased input of triglyceride into plasma. It is proposed that a limited capacity for removal of triglyceride from plasma, makes an individual susceptible to the triglyceride elevating effect of alcohol.

Diets rich in polyunsaturated fat and low in saturated fat, lowered serum cholesterol and especially serum triglyceride concentrations in normal subjects and hypertriglyceridaemic men. The reduction in triglyceride levels was due mainly to a change in very low density lipoprotein triglyceride. The fall in cholesterol was, however, associated with a significant reduction of both low density and very low density lipoprotein cholesterol; a change in the lipid composition of very low density lipoprotein was detected.

Neither K_2, nor post-heparin lipolytic activity changed significantly when triglyceride concentration fell on substituting a polyunsaturated fat diet for one in which the fat content was predominantly saturated. It was concluded that the fall in serum triglyceride concentration induced by polyunsaturated fat could not be accounted for by enhanced triglyceride uptake from plasma, and must, therefore, have been due to reduced input.

The relative rates of incorporation of a saturated and a polyunsaturated fatty acid into very low density lipoprotein triglyceride was measured during their simultaneous infusion into normolipaemic men. The results suggested that palmitic acid was preferentially incorporated compared with linoleic acid. As the feeding of polyunsaturated fat results in an increased ratio of free linoleate to free palmitate in plasma, this may imply that dietary polyunsaturated fat decreases the rate of secretion of very low density lipoprotein triglyceride into plasma.

Figure 39 shows the effect of diabetes, alcohol and dietary fat on serum triglyceride and on fractional removal rate of triglyceride from plasma, in diagrammatic form. In normal individuals, serum triglyceride levels appear to vary inversely with fractional removal rate. Low K_2 values are associated with high serum triglycerides. A similar situation appears to exist amongst diabetics. In the uncontrolled state, triglyceride levels tend to be higher and K_2 values lower than normal individuals. Alcoholic hypertriglyceridaemia tends to be associated with low K_2 values, which remain unchanged when alcohol withdrawal results in a reduction of serum triglycerides Dietary fat-induced changes in serum triglyceride also tend to occur without effect on fractional removal rate.

The alimentary lipaemia following ingestion of dietary fat by normolipaemic men, was due to an increase in both chylomicron and very low density lipoprotein triglyceride. After large fat loads, chylomicronaemia predominated, but after smaller more physiological loads, the increase in very low density lipoprotein triglyceride was similar to or even

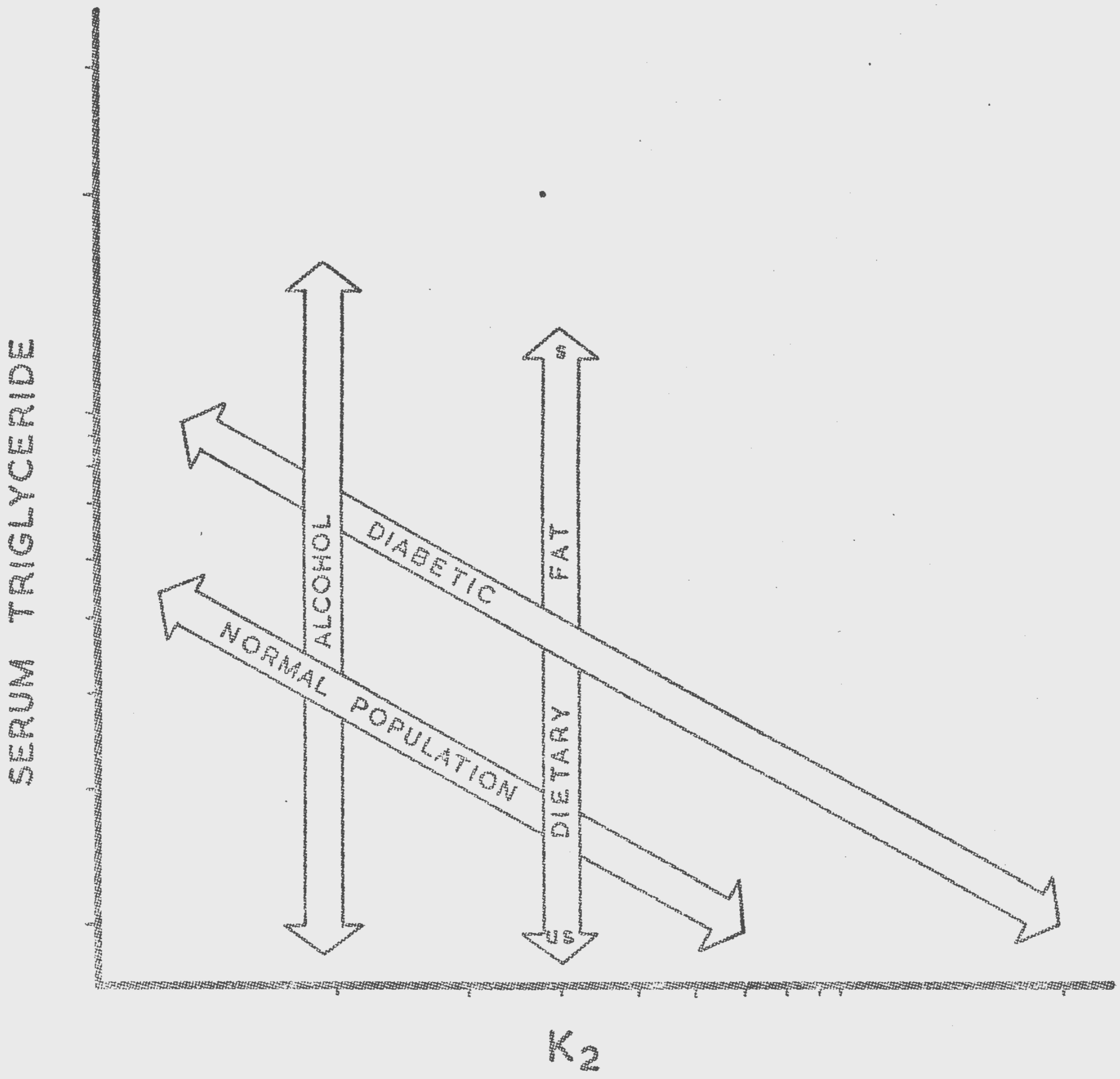

Fig. 39. Relationship between serum triglyceride and K_2 - effect of dietary factors, alcohol and diabetes mellitus

exceeded that in chylomicron triglyceride. It is concluded that both chylomicrons and very low density lipoprotein play an important role in dietary fat transport in man.

No evidence was found that either of these lipoprotein classes showed a differential contribution to alimentary lipaemia when saturated and polyunsaturated fat meals were compared. Serum triglyceride fluctuations throughout the day were, however, lower after a period of polyunsaturated fat feeding than after a period of feeding saturated fat. The 24 hour increment above base-line levels did not differ significantly on the two diets, yet the increment correlated significantly with the endogenous triglyceride level. It is suggested that the differences in levels throughout the day on the two diets could largely be attributable to differences in endogenous triglyceride levels.

ACKNOWLEDGEMENTS

I wish to acknowledge with sincere thanks, the help and encouragement of Dr. Barry Lewis, Senior Lecturer in the Department of Chemical Pathology, Royal Postgraduate Medical School, and Honorary Consultant in Medicine at the Hammersmith Hospital, London. His advice, discussion and assistance were a constant encouragement.

I would also like to acknowledge the assistance of Mr. A. February, Miss A. Nicoll and Dr. M. Mattock with some of the ultracentrifugation and analyses.

The diagrams were kindly drawn by Mr. A. February and the typing by my wife and Mrs. C. Eakins.

The help given by the Dietetics Department of the Hammersmith Hospital, especially by Miss E. Rabaya, is acknowledged with thanks.

During the time at which the studies were being performed, I was supported in part by a grant from Unilever Ltd., Holland. Payne and Byrne Ltd., England, kindly provided the Intralipid, Van den Berghs Ltd., the sunflower oil, and the Lister Institute, Elstree, Herts., the human albumin for injection.

Miss D. Sadler's expert assistance with the production was greatly appreciated.

Finally, I would like to express my gratitude to the many members of hospital and laboratory staff, and patients, who volunteered to participate in the experiments, and to my many colleagues for their helpful suggestions and discussions. In particular, I would like to express my thanks to Drs. M. Mattock, G. Sigurdsson, J. Davies, G. Onitiri, N. Myant, B. Tulloch and Professor R. D. Cohen.

APPENDIX 1

1000 CALORIE BREAKFASTS CONTAINING DIFFERENT AMOUNTS OF FAT

"0"g. fat breakfast (fat content ± 1.7g.)

1 piece fruit
1 packet marmalade
4 packets sugar
1 oz. cornflakes
7 oz. skimmed milk
2 slices bread
1 bottle 'Hycal'
½ oz. 'Marvel'

20g. fat breakfast

7 oz. milk
1 oz. cornflakes
4 packets sugar
1 slice bread
½ oz. butter
1 packet marmalade
1 bottle 'Hycal'

50g. fat breakfast

11 oz. milk
1 oz. cornflakes
4 packets sugar
2 slices bread
1 packet marmalade
1 oz.double cream
1 piece fruit
1 oz. butter

100g. fat breakfast

1½ slices bread
5 oz. double cream
4 oz. milk
1 oz. butter

APPENDIX 2

750 CALORIE, 50g. FAT EXPERIMENTAL MEALS

Saturated

1 pint milk)
60g. double cream) Homogenised
Artificial flavouring 10 ml)
1 slice bread
1 packet marmalade

Unsaturated

2 oz. 'Marvel')
50 ml. pure sunflower oil) Homogenised with 500 ml water
Artificial flavouring 10 ml)
1 slice bread
1 packet marmalade

APPENDIX 3

1000 CALORIE EXPERIMENTAL BREAKFASTS CONTAINING DIFFERENT AMOUNTS OF SATURATED AND POLYUNSATURATED FAT

20g. saturated fat

7 oz. milk (210 ml)
1 oz. cornflakes
4 packets sugar
1 slice bread
½ oz. butter (15g.)
1 packet marmalade
1 bottle 'Hycal'
Tea or coffee with sugar and milk from allowance

50g. saturated fat

11 oz. milk (330 ml)
1 oz. cornflakes
4 packets sugar
2 slices bread
1 oz. butter (30g.)
1 packet marmalade
1 oz. double cream (30g.)
1 piece fruit
Tea or coffee with sugar and milk from allowance

100g. saturated fat

1½ slices bread
1 oz. butter (30g.)
3 oz. double cream (taken with coffee) (90g.)
4 Oz. milk (120 ml) + 2 oz. double cream (60g.) mixed with a drink

20g. unsaturated fat

Soup containing 20 ml pure sunflower oil and 1 bouillon cube
2 slices bread
2 packets marmalade
1 bottle 'Hycal'
3 packets sugar
7 oz. fruit juice (210 ml)

50g. unsaturated fat

50 ml pure sunflower oil - 25 ml as soup with one bouillon cube
- 25 ml mixed with tomato juice
1½ slices bread
1 packet marmalade
1 bottle 'Hycal'

100g. unsaturated fat

100 ml pure sunflower oil - made into a soup with bouillon cube
1 slice bread
1 packet jam