

**THE REGULATION OF POSTPRANDIAL
LIPEMIA IN MAN**

Submitted in fulfillment of the degree

Doctor of Philosophy in Physiology

By Jonathan Cohen

University of Cape Town, 1988

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To my parents

CONTENTS

Acknowledgements	I
Declaration	II
List of Tables	III
List of Figures	IV
List of Publications	VI
List of Abbreviations	VII
Preface	VIII
Abstract	IX
Introduction	1
Chapter 1. General Methodology	8
Chapter 2. A Comparison of Three Methods for Assessing Chylomicron-Triglyceride Clearance.	15
Chapter 3. The Effects of Meal Fat Content on Postprandial Lipemia.	29
Chapter 4. The Effects of Protein Ingestion on Postprandial Lipemia and Chylomicron Triglyceride Clearance.	38
Chapter 5. The Effects of Carbohydrates and Insulin on Postprandial Lipemia and Chylomicron Clearance.	42
Chapter 6. The Effects of Chronic Endurance Exercise Training on Postprandial Lipemia and Chylomicron-Triglyceride Clearance	64
General Conclusions	74
References	76

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DECLARATION

I hereby declare that the work described in this thesis was conceived and executed by myself except where otherwise indicated.

LIST OF TABLES:

Table 1. Reproducibility data for oral fat tolerance tests using 40g, 80g, and 120g fat loads.	9
Table 2. Reproducibility data for intravenous fat tolerance tests.	10
Table 3. Clinical data and triglyceride clearance measurements of 12 normolipidemic men.	21
Table 4. Pearson correlation coefficients (r) between postprandial lipemia, Intralipid clearance, chylomicron clearance, and fasting triglyceride concentration.	22
Table 5. Fasting serum cholesterol and triglyceride concentrations, postprandial lipemia and maximum postprandial serum triglyceride concentrations in 12 men fed 3 doses of dairy cream.	33
Table 6. Serum triglyceride responses of 12 men to meals containing saturated, monounsaturated, and polyunsaturated fat.	34
Table 7. Clinical data of 21 subjects in whom 5 different carbohydrate treatments were compared.	48
Table 8. Effects of 5 different carbohydrate treatments on the serum triglyceride responses to a fat meal.	48
Table 9. Age and anthropometric data of 18 men and women in whom the effects of glucose were studied.	49
Table 10. Plasma insulin and triglyceride responses of sedentary subjects to meals containing glucose and fat.	49
Table 11. Serum triglyceride responses to meals containing cream alone, urea + cream, and starch + cream.	50
Table 12. Fasting and postprandial serum triglyceride concentrations in men with type I hyperlipoproteinemia.	53
Table 13. Age, anthropometry and clinical data of male and female Type I diabetics.	54
Table 14. Fasting metabolite and hormone concentrations and postprandial lipemia in Type I diabetics.	54
Table 15. Ages, body weights, fasting TG concentrations and chylomicron-TG clearance in athletes and sedentary men.	67

LIST OF FIGURES

Figure 1. Linear regression analysis comparing serum optical density with serum Intralipid-triglyceride concentration.	12
Figure 2. Linear regression analysis comparing serum optical density with serum Intralipid-triglyceride concentration determined by ultracentrifugation.	13
Figure 3. Mean serum triglyceride concentrations of 12 normolipidemic men after ingesting 100ml dairy cream.	19
Figure 4. Mean serum optical density of normolipidemic men after intravenous Intralipid administration.	20
Figure 5. Mean serum triglyceride concentrations of 12 normolipidemic men during duodenal fat perfusion.	21
Figure 6. Plasma Intralipid concentrations of subjects during infusion with normal saline or heparin.	23
Figure 7. Serum triglyceride concentrations of 5 normolipidemic subjects during heparin infusion.	23
Figure 8. Serum triglyceride responses to 40g, 80g, and 120g fat ingestion in 12 normolipidemic men.	33
Figure 9. Mean serum triglyceride concentrations of 15 normolipidemic subjects after meals containing 23g protein and 40g fat or 40g fat alone.	40
Figure 10. Mean serum triglyceride concentrations after meals containing 40g fat, 40g fat + 50g glucose, or 40g fat + 100g glucose in 18 normolipidemic subjects.	50
Figure 11. Mean serum triglyceride concentrations of 8 normolipidemic subjects after the ingestion of 40g fat, 40g fat + 50g glucose, and 40g fat + 16.6g urea.	51
Figure 12. Mean serum triglyceride concentrations of 8 normolipidemic subjects after the ingestion of 40g fat, 40g fat + 100g glucose, and 40g fat + 100g starch.	51
Figure 13. Mean serum optical density after Intralipid injection in 17 normolipidemic men before and 2 hours after 50g glucose ingestion.	52
Figure 14. Mean serum triglyceride concentration of 10 men during duodenal perfusion with 10% olive oil, and 10% olive oil + 20g glucose/hour.	53
Figure 15. Blood Beta-Hydroxybutyrate responses to fat ingestion in diabetic patients with and without concomitant insulin administration.	55
Figure 16. Blood glucose responses to fat ingestion in diabetic patients with and without concomitant insulin administration.	55

Figure 17. Serum triglyceride responses to fat ingestion in diabetic patients with and without concomitant insulin administration.	56
Figure 18. Plasma optical density after Intralipid administration in diabetic patients with and without concomitant insulin administration.	57
Figure 19. Serum triglyceride responses to 140g fat ingestion in athletes and in sedentary men.	68
Figure 20. Serum triglyceride responses to 40g fat ingestion in athletes and in sedentary men.	68
Figure 21. Serum triglyceride responses of sedentary men to 120g fat ingestion before and after exercise.	69
Figure 22. Serum optical density after Intralipid administration in athletes and in sedentary men.	69
Figure 23. Serum triglyceride responses to duodenal perfusion in athletes and in sedentary men.	70

List of Publications

This thesis is based on the following publications:

- 1) Cohen JC. A comparison of three methods for assessing chylomicron triglyceride clearance. *Am J Clin Nutr* (in press)
- 2) Cohen JC, Noakes TD, Benade AJS. Serum triglyceride responses to fatty meals: Effects of meal fat content. *Am J Clin Nutr* 1988;47:825-827
- 3) Cohen JC, Schall R. Reassessing the effects of simple carbohydrates on the serum triglyceride responses to fat meals. *Am J Clin Nutr* (in press)
- 4) Cohen JC, Benade AJS, Noakes TD. Chylomicron clearance in athletes and in sedentary men. *Am J Clin Nutr* (in press).
- 5) Cohen JC. Protein ingestion does not affect postprandial lipemia or chylomicron-triglyceride clearance. *Eur J Clin Nutr* (in press).

List of Abbreviations:

very low density lipoproteins; VLDL

low density lipoproteins; LDL

high density lipoproteins; HDL

triglyceride; TG

lipoprotein lipase; LPL

PREFACE

Although it has been recognized for decades that fat ingestion is associated with an acute increase in the plasma triglyceride pool size (1), the factors which determine the magnitude of the postprandial plasma triglyceride excursion remain poorly understood. Several investigators have sought to determine the importance of postprandial lipemia in the pathogenesis of atherosclerosis (2-4), but the physiological factors which influence postprandial plasma triglyceride concentrations have received little study. An early, but comprehensive review of the literature by Angervall (5) included fewer than 15 references to the topic. The paucity of systematic investigations into the regulation of postprandial triglyceride metabolism has also been noted in a recent review by Tall (6). The work described in this thesis was accordingly undertaken to identify factors which may play a role in the acute regulation of postprandial plasma TG concentrations. The thesis comprises a series of related but independent chapters, each of which describes an investigation into the putative role of one such factor. Each chapter includes a review of the appropriate literature, an explanation of why the experiment was undertaken, a description of methods and results, and a discussion of the results in the context of current knowledge. To minimize redundancy, the laboratory and clinical procedures used throughout the study have been described in detail in the chapter entitled "General Methodology".

ABSTRACT

The regulation of the serum triglyceride responses to fat ingestion have been examined in normolipidemic men. To evaluate the existing methods for comparing chylomicron-triglyceride clearance, the oral and intravenous fat tolerance tests and a steady state duodenal perfusion method were compared. Good correlations ($r > 0.8$) were found between each of these methods. Since the intravenous fat tolerance test is independent of fat absorption, these data suggested that the serum triglyceride response to fat feeding was largely determined by the rate of chylomicron-triglyceride clearance. To determine the influence of the quantity and type of meal fat on postprandial serum triglyceride concentrations, the serum triglyceride responses to three different doses of dairy cream, and to standard doses of olive and sunflower oil were examined. For a given type of fat, the magnitude of postprandial lipemia (the integrated serum triglyceride excursion) varied directly with the quantity of fat in the meal. This finding suggested that the chylomicron-triglyceride clearance system(s) did not become saturated even after large fat meals. In addition, it appeared that the hormonal factors released in response to fat ingestion (some of which are known to increase lipoprotein lipase activity *in vitro*) did not increase the rate of chylomicron-triglyceride clearance. If the quantity of fat in a meal was fixed, then postprandial lipemia increased with increasing saturation of the triglyceride fatty acids. These differences did not appear to reflect differences in triglyceride absorption.

Since acute fat feeding per se did not appear to stimulate chylomicron-triglyceride clearance, the effects of dietary proteins and carbohydrates were studied. The addition of up to 35g protein to a standard test meal did not affect postprandial lipemia. These results were supported by the observation that protein ingestion did not affect intravenous fat tolerance.

Postprandial serum triglyceride concentrations were significantly influenced by carbohydrate ingestion. Fructose (50g) and sucrose (100g) markedly increased postprandial lipemia, although glucose ingestion did not. In agreement with earlier studies, glucose ingestion decreased serum triglyceride concentrations 2 hours after the meal. This effect was abolished by intraduodenal fat administration and by substituting starch for glucose in the test meal. The effects of glucose could be reproduced by iso-osmotic quantities of urea, however. These findings suggested that glucose ingestion did not increase chylomicron-triglyceride clearance. It is more likely that glucose delayed the absorption of triglycerides by slowing gastric emptying, and that this effect was partly related to the increased osmolarity of glucose-containing meals.

The effects of chronic exercise on postprandial lipemia and chylomicron-triglyceride clearance were determined in endurance-adapted athletes. The serum triglyceride responses to large and small fat meals were lower in athletes than in sedentary men with comparable fasting triglyceride concentrations. These differences were not eliminated by a single bout of acute exercise in the sedentary men. The clearance of intravenously administered Intralipid, and chylomicron-triglyceride clearance assessed from steady state chylomicron-triglyceride concentrations during duodenal fat perfusion were faster in athletes than in the sedentary men. These data suggested that the low postprandial lipemia in athletes reflects increased chylomicron-triglyceride clearance caused by increased activity of the triglyceride clearing system(s).

Given these considerations, it appears that the pathway(s) for chylomicron triglyceride clearance are extremely efficient in normal men and that these pathways are not subject to acute physiological regulation.

INTRODUCTION

In men consuming Western diets, fats comprise some 40% of the daily caloric intake (7). The bulk of these fats (90%) are ingested as long-chain triglycerides (TG), containing primarily C16 and C18 fatty acids (8,9). Other lipids such as cholesterol and phospholipids generally contribute less than 10g to the total weight of the diet. Accordingly, a Western man consuming 3000 Calories per day ingests some 130g fat daily, of which approximately 120g is TG.

Digestion and absorption of dietary triglyceride:

The digestion and absorption of TG has been extensively studied, and several reviews of the topic have been published. A brief description of the absorptive processes is presented here. For more detailed information the reader is referred to reviews by Friedman (10), Carey (11), and Tso (12).

Triglyceride digestion comprises 3 sequential steps:

- i) The dispersion of bulk fat globules into emulsion particles,
- ii) The enzymatic hydrolysis of fatty acid esters, and
- iii) The desorption and dispersion of insoluble lipid products into an absorbable form.

The emulsification of ingested fat begins in the stomach, where long-chain TG may be stored for 2-4 hours. During this time, peristaltic contractions facilitate the intra-gastric mixing of bulk phase fat globules with complex polysaccharides, membrane derived phospholipids, and peptic digests of dietary proteins which constitute potential emulsifiers (11). Enzymatic hydrolysis of TG also begins in the stomach. Most gastric lipolysis is catalyzed by lingual lipases which originate in the serous glands (von Ebner) beneath the circumvallate papillae of the tongue (13). Some authors have suggested that lingual lipases digest as much as 30% of the total dietary TG (11).

When gastric chyme is propelled through the pylorus into the duodenum, the shear forces exerted on the mixture promote the dispersion of dietary TG into small, stable emulsion particles (14). Peristaltic contractions facilitate the mixing of these particles with biliary lipids and bile salts, which aid fat emulsification (11). The hydrolysis of dietary TG is largely completed in the upper small intestine by a pancreatic lipase which cleaves the primary (sn-glycerol 1- and 3-) ester bonds of the TG molecules, generating from each a 2-monoglyceride and 2 free fatty acids (12). Pancreatic lipase is present in intestinal chyme at concentrations some 100-1000 fold in excess of that needed for complete hydrolysis of the intestinal TG (11). This finding is consistent with the observation that TG absorption is extremely efficient in normal men.

It is not known how the products of TG digestion desorb from the micelles of intestinal chyme and enter the absorptive enterocytes. There is general consensus that newly absorbed fatty acids and monoglycerides cross the microvillus membrane by passive diffusion and are re-esterified to TG within the smooth endoplasmic reticulum (SER) of the enterocyte. The predominant pathway within the SER is direct synthesis of TG from 2-monoglyceride and 2 molecules of activated fatty acid (15). Morphological studies have indicated that the re-esterified TG forms oil droplets in the SER and that these droplets are associated with phospholipids, cholesterol and cholesterol esters. These intracellular lipid aggregates are referred to as pre-chylomicrons (16). Several apolipoproteins (A-I, A-IV, and B) are added to the pre-chylomicron in the enterocyte, but it is not known whether these apolipoproteins are added

at the same time, or where the association of lipid and protein occurs. Apo B is required for the synthesis and secretion of TG-rich lipoproteins by the intestine (17), but the role of other apolipoproteins in these processes is less clear. Maturing chylomicrons move from the SER to the Golgi apparatus, where glycosylation of proteins takes place. During this time, the pre-chylomicrons become considerably enriched in phospholipid (16).

During active lipid absorption, the Golgi cisternae become distended with pre-chylomicron droplets, and bud off from the Golgi apparatus to form vesicles. The vesicles containing pre-chylomicrons migrate towards the plasma membrane and release their contents into the intercellular space by exocytosis. The TG-rich lipoproteins which contain dietary lipids are now referred to as chylomicrons.

Structure of lymph chylomicrons:

In intestinal lymph, chylomicron particles range in size from 80 to 500nm. Chylomicron size is believed to vary as a function of the rate of TG absorption (18). The chemical composition of lymph chylomicrons may also vary, although TG invariably comprise the bulk of the particle weight. Zilversmit (19) reported that chylomicrons isolated from the intestinal lymph of a dog fed corn oil contained 95-96% TG, 3-4% phospholipid, 0.6% cholesterol, 0.2% cholesterol ester, and 0.6% protein. Comparable data were reported for human chylomicrons isolated from chyluric patients by Green et al (20).

The gross architecture of the chylomicron particle is determined by its chemical composition. The particles are spherical, comprising a hydrophobic core of TG and cholesterol esters surrounded by a closely packed monolayer of cholesterol and phospholipid. The gaps between the polar heads of this monolayer are filled by apoproteins. Lymph chylomicrons have been shown to contain all major apoproteins (including A-I, A-II, A-IV, B, the C peptides, and E). The apoprotein composition of chylomicrons isolated from chyluric patients (20) comprised 3.4% apo B, 10% apo A-IV, 4.4% apo E, 15% apo A-I, and 47.3% apo A-II and C. Animal studies have demonstrated that the small intestine is the site of synthesis of apo A-I, A-IV and B (21), but the C peptides and apo E do not appear to be synthesized in the intestinal cells to any significant extent, and are probably transferred to the chylomicron surface after secretion (24). Therefore the presence of these apolipoproteins in intestinal lymph samples indicates contamination by hepatic lymph and filtered plasma.

Tissue specific heterogeneity of apo B has been shown in several species including man. These forms have been separated by polyacrylamide gel electrophoresis and are conventionally described in terms of the nomenclature proposed by Kane (22). At least 2 forms, B48 and B100, are believed to occur in native lipoproteins. There is some evidence that these proteins are transcribed from the same gene. A post-transcriptional modification of the apo B mRNA in intestinal cells results in incomplete translation of the gene product (23), resulting in a smaller protein (apo B48). To date, this modification has not been described in human liver cells. Accordingly in the human, apo B48 appears to be produced exclusively in the intestine and may prove to be a useful marker of intestinal lipoproteins.

Intravascular metabolism of chylomicrons:

The intravascular metabolism of chylomicrons proceeds in 2 stages. Initially, the particles undergo sequential delipidation within the circulation. The TG component is hydrolyzed by an endothelial-lipase (lipoprotein lipase) which is synthesized in most extrahepatic tissues, including adipose, skeletal muscle, heart muscle, lung, brain and adrenal gland (25). The pivotal role of this enzyme in chylomicron-TG clearance is clearly illustrated by the florid chylomicronemia which arises in patients with congenital lipoprotein lipase (LPL) deficiency (26), and by animal studies in which immunological blockade of LPL function causes a dramatic rise in plasma TG concentrations (27). The extrahepatic localization of the lipase suggests that most dietary TG is cleared from the circulation by peripheral (non-hepatic) tis-

sues. This hypothesis is supported by animal studies in which the major fraction of radiolabelled chylomicron-TG is recovered from adipose tissue and skeletal muscle after intravenous infusion (28,29).

If LPL hydrolysis is the rate-limiting step for chylomicron-TG clearance then it seems likely that postprandial serum TG concentrations will be determined largely by the aggregate LPL activity of the vascular endothelium. While the qualitative aspects of this relationship have been established, attempts to define a quantitative relationship between LPL activity and postprandial lipemia have been unsuccessful.

In contrast to the high correlation between postprandial lipemia and chylomicron-TG clearance reported by Nestel (30), several investigators have found weak correlations between postprandial serum TG concentrations and LPL activity measured during the fasted state in postheparin plasma (31) or in adipose tissue (32,33). It is not clear whether this finding reflects an inconsistency in the generally accepted model of chylomicron-TG removal, or an error in the extrapolation from *in vitro* determinations of LPL activity to the functional activity of LPL in the whole animal. Uncertainty regarding the relationship between LPL activity and postprandial lipemia is compounded by 2 further considerations:

a) Passive regulation of triglyceride clearance:

The relationship between plasma TG flux and plasma TG concentration is usually analysed in terms of the classical theory of enzyme kinetics proposed by Michaelis and Menten. According to this theory, the curve described by plasma TG transport plotted against TG concentration assumes a hyperbolic shape. At low plasma TG concentrations, the rate of TG clearance is concentration dependent. Under these conditions (first order kinetics), the fraction of the plasma TG pool removed per unit time is independent of the pool size because the probability of any given productive particle-enzyme interaction occurring is not diminished by the presence of other particles. The disappearance of a tracer or of a bolus of particles injected into this system conforms almost exactly to a monoexponential decay.

As plasma TG concentrations increase further, however, the corresponding increase in the rate of TG clearance becomes smaller. At these rates of plasma TG clearance, TG removal no longer proceeds as a first order reaction. At very high concentrations of plasma TG, the clearance mechanism approaches saturation. At this point the rate of change of plasma TG clearance approaches zero order and further increases in the plasma TG concentration produce little or no change in the absolute rate of TG removal.

In normolipidemic men, the TG clearance mechanism does not appear to become saturated during the postprandial phase (34). Brunzell (35) and others (36) have reported that the TG clearance mechanism is saturable, but this condition occurs at plasma TG concentrations well above the normal level. While the TG clearance mechanism operates below its saturation threshold, variations in the absolute rate of removal of TG can be achieved without any alteration in LPL activity or in the affinity of the enzyme for its substrate. This system will accommodate the waves of chylomicron-TG which enter the circulation during the postprandial phase, and will maintain the plasma TG concentrations within a relatively narrow range. For example, Grundy and Mok (37) reported that a 10 to 20 fold increase in the rate of influx of TG into the circulation induced by intraduodenal infusion of TG led to a concomitant rise in the plasma TG pool size of less than 60% in normolipidemic men.

Passive modulation of TG removal may also be effected by heterogeneity of the chylomicron structure which influences its interaction with LPL. Quarfordt and Goodman (38) reported that the TG of large chylomicrons have a shorter half life in the circulation than do the TG of small chylomicrons. Since chylomicron size appears to be proportional to dietary fat load (39), it is possible that the TG entering the circulation after a high fat meal are cleared more efficiently than those following a low fat meal.

For normal function against lipoprotein substrates, LPL requires an activator peptide, apo C-II (40). Homozygous apo C-II deficiency confers Type I hyperlipoproteinemia, the lipoprotein profile found in genetic LPL deficiency (41). This observation illustrates the absolute requirement for apo C-II in normal chylomicron metabolism. Although it is generally accepted that the plasma concentration of apo C-II is not a determinant of chylomicron clearance rate (42), it is possible that the distribution of apo C-II between plasma lipoproteins may influence chylomicron clearance. Havel et al (43) reported that apo C-II are transferred from HDL to chylomicrons during the postprandial phase. The transfer appeared to be proportional to the size of the acceptor particles. The larger the chylomicrons, the more apo C-II they received. The apo C-II content of chylomicrons may also be determined by the apo A-I content of the nascent particles. Erkelens and Mocking (44) reported that apo C binding to artificial TG-rich particles (Intralipid) in vitro could be increased by prior incubation of the Intralipid with apo A-I. Furthermore, incubation with apo A-I in vitro increased both apo C-II binding and Intralipid clearance in vivo. These studies suggest that chylomicron apolipoprotein composition may influence the metabolism of dietary TG.

The site of uptake of chylomicron-TG may also be passively modulated as a consequence of tissue-specific heterogeneity of the affinity of LPL for TG substrate. Fielding et al (45) have reported that the LPL isolated from rat heart has a K_m of 0.07mM, while the corresponding value for rat adipose tissue is 0.70mM. These data indicate that normal plasma TG concentrations provide saturation of the heart lipase, but not of the adipose tissue enzyme. Nilsson-Ehle (46) has suggested that under fasting conditions when plasma TG levels are relatively low, TG uptake into adipose tissue will be comparatively small, while uptake into heart will proceed at a high rate. After the ingestion of a fatty meal, TG uptake into adipose tissue will increase with increasing plasma TG concentrations, while uptake into heart will remain constant as the lipase in this tissue is already saturated. This heterogeneity would facilitate the metabolic channeling of TG in accordance with the energy requirements of different tissues.

b) Active regulation of TG clearance:

In contrast to passive modulation, where the amount of substrate hydrolyzed varies without accompanying changes in LPL activity, active regulation of TG hydrolysis is accomplished by changes in enzyme activity. For example, adipose tissue LPL activity is increased postprandially in several strains of rats and in cattle (see 47 for review). This regulation is believed to be under hormonal control. Several hormones have been shown to influence LPL activity in adipose tissue.

i) Insulin: Given the primacy of insulin in the regulation of glucose homeostasis, the concept that this hormone co-ordinates fuel substrate homeostasis during the postprandial state is teleologically attractive. In the metabolic scenario proposed by Nilsson-Ehle (46) the active regulation of LPL activity by insulin augments the tissue specific uptake of plasma TG which occurs passively as a consequence LPL heterogeneity. Accordingly, under fasting conditions, low plasma insulin concentrations lead to low adipose tissue LPL activity, and hence a comparatively limited uptake of plasma TG into adipose tissue. The postprandial rise in plasma insulin concentrations increases adipose tissue LPL activity and accelerates the rate of uptake of plasma TG into this tissue. The role of insulin in the acute regulation of adipose tissue LPL activity has been extensively studied in animals and in man (25,47,48). In the rat, abundant evidence in support of a physiological role for insulin in the regulation of adipose tissue LPL activity has been provided by in vivo and in vitro studies (25,47). The evidence for a similar role in man is less clear, however.

In vivo studies in man have indicated that the administration of glucose (49) or insulin and glucose (50) increases adipose tissue LPL activity, while prolonged insulin deficiency such as may occur in Type I diabetes diminishes the activity of this enzyme (51). The physiological significance of this observation is dubious, as little (33) or no (52) increase in adipose tissue LPL activity occurs after mixed meals, despite substantial postprandial increments in postprandial lipemia. The discrepancy between the effects of glucose and mixed meals may be explained by the finding that insulin-induced increases in

adipose tissue LPL activity are significantly blunted by the concomitant ingestion of fat (53). Experiments in which the source of dietary carbohydrates was varied to provide varying insulin responses raised further doubts regarding the role of insulin as a primary regulator of adipose tissue LPL activity. Paired feeding studies performed in hypertriglyceridemic patients indicated greater increases in adipose tissue LPL activity in response to fructose than to glucose, despite substantially higher plasma insulin concentrations after glucose meals (54). On the basis of these studies Brunzell et al (48) concluded that insulin plays a permissive role, but not a primary role in the regulation of adipose tissue LPL activity.

The effect of insulin on skeletal muscle LPL activity is uncertain. Some studies in the rat (55) have indicated an inverse association between plasma insulin concentrations and LPL activity of heart and skeletal muscle, but no similar correlation has been found in normal or insulin-deficient man (56). More recent studies suggest that skeletal muscle LPL activity is maintained at low levels by normal carbohydrate intake (57). Reduction of the carbohydrate content of the diet leads to increased LPL activity in skeletal muscle within 2 weeks.

ii) Gastric inhibitory polypeptide (GIP): A possible role for GIP in the postprandial regulation of LPL activity and chylomicron TG clearance is suggested by *in vitro* studies (58) which indicate increased LPL activity in adipocytes exposed to this hormone. This hypothesis was supported by studies in dogs in which GIP administration in doses calculate to reproduce postprandial plasma GIP concentrations augmented the clearance of intravenously administered lymph chylomicrons (59). Jorde, using the intravenous fat tolerance test, failed to reproduce these findings in humans (60). It is not known whether this discrepancy reflects species (dog versus human) or methodological (chylomicrons versus Intralipid) differences.

iii) Other gastrointestinal hormones: *In vitro* studies using adipose tissue from fasted rats indicate that the gastro-intestinal hormones gastrin and pancreaticozymine increase LPL activity at concentrations which simulate the fed state *in vivo* (61). The effect of gastrin appears to be glucose dependent, but that of pancreaticozymine is glucose-independent and cumulative with that of insulin. Eckel has suggested a possible role for these hormones, particularly pancreaticozymine, in regulating adipose tissue LPL *in vivo* (47) but the effects of these hormones on chylomicron clearance has not been studied.

Other hormones including growth hormone, thyroid hormone, prolactin, the adrenergic hormones and the glucocorticoids have been shown to influence adipose tissue LPL activity (47). The effects of these hormones are likely to result from chronic administration, and their role in the acute regulation of plasma TG concentrations is doubtful. Accordingly the effects of these hormones will not be considered further here.

Fate of redundant surface material:

The hydrolysis of TG in the chylomicron core causes a reduction in the volume of the particle, and consequently, the redundancy of polar surface material. Several studies have indicated that the redundant surface components are transferred to (or form) high density lipoproteins (see 62 for review). Accordingly, high density lipoproteins become enriched with phospholipids and Apo A-I during the hours following a fatty meal (63).

Chylomicron remnants:

The end-products of the delipidation cascade are remnant particles which are largely depleted of TG (64). Under normal conditions, chylomicron remnants are efficiently removed from the circulation by the liver (65). There is overwhelming evidence from *in vitro* and *in vivo* studies to suggest that apo E is

essential for normal uptake of chylomicron remnants (66). In subjects homozygous for apo E2, a form of apo E which exhibits grossly impaired binding to hepatic membranes *in vitro* (67), the half life of chylomicrons is increased about 4 fold (68).

The nature of the apo E binding site on hepatic cells has not been clearly elucidated. One possibility is that chylomicron remnants are cleared by the apo B,E (low density lipoprotein) receptor described by Goldstein and Brown. The observation that chylomicron remnant clearance is normal in homozygous Watanabe heritable hyperlipidemic rabbits (69), a mutant in which apo B,E receptors are not functional, led some authors to propose a specific "remnant" receptor which binds apo E but not apo B (see 70 for review). *In vitro* studies suggested that this putative receptor had a very high affinity for apo E-containing lipoproteins (71). Recent efforts to clone this receptor have been unsuccessful, however. Furthermore, Windler *et al* (72) have reported that apo B,E receptors account for at least 50% of chylomicron-remnant uptake in the perfused rat liver.

The clearance of chylomicron remnants is believed to occur almost entirely within the d1.006 lipoprotein fraction. Studies in which intestinal lipoproteins have been labeled with retinyl palmitate (73) or apo B48 (74) have indicated that the chylomicron-remnants do not form lipoproteins of higher density. This is in contrast with the TG-rich lipoproteins of hepatic origin, some of which give rise to apo B100 containing lipoproteins in the LDL range. The mechanism responsible for the distinct metabolic fates of hepatic- and intestine-derived TG- rich lipoproteins is not known.

Aims and scope of this study:

The primary aim of the work described in this thesis was to determine whether postprandial increases in plasma TG concentrations are actively damped by increases in TG clearance. Although the regulation of the clearance mechanism(s) has been extensively investigated, the regulation of chylomicron-TG clearance per se has received little study. Therefore, while it is clear that several hormones can potentially increase TG clearance (as described above), the composite effects of these factors under physiological conditions are not known.

One reason for the paucity of *in vivo* investigations into the postprandial regulation of TG metabolism is the lack of a definitive method whereby the disappearance of diet-derived TG can be measured. Nascent lymph chylomicrons are not readily available from normal donors, therefore various indirect methods to assess chylomicron clearance have been devised. Little *in vivo* validation of these methods has been attempted, and each has been criticised on theoretical grounds. Furthermore, no comparative studies of the different methods are available. Accordingly, the three most commonly used methods were compared and validated in this study. Further discussion of this work is presented in Chapter 2.

Intuitively, it seems likely that active regulation of chylomicron clearance would be triggered in response to feeding. Since few studies have examined the effects of different nutrients (fats, proteins and carbohydrates) on postprandial serum TG concentrations, it is not clear whether such a response occurs, or how it is mediated. In the present study, the effects of these factors have been evaluated. Chapter 3 describes the effects of varying the amount and type of dietary fat on postprandial lipemia. In Chapter 4, the effects of dietary protein are considered. The possible roles of different carbohydrates are evaluated in Chapter 5.

Physiological perturbation of plasma TG metabolism can be induced by chronic endurance exercise training. The effects of chronic exercise training on the plasma TG response to fat feeding, and on chylomicron clearance have not been extensively studied. In the present study, postprandial lipemia and chylomicron clearance have been evaluated in endurance trained athletes. These results are presented in Chapter 6.

CHAPTER 1

GENERAL METHODOLOGY

CHAPTER 1: GENERAL METHODOLOGY

1) Procedures for assessing chylomicron-triglyceride clearance:

General considerations:

All procedures used to assess chylomicron-TG clearance were approved by the Ethics and Research Committee of the University of Cape Town. The studies were performed on an outpatient basis on subjects following their self-selected diets. Subjects were asked to refrain from alcohol use for at least 72 hours, from exercise for 24 hours, and from food and beverages (excluding water) for 12 hours before each test.

i) Oral fat tolerance testing:

a) Procedure:

Oral fat tolerance testing was performed according to the procedure described by Patsch et al (75). On arrival at the laboratory the subjects were seated, and blood samples were drawn from an antecubital vein into plain tubes. The appropriate test meal was then administered. In all cases, the meals were presented in liquid form, and were consumed within 10 minutes. The subjects were then allowed to leave the laboratory in order to continue with their customary daily activities. No further food or beverage (excepting water) was consumed, and no exercise was permitted during the tests. Subsequent blood samples were drawn at 2, 4, 6 and 8 hours after the meal, except where otherwise indicated. In order to determine whether significant quantities of TG appeared in the plasma within 2 hours after the meal, blood samples were drawn from 22 subjects 1 hour after the ingestion of 100mL cream (40g fat). In these samples, the average TG increment above the fasting value was 10%.

b) Analysis of data:

A curve describing changes in serum TG concentration with respect to time was constructed using linear interpolation. The curve was normalized to zero by subtracting the fasting serum TG concentration from each Y-coordinate. Postprandial lipemia ($\text{mg.dL}^{-1} \cdot \text{hr}$) was calculated by numerical integration of the area under the curve.

c) Reproducibility:

The reproducibility of the oral fat tolerance test was estimated using test meals comprising 100, 200 and 350mL fresh cream (40% fat w/v). In 8 subjects (5 men and 3 women) tested on 2 occasions with 100mL cream, mean inter-test variation was 16%. In 8 men tested on 2 occasions with 200mL cream, mean inter-test variation was 18%. In 20 men tested on 2 occasions with 350mL cream, mean inter-test variance was 20%. In each case the test procedures were separated by an interval of 3 weeks. These data compare favorably with the inter-test variation of 20% reported by Angervall (5) in subjects given 200mL heavy cream. Raw data are given in Table 1.

Table 1. Reproducibility data of oral fat tolerance tests using three fat loads.

No.	350ml Cream (140g fat)			200ml Cream (80g fat)			100ml Cream (40g fat)		
	Test1 (mg.dL ⁻¹ .8hr)	Test2 (mg.dL ⁻¹ .8hr)	(d)	Test1 (mg.dL ⁻¹ .8hr)	Test2 (mg.dL ⁻¹ .8hr)	(d)	Test1 (mg.dL ⁻¹ .8hr)	Test2 (mg.dL ⁻¹ .8hr)	(d)
1)	1072	1007	6	305	350	14	46	60	26
2)	340	270	23	546	686	23	315	371	16
3)	232	173	29	507	405	22	143	116	21
4)	303	356	16	819	871	6	193	223	14
5)	199	383	63	403	536	28	262	248	5
6)	479	550	14	580	521	11	176	159	10
7)	406	294	32	445	310	36	250	270	8
8)	519	576	10	538	560	4	116	90	25
9)	288	224	25						
10)	380	308	21						
11)	619	545	13						
12)	1293	1387	7						
13)	629	466	30						
14)	503	558	10						
15)	662	577	14						
16)	239	200	18						
17)	584	569	3						
18)	604	483	22						
19)	832	695	18						
20)	779	623	22						
-									
x	548	512	20	518	530	18	188	192	16

Abbreviations: No. = Subject number, (d) = percentage difference between test 1 and test 2.

Note: No. denotes a different subject for each test dose.

ii) Intravenous fat tolerance testing:

a) Procedure:

Intravenous fat tolerance testing was performed according to the procedure described by Rossner (76). On arrival at the laboratory, subjects were seated and an indwelling cannula was placed in an antecubital vein and kept patent by slow saline infusion. A fasting blood sample was drawn and a bolus of 20% Intralipid (0.5mL/kg) was then administered within 15 seconds via the cannula. To remove residual Intralipid, the cannula was flushed with 25mL of saline. Three mL of blood were then drawn and discarded. Further blood samples were drawn at 5, 7.5, 10, 12.5, 15, 20, 25, and 30 minutes after Intralipid infusion unless otherwise indicated.

b) Analysis of data:

The rate of disappearance of Intralipid is expressed as the first order rate constant (k_2) as described by Hallberg (35). The data were assumed to follow a monoexponential decay, and k_2 was calculated from the log-linear plot of plasma Intralipid concentration versus time. An estimate of the fit of the data to a monoexponential decay curve (r) is given for each set of data. The k_2 was expressed as percentage disappearance of Intralipid-TG per minute (%/min).

c) Reproducibility:

Intravenous fat tolerance was determined on 2 occasions in 14 normolipidemic young men. Mean inter-test variation was 12.1% (see Table 2).

Table 2. Reproducibility data for Intravenous fat tolerance tests.

	Test1 k2 (min)	Test2 k2 (min)	(d)
1)	6.6	7.6	13.6
2)	4.7	4.6	2.2
3)	6.0	6.6	9.5
4)	6.3	5.7	10.0
5)	4.1	3.3	22.2
6)	2.8	3.2	13.3
7)	2.8	3.3	16.4
8)	4.6	5.1	10.3
9)	5.1	5.8	12.8
10)	4.3	4.7	9.4
11)	4.3	3.7	15.0
12)	6.3	5.1	21.1
13)	7.7	8.3	7.5
14)	4.7	4.5	4.3
\bar{x}	5.0	5.1	12.1

Key: (d) = percentage difference between Test 1 and Test 2

iii) Duodenal perfusion studies:

a) Procedure:

Duodenal perfusion studies were performed in the gastro-intestinal clinic at Groote Schuur Hospital, using the method described by Grundy and Mok (37). On arrival at the clinic, a naso-duodenal tube was positioned in the third portion of the duodenum under fluoroscopic guidance. Subjects were then confined to bed, and a fat emulsion was infused at a rate of 175mg fat/kg body weight/hour for 10 hours. Blood samples were taken just before, and at 5,6,7,8,9, and 10 hours after the start of the infusion. The blood was collected into tubes containing EDTA (1.2mg/mL) and aprotinin (500 units/mL). Plasma was collected by low speed centrifugation. From each hourly sample drawn during the steady state period 1mL plasma was taken and pooled. Mean steady-state chylomicron-TG concentration was determined from the pooled sample.

b) Analysis of data:

Chylomicron-TG half life was calculated using the formula¹

$$t_{1/2} = 0.693/k$$

where k = input rate of chylomicron-TG/plasma chylomicron-TG pool size.

Chylomicron pool size was calculated from the plasma volume and the plasma concentration of chylomicron-TG. Plasma volume was calculated from the formula¹

$$\text{Plasma volume (mL)} = 927 + 31.47 \times \text{body weight(kg)}$$

2) Laboratory methods:

a) Lipid analyses:

i) Triglyceride concentration:

The TG concentrations of serum and plasma, and of chylomicron fractions isolated by ultracentrifugation, were determined using a commercial enzyme kit (kit 240052, Boehringer Mannheim GmbH, Mannheim, West Germany). The TG assays were standardized against curves determined from serial dilutions (range 46 to 368mg/dL) of a lyophilized control serum (Precilip, Boehringer Mannheim). The assays were invariably linear within this range (typical regression values were $r = 0.999$, $y\text{-intercept} = -3.2$) and intra-assay variance estimated from duplicate aliquots of 30 samples was less than 5%. Samples to be analysed within 24 hours of collection were stored at 4 degrees C. Samples to be stored for longer periods were kept frozen at -20 degrees C. All samples from a single experiment were analysed in a single batch.

1. See reference 37

ii) Intralipid-TG concentration:

Most previous studies (76,77) of intravenous fat tolerance have used photometric measurements to determine Intralipid-TG concentration. These procedures are performed on whole serum or plasma samples and therefore measure not only exogenous lipids (Intralipid) but also all endogenous lipoproteins which produce light diffusion in serum. The significance of the contribution of endogenous lipoproteins to the optical properties of plasma is not clear. While Rossner et al (76) reported an excellent correlation between plasma Intralipid-TG concentration and light scattering index determined by nephelometry, a comparative study by Lambert et al (78) indicated that Intralipid-TG concentrations estimated by spectrophotometric methods were poorly correlated with those determined by ultracentrifugation or by decantation in a polyvinylpyrrolidone gradient.

To determine whether spectrophotometry could be used to provide a reliable index of serum Intralipid-TG concentration, the relationship between serum optical density and Intralipid-TG concentration was investigated as follows:

a) Intralipid was added to a fasting serum sample to give a final concentration of 500mg Intralipid-TG/dL plasma. A standard curve was calculated from 10 serial dilutions of 10% each. Serum optical density and Intralipid-TG concentration were linearly related over this range ($r = 0.99$), and the regression line included the origin (see Figure 1)

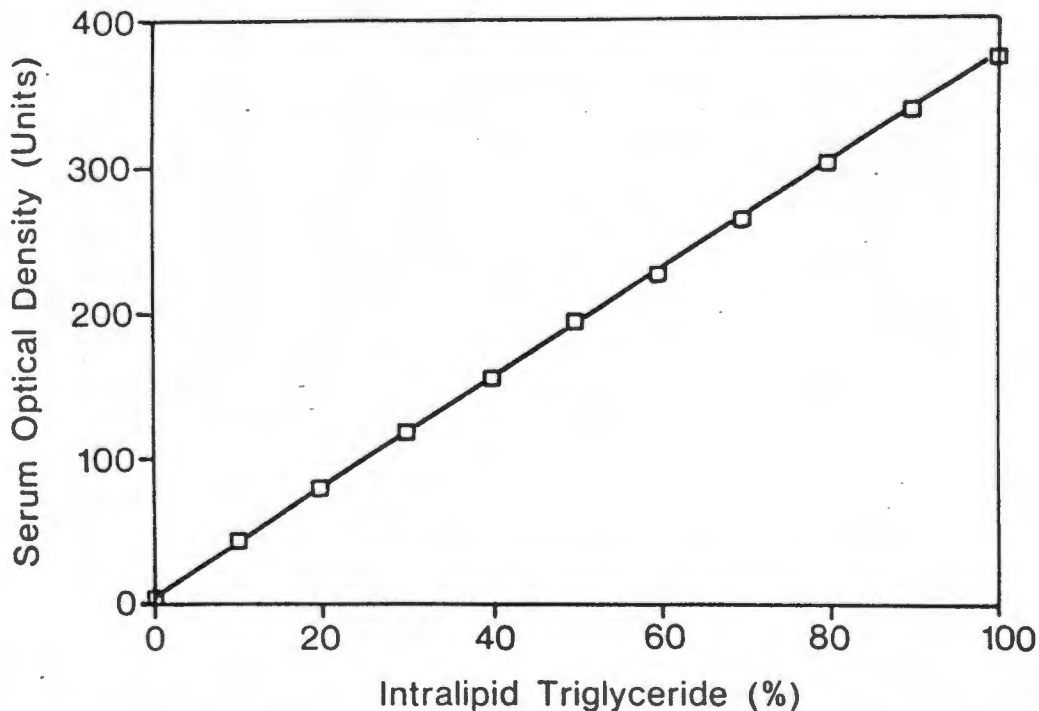


Figure 1. Linear regression analysis comparing serum optical density with serum Intralipid-triglyceride concentration.

b) Twenty samples of plasma drawn at varying times during intravenous fat tolerance tests were diluted 1:20 with normal saline. The optical density of these samples was then determined (in duplicate) by spectrophotometry at 650nm within 12 hours of sample collection. Intra-assay variation of the spectrophotometric procedure was less than 1%. In duplicate aliquots of these samples, Intralipid particles were isolated by preparative ultracentrifugation (as described below). The TG concentration of the supernatant was measured enzymatically. Correlation (r) between the TG concentration of the supernatant (Intralipid TG) and the optical density of serum was 0.98 (see Figure 2).

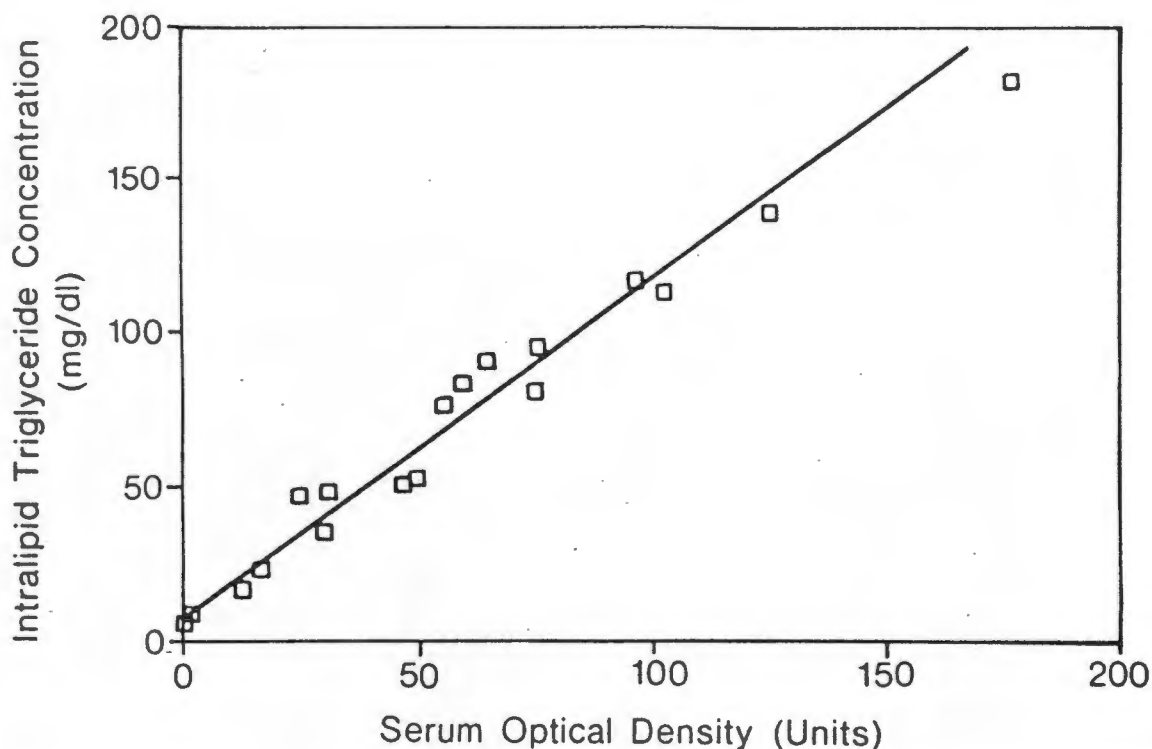


Figure 2. Linear regression analysis comparing serum optical density with serum Intralipid-triglyceride concentration determined by ultracentrifugation.

These findings indicate that the spectrophotometric method provides an indication of plasma Intralipid-TG concentration which is both accurate and precise. Therefore in all further experiments, serum optical density was considered to reflect Intralipid-TG concentration.

iii) Cholesterol concentration:

The cholesterol concentrations in serum or plasma, or in high-density lipoprotein (HDL) fractions isolated by precipitation were determined enzymatically (Boehringer Mannheim kit no. 237574). The assay was standardized against lyophilized control sera (Precilip and Precilip E.L.) containing 166mg and 397mg cholesterol per decilitre respectively. For assay of HDL cholesterol, the 166mg/dL standard was diluted fourfold. Samples were stored at 4 degrees C and analysed within 24 hours of sample collection. Intra-assay variation estimated from 30 samples was less than 5%.

iv) Fatty acid analyses:

Plasma- or VLDL lipids were separated by thin layer chromatography using the solvent system petroleum ether:diethyl ether (peroxide free):acetic acid (85:15:2 v/v/v) (14). Lipids were located on the plates by spraying with 2,5-di(5-tert-butyl-2-benzoxazolyl)-thiophene and viewing under ultraviolet light. Spots containing TG were scraped off and trans-methylated by heating with 5% H₂SO₄-methanol for 2 hours at 70 degree C. Fatty acid methyl esters were then extracted with n-hexane and separated in a Varian 4600 gas liquid chromatograph on 2m glass columns of 2mm internal diameter, packed with 10% SP 2330 Chromosorb W, 100-200 mesh. Flow rates were: hydrogen 20mL/min, air 200mL/min, nitrogen 20mL/min. Temperature programming was linear at 3 degrees C/min (initial temperature 150 degrees C, final temperature 220 degrees C, injector temperature 260 degrees C, detector temperature 270 degrees C). Fatty acid peaks were detected by flame ionization. Fatty acids were identified by comparison with known standards. Quantitation was achieved by the area-ratio method using a Varian 402 integrator.

b) Lipoprotein analyses:**i) Isolation of chylomicrons:**

Two mL of serum or plasma were overlaid with sodium chloride solution ($d = 1.006\text{gm/mL}$) and subjected to preparative ultracentrifugation in a Beckman SW-41 rotor at $8.5 \times 10^9 \text{ rad}^2/\text{sec}$. During this procedure, lipoproteins of $S_f > 1000$ move to within 0.5cm of the top of the tube (79). Two mL of the supernatant were carefully aspirated using a modified Pasteur pipette (80). The TG content of the aspirate was considered to represent chylomicron TG. Intra-assay variance for chylomicron TG determination (estimated from 20 samples) was less than 10%. Chylomicron TG concentration was determined in plasma samples taken from 3 fasted subjects. In two of the subjects chylomicrons were not recovered in fasting plasma, while in the third subject, less than 3% of the plasma TG was recovered in the chylomicron fraction.

ii) Separation of high-density lipoproteins:

High density lipoproteins (HDL) were separated from lipoproteins of lower density using the methods described by Gidez et al (81), and by Warnick et al (82). These methods are based on selective, polyanionic precipitation of the apo B- and apo E-containing lipoproteins (LDL and VLDL), leaving HDL in the infranatant. The method of Gidez et al (81) includes a second precipitation which facilitates the separation of HDL into subclasses resembling the HDL2 and HDL3 achieved by preparative ultracentrifugation. Intra- assay variance estimated from duplicate analysis of 30 samples was less than 5% for the HDL estimation and less than 8% for HDL2 estimation.

c) Other Analyses:

i) Glucose: Plasma glucose concentrations were measured in duplicate on a Beckman glucose analyser (Model ERA 2001). Intra-assay variation was less than 5%.

ii) Insulin: Plasma insulin concentrations were measured in duplicate by radioimmunoassay using a kit obtained from Pharmacia Diagnostics. Intra-assay variation was less than 4%

CHAPTER 2

A COMPARISON OF THREE METHODS FOR ASSESSING CHYLOMICRON TRIGLYCERIDE CLEARANCE

CHAPTER 2: A COMPARISON OF THREE METHODS FOR ASSESSING CHYLOMICRON TRIGLYCERIDE CLEARANCE

INTRODUCTION:

Several methods have been used to assess the rate of clearance of diet-derived, or "exogenous" TG. In some studies, direct measurements of chylomicron-TG clearance have been obtained by measuring the rate of disappearance of a bolus of intravenously injected chylomicrons (30,83). This method involves the homologous administration of endogenously labeled chylomicrons isolated from thoracic duct lymph, and its use has been limited by ethical and practical considerations. Therefore in most studies, chylomicron-TG clearance has been assessed by indirect methods such as oral (5,75) or intravenous (76,84) fat tolerance tests, which do not provide quantitative estimates of chylomicron-TG clearance.

The oral fat tolerance test is based on the assumption that the magnitude of triglyceridemia which follows the ingestion of a fatty meal (postprandial lipemia) is inversely related to the rate of chylomicron-TG clearance. Indirect support for this hypothesis is provided by the observation that, in the absence of gastro-intestinal pathology, TG absorption is extremely efficient (85). If it is assumed that the bulk of TG absorption occurs within 7-8 hours postprandial, the integrated area under the curve of plasma TG concentration plotted against time (postprandial lipemia) should reflect chylomicron TG half-life. Accordingly, in several recent studies (31,32,75), postprandial lipemia was considered to be analogous to chylomicron-TG half life. More direct evidence that postprandial lipemia is determined by chylomicron-TG half life is limited to a single study by Nestel (30), who reported a strong inverse correlation between postprandial lipemia and the rate of clearance of intravenously administered chylomicrons in a mixed group of normo- and hypertriglyceridemic men. Since the rate of TG absorption is not controlled during the oral fat tolerance test, chylomicron-TG clearance cannot be quantitatively determined by this method. Rossner (76) has suggested that vagaries in the rate of fat absorption severely limit the utility of the oral fat tolerance test, but no relationship between any parameter of fat absorption and postprandial lipemia has been reported in normal men.

The intravenous fat tolerance test was devised in order to eliminate TG absorption as a confounding factor in the estimation of chylomicron-TG clearance. For this test, an artificial fat emulsion is used to model the intravenous decay kinetics of chylomicron-TG. Early studies (86) indicated that many artificial fat emulsions are taken up by the reticuloendothelial system and are therefore unsuitable for use as tracers of chylomicron metabolism. In 1963, Schuberth (87) reported that a fat emulsion comprising soya oil and egg lecithin in a glycerol base (Intralipid, Vitrium AB Stockholm, Sweden) showed excellent clinical tolerance. Moreover, this emulsion appears to resemble chylomicrons in several respects: *In vitro* studies have indicated that Intralipid particles associate with apo CII (88) and act as a substrate for LPL in postheparin plasma (89). Hallberg (35) reported that Intralipid- and chylomicron-TG had similar decay kinetics after intravenous injection in man.

On the basis of these studies, the intravenous fat tolerance test has been widely used, both in cross-sectional studies such as comparisons of normal and hypertriglyceridemic men (76), and in longitudinal investigations into the effects of experimental perturbations involving diet (84) or exercise (90). In most studies of normolipidemic men, however, the mean half life of intravenously injected Intralipid-TG is about 15 minutes (76), whereas the half life of chylomicron-TG determined under similar conditions is about 6 minutes (30,83). Furthermore, the sensitivity of the intravenous fat tolerance test to intra- individual changes in plasma TG clearance is not known, as the responsiveness of the test to small, but unequivocal changes in the rate of intravascular lipolysis has not been tested.

More recently, Grundy and Mok (37) have developed a method whereby chylomicron-TG clearance can be calculated from steady-state plasma chylomicron-TG concentrations. These conditions are achieved by perfusing the duodenum with TG at a constant rate. This method is based on two assumptions: Firstly, the rate at which chylomicron-TG enter the blood is considered to be equal to the rate of influx of perfusate-TG into the duodenum. Secondly, it is assumed that the chylomicron fraction isolated using ultracentrifugation contains all of the chylomicron-TG, but none of the "endogenous" TG of plasma. While these assumptions remain unproven, chylomicron-TG clearance estimated using this method in normolipidemic men (37) is similar to the rate of disappearance of intravenously injected chylomicron-TG (30).

Although collation of results from different studies suggests that these indirect methods yield generally consistent results in the cross-sectional comparison of normolipidemic and hyperlipidemic individuals (5,30,37), a comparative study of the different methods has not been undertaken. In the absence of such data, and given the different assumptions on which each of these tests are based, the degree to which observations concerning the clearance of chylomicron-TG are affected by the measuring technique employed is unknown. The present study was undertaken in order to compare the oral, intravenous, and intraduodenal methods of estimating chylomicron-TG clearance, and to determine the sensitivity of the intravenous fat tolerance test to experimentally-induced changes in plasma TG clearance.

METHODS:

1) Subjects:

Three methods for assessing chylomicron TG clearance were compared in twelve normolipidemic, male medical students who were apparently in good health (Group A, see Table 3 for clinical data). These men followed self-selected diets, and all had been weight stable for the 6 months preceding the study. One subject was mildly obese (24% body fat) as determined from skinfold measurements (91). None of the subjects smoked or used any medication known to affect lipoprotein metabolism. Three subjects were competitive marathon runners.

The effects of heparin on intravenous fat tolerance were examined in ten normolipidemic young men (mean age = 29 ± 4 yr) and 2 women (aged 30) who did not smoke and who did not regularly use any medication known to affect lipoprotein metabolism (Group B). These subjects were apparently healthy, and all performed regular, moderate exercise (1 to 3 hours per week).

2) Procedures:

i) Oral fat tolerance test:

Oral fat tolerance testing was performed in subjects in Group A as described in Chapter 1 using meals comprising 100mL cream (40g fat), 1 teaspoon (3g) chocolate flavouring, and 300mL water. Serum was collected by low speed centrifugation (3000g for 15 minutes), stored at 4 degrees C, and analysed for TG concentration within 24 hours of sample collection.

ii) Intravenous fat tolerance test:

Intravenous fat tolerance testing was performed as described in Chapter 1. To compare the intravenous fat tolerance test with other tests of chylomicron clearance, a single fat tolerance test was performed in the 12 men in Group A. To determine the effects of heparin on Intralipid clearance, a baseline intravenous fat tolerance test was performed in 12 subjects in Group B. Ninety minutes after the completion of the baseline test, an intravenous infusion of heparin was started. Six subjects received 12.5 units of heparin per minute, while the other 6 received 25 units per minute. Fifteen minutes after commencing the heparin infusion, a second intravenous fat tolerance test was performed. The heparin infusion was maintained for the duration of the test. In each case, the same batch of Intralipid was used for both tests.

To control for systematic errors which might be incurred by performing the tests at different times during the morning, intravenous fat tolerance was examined in 3 subjects at 8.00 am and again at 11.30 am on the same day. During these procedures, no heparin was given.

iii) Effects of heparin on endogenous serum TG concentrations:

Heparin (12.5 or 25 units per minute for 45 minutes) was infused into fasting subjects via a cannula placed in an ante-cubital vein. Five subjects received the higher dose and 2 subjects the lower dose infusion. Blood samples were drawn into plain tubes before, and at 5 minute intervals during the infusion. Serum TG concentrations were measured enzymatically using a method which discriminates between free and esterified serum glycerol (Test kit 877557, Boehringer Mannheim).

iv) Duodenal perfusion:

Duodenal perfusion was performed in subjects in Group A as described in Chapter 1 using a fat emulsion comprising 100g olive oil and 7g gum acacia per litre of water. A previous study has indicated that fat given orally in this form is efficiently absorbed in normal men (92). In 7 subjects, chylomicrons were isolated from each sample drawn during the perfusion (see Chapter 1). The remaining d.006 lipoproteins were then isolated from these samples by ultracentrifugation at 41000 rpm for 22 hours. Chylomicron and VLDL lipids were extracted (93), and separated by thin layer chromatography. The fatty acid composition of the TG spots was determined by gas chromatography (see Chapter 1).

3) Analysis of data:

Postprandial lipemia, k_2 of Intralipid decay, and the $t_{1/2}$ of chylomicron-TG were calculated as described in Chapter 1.

In another study performed in this laboratory (see Chapter 5 and reference 94), the magnitude of postprandial lipemia appeared to be normally distributed in normolipidemic subjects. Therefore the correlations between the parameters measured in the present study were determined using Pearson's method (95). To exclude the possibility of spurious correlations arising as a consequence of non-normal distribution of the data, correlations were also determined using a non-parametric method (Spearman's ranked correlation procedure). The mean values of the Intralipid decay constants obtained before and after heparin infusion were compared using Wilcoxon's signed rank test for paired data.

RESULTS:

The procedures were well tolerated and none of the subjects developed nausea or diarrhoea during, or after any of the tests. The results are reported as the mean \pm standard deviation. Detailed test results are shown in Tables 3 and 4.

i) Oral fat tolerance:

Fat ingestion increased the serum TG concentration in all subjects. On average, serum TG concentrations were maximally elevated at 2 hours (mean maximum increase = 55 ± 42 mg/dL), and had returned to fasting values within 8 hours after the meal (see Figure 3).

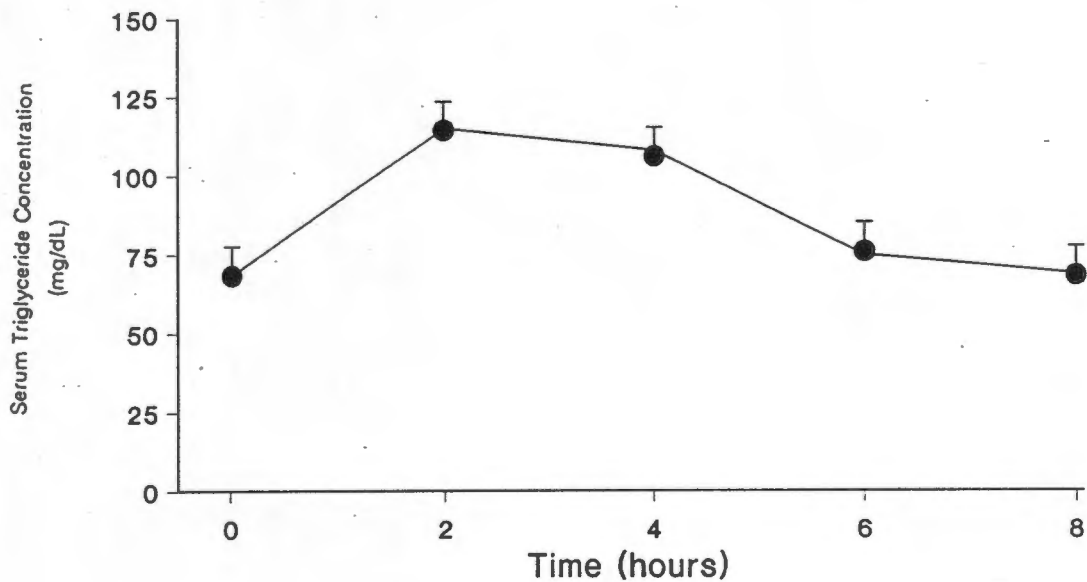


Figure 3. Mean serum triglyceride concentrations of 12 normolipidemic men after the ingestion of 100mL dairy cream.

ii) Intravenous fat tolerance:

Intralipid clearance was adequately fitted by a single exponential in all subjects in group A (mean $r = 0.97$, Figure 4).

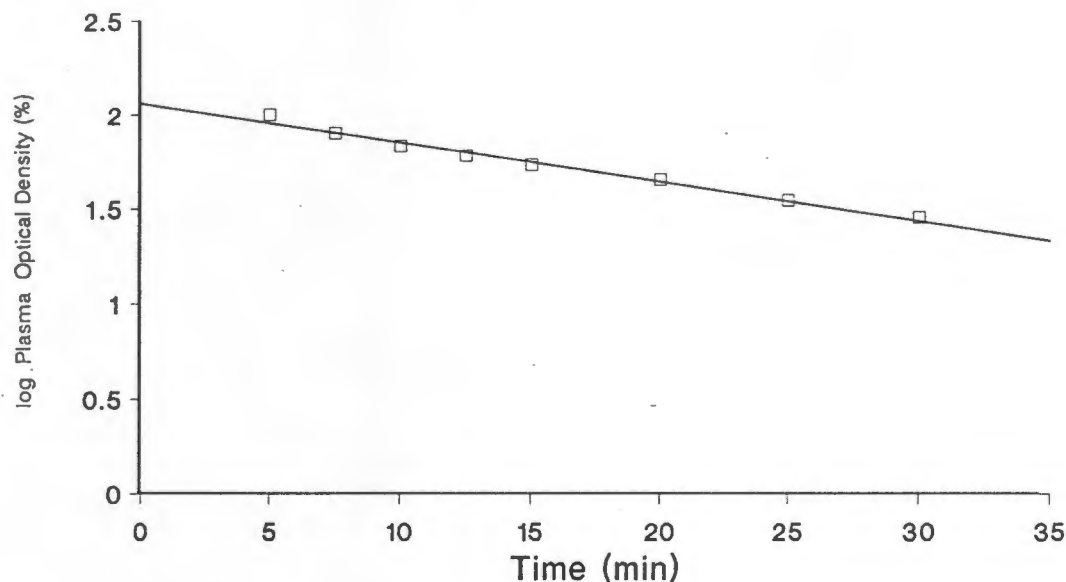


Figure 4. Mean serum optical density of 12 normolipidemic men after intravenous Intralipid administration (0.5mL Intralipid/kg body weight).

iii) Duodenal perfusion:

In all subjects, plasma TG concentrations were increased by duodenal TG perfusion (mean increase in plasma TG = 69 ± 37 mg/dL, see Figure 5). Sixty percent of the increment in plasma TG concentration was recovered in the chylomicron fraction, and a further 30% was recovered in the VLDL fraction ($d < 1.006$, $S_f < 1000$). The mean TG concentration of the samples drawn during the 5 hour steady state period was considered to represent the steady state TG concentration. The average variation of plasma TG concentrations around the steady state value was less than 11%. Variation of the chylomicron TG concentration around the steady state concentration averaged 17%. Oleic acid (C18:1) constituted 81% of the TG fatty acids in the perfusate, and $41 \pm 5\%$ of the TG fatty acids extracted from VLDL fraction obtained from 7 fasted subjects. The mean oleic acid content of the chylomicron-TG remained constant throughout the perfusion ($75 \pm 3\%$ at 5, and at 10 hours).

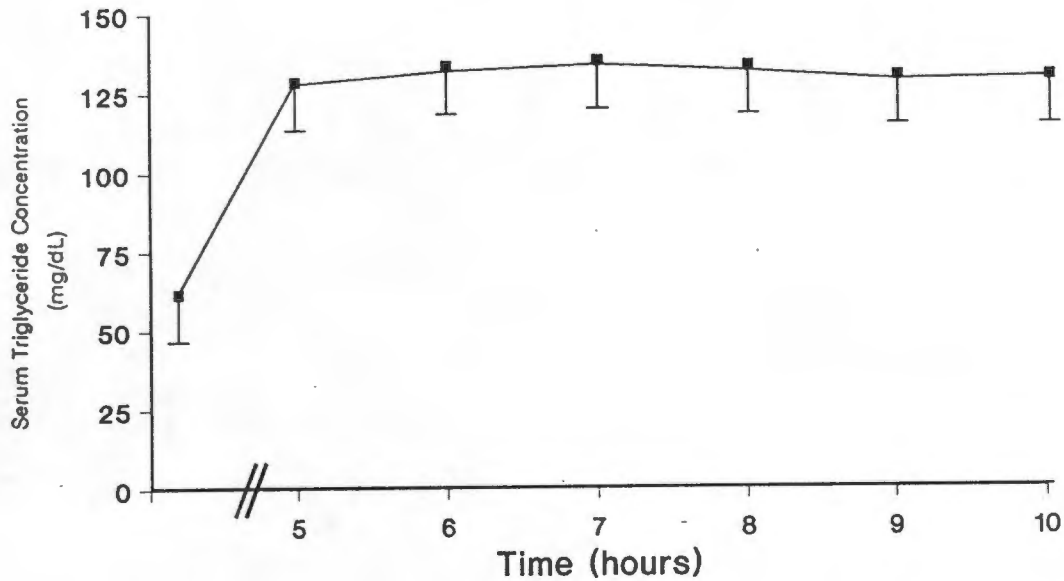


Figure 5. Mean serum triglyceride concentrations of 12 normolipidemic men during duodenal fat perfusion (175mg triglyceride/kg body weight/hour).

iv) Correlations:

Pearson correlation coefficients between postprandial lipemia, Intralipid half life, chylomicron-TG half life and fasting serum TG concentration are shown in Table 3. Correlation coefficients determined using Spearman's ranking method were lower than the Pearson correlation, but were significantly different from 0 ($P < 0.05$.) for all parameters tested.

Table 3. Pearson correlation coefficients (r) between postprandial lipemia (PPL), t1/2 for Intralipid clearance (It1/2), t1/2 for chylomicron triglyceride clearance (Ct1/2), and fasting triglyceride concentration (FTG).

	PPL	It1/2	Ct1/2
FTG	.79	.80	.77
Ct1/2	.84	.82	
It1/2	.84		

$P < .0025$ for $n = 12$ and $r = 0.75$

Table 4. Clinical data and triglyceride clearance measurements of subjects in Group A.

	AGE	BWT	%BF	TC	FTG	PPL	It1/2	Ct1/2
1	21	90	18	207	89	269	16.1	4.8
2	30	93	24	190	106	584	27.7	10.3
3	21	70	12	175	68	99	16.1	2.2
4	20	74	11	185	97	214	17.3	8.7
5	21	67	12	160	91	384	13.9	6.2
6	20	64	15	148	46	102	10.3	3.8
7	20	68	12	148	45	221	12.8	3.2
8	19	68	15	170	29	33	7.5	2.4
9	21	65	10	160	41	46	10.5	2.5
10	21	55	11	168	72	105	11.4	2.5
11	27	66	14	172	53	218	11.8	3.6
12	26	70	12	155	60	121	9.6	3.3
x	22	71	14	170	66	200	13.7	4.5
s.d.	3	11	4	18	25	158	5.2	2.6

Abbreviations: BWT = body weight (kg), %BF = percent body fat, TC = total cholesterol concentration (mg/dl), FTG = fasting triglyceride concentration (average of three test days, mg/dl), PPL = postprandial lipemia (mg.dL⁻¹.8hr), It1/2 = Intralipid half life (minutes), Ct1/2 = chylomicron-triglyceride half life

v) Effects of heparin on serum TG concentrations and intravenous fat tolerance:

Heparin infusion decreased serum TG concentrations in all subjects (see Figure 6). The mean maximum decrease from the fasting value was 16 ± 10 mg/dL ($24 \pm 15\%$, range 12 to 54%) during the 25 unit/minute infusion. In the 2 subjects who received the 12 unit/minute infusion, serum TG concentrations were decreased by 22 and 24%. In 4 subjects, serum TG concentrations decreased progressively throughout the infusion. The remaining subjects reached steady state serum TG concentrations within 30 minutes after commencing the infusion.

-Heparin infusion increased the k_2 of Intralipid disappearance in all subjects in Group B (see Figure 7). Mean k_2 was $5.3 \pm 1.8\%$ /minute before and $9.8 \pm 3.4\%$ /minute after the 25 unit/minute heparin infusion ($P < 0.025$). In the subjects who received the 12.5 unit/minute infusion, mean k_2 was $7.3 \pm 2.3\%$ /minute before, and $15.0 \pm 9.3\%$ /minute after heparin administration ($P < 0.05$). In all studies, the data were adequately fitted by a single exponential ($r = 0.96$). The large mean value for k_2 after the 12.5 unit/minute heparin infusion was due to disproportionately high responses in 2 subjects ($k_2 = 25$ and 27% /minute).

Since no clear dose response relationship was apparent in our study, data from the 2 groups have been combined for the purposes of the discussion. The k_2 values of the control studies were 6.6, 4.3, and 6.7 %/min at 8.00 am. The corresponding values were 6.5, 4.4, and 6.9 %/min at 11.30 am.

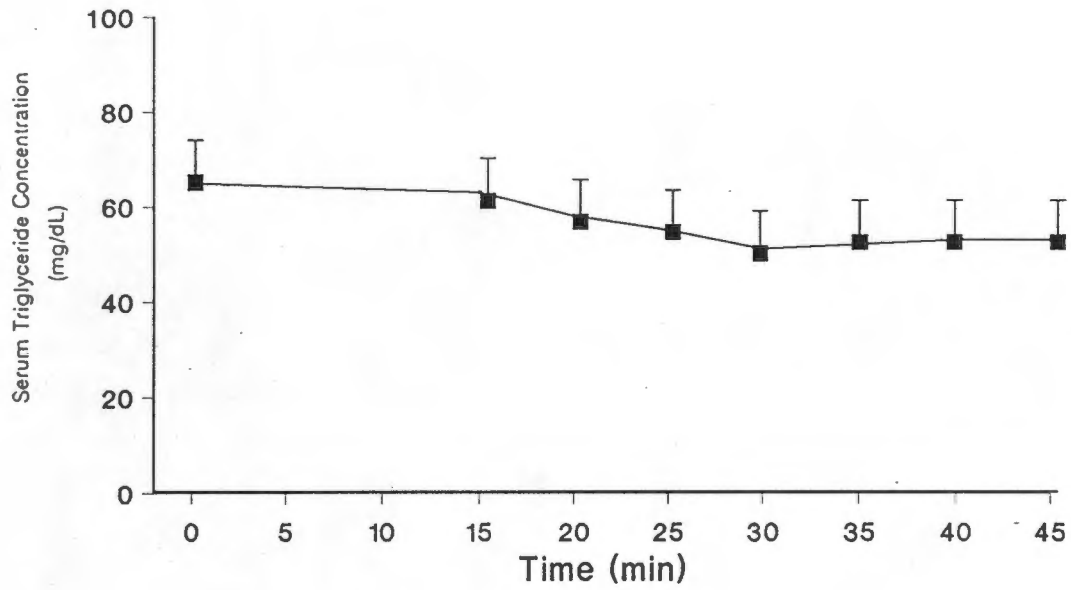


Figure 6. Serum triglyceride concentrations of 5 normolipidemic subjects during heparin infusion (25 units/minute).

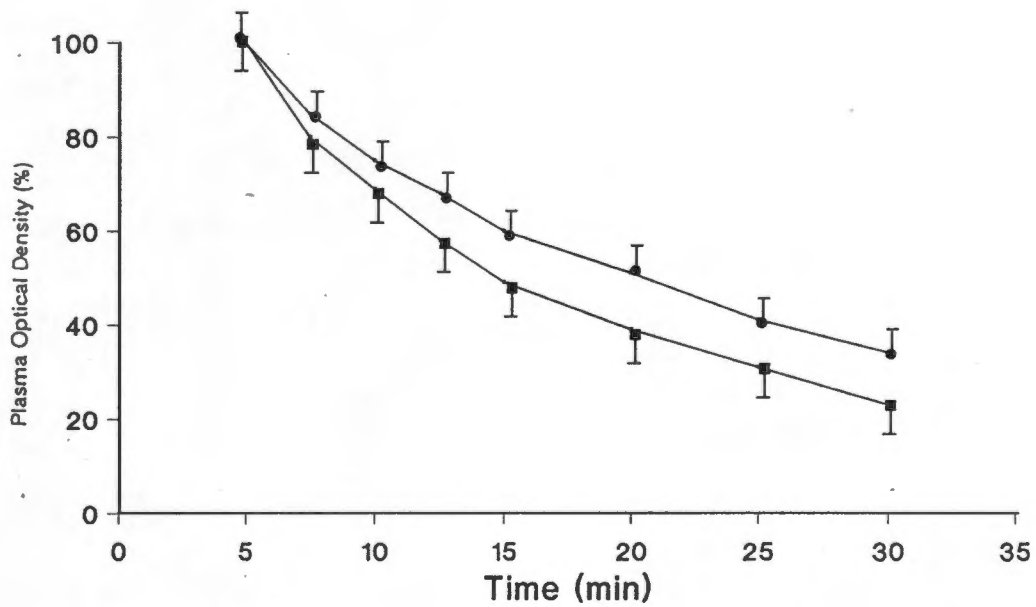


Figure 7. Plasma Intralipid concentrations of 6 normolipidemic men during infusion with normal saline (●) and heparin (25 units/minute) (■).

DISCUSSION:

The results of this study indicate that oral and intravenous fat tolerance tests, and the duodenal perfusion method of Grundy and Mok (37), provide qualitatively similar assessments of chylomicron-TG clearance in normolipidemic men. The mean values for postprandial lipemia, Intralipid half life, and the half life of chylomicron clearance of the men in this study were similar to those found in normolipidemic men in previous studies (37,76,96), and good correlations were obtained between these methods for estimates of chylomicron-TG half life ranging from 2.2 to 10.3 minutes (estimated using the duodenal perfusion method). The results of the non-parametric correlation procedure indicate that these correlations were not due to outliers or to other artifacts of data distribution.

It is possible that the correlations obtained between these methods were the fortuitous consequence(s) of factors unrelated to TG clearance. The tests are based on different theoretical assumptions, however, and it is difficult to formulate an alternative hypothesis to explain the correlations observed. For example, the oral and intraduodenal perfusion methods both rely on the efficient absorption of TG from the gastrointestinal tract. Therefore the correlation observed between these 2 methods may reflect absorptive processes, rather than clearance. This possibility must be considered unlikely for the following reasons:

1) Both the oral fat tolerance test and the duodenal perfusion method show good correlation with the intravenous fat tolerance test, which is clearly unrelated to TG absorption.

2) The inverse relationship between chylomicron-TG clearance and fasting serum TG concentration is believed to reflect a common clearance pathway for hepatic and intestine derived TG-rich lipoproteins (36). Therefore the strong correlations between postprandial lipemia, chylomicron-TG half life and fasting serum TG concentration observed in the present study suggest that the rise in serum TG concentrations measured after oral and after intraduodenal fat administration was limited by events within the intravascular compartment, rather than at the site of TG absorption.

3) Other studies performed in this laboratory (see Chapter 3 and reference 94) have indicated that postprandial lipemia (measured as area under the curve) increases in direct proportion to meal fat content after oral fat loads ranging from 40 to 120g. If postprandial lipemia was limited by the rate of fat absorption, then increasing fat loads would produce disproportionately small increases in the magnitude of postprandial lipemia.

4) A strong inverse correlation between the magnitude of postprandial lipemia and the rate of clearance of intravenously injected chylomicrons has been reported previously (30). This finding suggests that postprandial lipemia is limited by TG clearance, rather than by absorption.

Given these considerations, it seems likely that the correlations between the three methods used in this study reflect a common dependence on the rate of chylomicron-TG clearance.

Postprandial lipemia, chylomicron TG clearance and endogenous TG pool size:

The results of the present study support a previous observation (30) that the magnitude of postprandial lipemia is determined largely by the rate of chylomicron-TG clearance. The rate of chylomicron-TG removal is determined in part by the efficiency of the TG removal system(s) and possibly by the plasma endogenous TG pool size. Therefore the relationship between chylomicron-TG half life, Intralipid half life and postprandial lipemia observed in the present study may reflect a common dependence on fasting plasma TG pool size, as each of these methods showed strong correlation with fasting plasma TG concentration. Two longitudinal studies have provided support for this concept:

Using the duodenal-perfusion method, Grundy and Mok (37) found impaired chylomicron-TG clearance in subjects with endogenous hypertriglyceridemia. Dietary (caloric) restriction reduced endogenous TG concentrations and concomitantly increased chylomicron TG clearance in 7 out of 8 subjects. Similar changes were noted in most subjects following Clofibrate treatment.

Brunzell et al (36) found that fasting chylomicronemia (which was considered to reflect impaired chylomicron clearance) could be induced in hypertriglyceridemic subjects by increasing dietary carbohydrate intake, and hence endogenous TG production. The appearance of fasting chylomicronemia was not related to increased chylomicron formation, as dietary fat intake was held constant in these studies. Post-heparin plasma LPL activity was not diminished by carbohydrate ingestion in these men, therefore it seems unlikely that the diminution of chylomicron-TG clearance was secondary to impaired TG removal capacity.

If chylomicron TG clearance is dependent on the endogenous TG pool size, and if the endogenous plasma TG pool size is determined primarily by VLDL production, as suggested by Tobey et al (97), then the rate of chylomicron-TG clearance would not necessarily reflect the capacity of the TG removal system(s). This hypothesis would explain the poor correlation found in several studies (31,32,33) between LPL activity (which presumably reflects TG clearing capacity) and postprandial lipemia. A further implication of this argument is that both fasting and postprandial plasma TG concentrations are determined by the rate of VLDL production.

Some authors have suggested that measurements of chylomicron-TG clearance are not simply a reflection of endogenous TG pool size, however, and several studies have reported that the fractional clearance of chylomicron-TG and the fasting plasma TG concentration can be independently perturbed. Hallberg (98) reported that the fractional removal rate of Intralipid is not reduced by increasing rates of Intralipid infusion and hence Intralipid concentration. In healthy women, Rossner (99) found that the fractional clearance rate of Intralipid is not affected by estrogen administration, despite a significant increase in plasma TG concentration. Nestel (84) reported that acute exposure to diets comprising 80% carbohydrates increased fasting plasma TG concentrations by 100% but did not affect the fractional clearance rate of Intralipid. In another study performed in this laboratory (see Chapter 7 and reference 100) chylomicron-TG clearance measured by oral and intravenous tolerance tests and by duodenal perfusion was faster in endurance-trained athletes than in sedentary men matched for fasting TG concentration. These findings suggest that the relationships between postprandial lipemia, Intralipid and chylomicron half life, and fasting TG concentrations reflect a common dependence on the activity of the TG clearing system(s).

Since the rate of plasma TG removal is limited by the activity of LPL, measurements of the activity of this enzyme may provide an estimate of TG removal capacity which is independent of possible confounding factors such as TG pool size. Accordingly, the observation (101) that post-heparin plasma LPL activity is negatively correlated with fasting serum TG concentration, and with endogenous TG turnover in normolipidemic men and women supports the hypothesis that fasting TG concentrations and the parameters of TG clearance estimated in the present study are related by a common dependence on the activity of the TG removal system(s). This hypothesis is further supported by the study of Pagano Mirani-Oostdijk (102), in which diurnal changes in serum TG concentrations in normolipidemic men on high carbohydrate diets appeared to be related to changes in adipose tissue LPL activity.

Other population based studies (103) have failed to show significant correlation between LPL activity and serum TG concentrations, however, while studies in rats suggest that physiological variations in adipose tissue LPL activity are poorly correlated with changes in the kinetic behaviour of VLDL (104). Furthermore, factors such as the enzyme source (tissue or postheparin plasma), method of extraction from tissue, and dose of heparin administered may determine the apparent LPL activity, and it is not clear which of these methods best reflects the *in vivo* TG clearing capacity (105).

Validation of the duodenal perfusion method:

An estimate of chylomicron-TG half life obtained using the duodenal perfusion method (37), has been widely quoted as a standard for normolipidemic men. The mean chylomicron-TG half life reported by those workers (37) is in good agreement with that reported by Nestel et al (30) for the clearance of intravenously administered chylomicrons, but no further validation of the duodenal perfusion method has been described. In the present study, the mean chylomicron-TG half life of 12 normolipidemic men estimated using the duodenal perfusion method was identical to that reported by Grundy and Mok (37). In addition, the present data provide support for the assumptions underlying the duodenal perfusion method.

Firstly, Grundy and Mok (37) assumed that the absorption of intraduodenally administered TG was complete, on the basis of the extensive fat balance studies performed by Annegers, Boutwell and Ivy (85). The present data provide further support for this contention, as described in detail above.

The second assumption of the test, ie. that chylomicrons can be completely separated from endogenous lipoproteins by ultracentrifugation, is more contentious. Chylomicrons entering the intravascular compartment are subject to progressive delipidation by LPL. This process generates a heterogeneous population of partly catabolized intestine-derived lipoproteins, which shows considerable overlap with the density range of hepatic VLDL (39,74). The separation of chylomicrons from endogenous TG-rich lipoproteins is therefore based on an operational definition, rather than on a true physiological distinction. If the presence of endogenous VLDL in the chylomicron fraction leads to spuriously elevated estimations of chylomicron-TG concentration, then the calculated rate of chylomicron-TG clearance will underestimate the true value. Grundy and Mok (37) elected to use the $Sf > 1000$ lipoproteins to represent chylomicrons on the basis of electrophoretic studies which indicated good separation of exogenous and endogenous lipoproteins. In their study, less than 5% of the TG in fasting plasma samples was recovered in this fraction. This convention has recently been adopted by other authors (106). In the present study, the fatty acid composition of the TG in the $Sf > 1000$ lipoproteins was analysed in order to determine the relative proportions of endogenous and exogenous TG in this fraction.

This analysis indicated that 93% of the TG in the "chylomicron" fraction was derived from the perfusate. These data suggest that the chylomicron fraction isolated in this study was relatively free of contamination with endogenous lipoproteins, although some of the perfusate-derived TG recovered in this fraction may have already recycled through the liver. While the endogenous TG contribution to this fraction was minimal under fasted conditions, it is possible that hepatic VLDL with a flotation rate of $Sf > 1000$ are secreted under conditions of acute fat feeding.

Conversely, the ultracentrifugation procedure used in this study may yield spuriously low estimates of chylomicron-TG concentration, and consequently half life. In most studies, the lipoprotein fraction $Sf > 400$ isolated using the isopycnic ultracentrifugation method described by Havel et al (107) has been considered to represent chylomicrons. Redgrave and Carlson (108) reported that most of the postprandial increase in plasma TG concentrations occurred in the $Sf > 400$ fraction isolated by density gradient ultracentrifugation. Lewis et al (39) reported a direct relationship between dietary fat load and the fraction of plasma TG appearing in the $Sf > 400$ fraction for meals containing from 1 to 100g fat. After each meal, however, an appreciable proportion of the plasma TG increase appeared in the $Sf_{100-400}$ fraction. These data suggest that the $Sf > 400$ fraction may not contain the entire chylomicron fraction. In the present study, the fraction of the perfusion-induced increment in plasma total TG concentration recovered in the chylomicron ($Sf > 1000$) fraction averaged 60%, while a further 30% was recovered in the $d < 1.006$ lipoproteins which floated at $Sf < 1000$. The magnitude of the perfusion-induced increment in plasma TG concentration was strongly correlated with chylomicron-TG content ($r = 0.86$). Given the strength of this correlation, and since the distribution of the perfusate-derived TG is probably continuous in the $d_{.006}$, Sf_{d0} lipoprotein fraction, it seems unlikely that the apparent chylomicron-TG half life would

be qualitatively altered by the inclusion of almost any sub-population of this fraction in the estimation of total chylomicron-TG concentration. Furthermore, even if the perfusion-induced increment in plasma TG concentration was considered to be constituted entirely by chylomicrons, an assumption which ignores the TG content of chylomicron-remnants and probably of some hepatic VLDL, mean chylomicron half life would be 7.9 minutes. This value, which represents the most extreme possible overestimate of chylomicron-TG half life obtainable with the duodenal perfusion method, is still in good agreement with the value obtained by direct methods (30). Therefore it appears reasonable to conclude that the duodenal perfusion method yields values for chylomicron-TG half life which are consistent with those achieved by other methods, and which do not overestimate, nor significantly underestimate the true value.

Validation of the intravenous fat tolerance test

The results of the heparin infusion studies indicate that the intravenous fat tolerance test is sensitive to experimentally induced increases in plasma TG clearance. Heparin infusions that decreased the endogenous plasma TG concentration by as little as 12% caused significant increases in the rate of Intralipid clearance. In the 3 control studies in which no heparin was given, the rate of Intralipid disappearance remained constant during the study period. Therefore, while the resolving power of the intravenous fat tolerance test was not precisely quantitated in this study, the data suggest that the test will readily detect small changes in the rate of plasma TG clearance.

The magnitude of the heparin-induced changes in plasma TG clearance was assessed from measurements of endogenous TG concentrations. This estimate is based on the assumption that the decrease in endogenous TG concentration measured during heparin infusion is due almost entirely to a heparin-induced increase in plasma TG clearance. Since the plasma TG clearing action of heparin is secondary to the liberation of endothelial lipases into the circulation (109), it might be argued that *in vitro* measurements of plasma lipolytic activity would provide a more direct estimate of changes in intravascular lipolytic activity, and thus of plasma TG clearance. The doses of heparin used in the present study are much lower than those employed for maximal release of endothelial lipases into the circulation (36), however, and the proportion of intravascular lipolytic activity remaining on the endothelium (and consequently inaccessible to *in vitro* assay) under these conditions is not known. Accordingly, changes in intravascular lipolytic activity could not be reliably determined from measurements of plasma lipolytic activity.

Previous studies have suggested that exogenous and endogenous TG- rich lipoproteins may compete for a common clearance pathway (36). Consequently, the rate of Intralipid clearance is determined in part by the size of the endogenous plasma TG pool. Since heparin infusion decreases the endogenous TG concentration, the increased rate of Intralipid clearance measured in the present study may be partly attributable to a reduction in the number of endogenous TG-rich lipoprotein particles. To evaluate the fraction of the heparin-induced increase in Intralipid clearance which can be attributed to reduced TG-pool size, we calculated the regression of k_2 on fasting serum TG concentration in the 12 normolipidemic young men who comprised group A. These data were chosen because of the high correlation ($r = 0.80$) between these 2 parameters in this sample. The regression coefficient (b) was -0.603 in this sample. From these data, it can be calculated that a 16mg/dL decrease in endogenous plasma TG concentration (the average decrease in the present study) would result in a 1%/minute increase in the rate of Intralipid clearance. Since the heparin-induced increase in k_2 averaged 6.1%/minute it can be concluded that less than 17% of this increase is attributable to changes in endogenous TG concentration.

The observation that Intralipid clearance is sensitive to small changes in the rate of plasma TG clearance provides further support for the validity of this emulsion as a tracer of the lipolytic phase of chylomicron metabolism. In addition, these findings suggest that the intravenous fat tolerance test can

be used to determine whether experimental perturbations of serum TG concentrations are secondary to increased intravascular lipolytic activity.

In summary, the results of the present study indicate good agreement between 3 indirect methods for assessing chylomicron-TG clearance. The correlations between these methods reflect a common dependence on chylomicron-TG clearance, rather than on unrelated factors such as TG absorption. The rate of chylomicron clearance is related to fasting TG pool size. It is not clear, however, whether, or to what extent the relationship between clearance and pool size reflects a common dependence upon the TG removal system(s).

CHAPTER 3

THE RELATIONSHIP BETWEEN MEAL FAT CONTENT AND POSTPRANDIAL LIPEMIA

CHAPTER 3: THE RELATIONSHIP BETWEEN MEAL FAT CONTENT AND POSTPRANDIAL LIPEMIA

INTRODUCTION:

Dietary TG is almost completely absorbed in healthy men (85), therefore postprandial serum TG concentrations are likely to be determined, at least in part, by the quantity of TG consumed in a meal. Postprandial plasma TG concentrations may also vary according to the type of fat consumed. Despite widespread interest in the effects of dietary fats on plasma lipids, the relationship between the TG content of a meal and the magnitude of postprandial lipemia induced by the meal has not been systematically studied.

Postprandial plasma TG concentrations may be acutely regulated by fat-induced increases in plasma TG clearance. Since postprandial lipemia is largely determined by the rate of plasma TG clearance (see Chapter 2), it is likely that putative regulation of postprandial TG concentrations would be effected within the circulation. Fat ingestion may augment plasma TG clearance by 2 mechanisms:

Firstly, it is possible that fat ingestion may stimulate the release of factors which increase the activity of the TG clearing system(s). Eckel et al (58), and Bourdeaux et al (61) have reported that the gastrointestinal polypeptides GIP and pancreaticozym increase the synthesis of LPL by adipocytes in vitro. The physiological significance of these factors in the regulation of chylomicron clearance is uncertain, however. While Wasada et al (59) reported that exogenous GIP administration increases chylomicron clearance in dogs, GIP does not appear to increase intralipid clearance in humans (60).

Secondly, since the rate of clearance of chylomicron-TG appears to be proportional to chylomicron size (38), and since the proportion of large chylomicrons secreted after a meal appears to be related to meal fat content (39), it is possible that the postprandial serum TG excursion following large fat meals may be passively ameliorated as a consequence of the structure of the resulting chylomicrons.

In a study of a single individual, Moreton (110) reported that the postprandial increment in the amount of Tyndall light scattered by serum (which was considered to be an index of alimentary lipemia) was directly proportional to the quantity of fat ingested. This finding suggests that acute fat feeding does not stimulate the rate of clearance of plasma TG, an hypothesis consistent with the subsequent observation that fat ingestion does not stimulate the activity of adipose tissue LPL (49), which is believed to represent the principal removal pathway for chylomicron-TG clearance (46).

Conversely, Kingsbury et al (111) reported that fat loads in excess of 70g did not produce proportionate increases in postprandial serum TG concentrations. If it is assumed that fat absorption was complete in these subjects then disproportionately low increases in postprandial lipemia with increasing fat loads suggest increased fractional clearance of plasma TG at higher fat loads.

Few investigators have examined the effects of TG-fatty acid saturation on postprandial lipemia. In a study of the acute effects of a variety of oils on plasma lipids Kingsbury et al (111) noted differences in the plasma glyceride responses to different oils but the importance of TG-fatty acid composition was not examined. Engelberg (112) reported that TG-fatty acid saturation did not affect postprandial lipemia. This observation was supported by Kashyap et al, who reported no systematic effect of P/S ratio on postprandial lipemia induced by mixed meals in 6 subjects (113). Conversely Harris et al (114) reported that the postprandial rise in plasma TG levels was markedly lower after salmon oil than after a saturated fat meal. Since salmon oil did not appear to be malabsorbed, these authors suggested that dietary salmon oil increased the rate of clearance of chylomicrons.

The present experiments were undertaken to investigate the relationship between meal fat content and postprandial lipemia. My interest in this relationship arose from the observation that in many recent studies (31,75) postprandial lipemia has been measured after the consumption of very large fat loads (350mL heavy cream). Clearly the consumption of this quantity of TG in a single meal does not reflect the dietary custom of Western man, and the relevance of such studies to conventional dietary practice depends upon a linear relationship between fat load and postprandial lipemia. If the magnitude of postprandial lipemia does not vary linearly with the TG dose administered, then conclusions made from studies using a standardized fat load can only be applied to that particular fat load. In order to exclude possible variation due to the type of fat consumed, or to non-fat components in the meal, the effects of three different quantities of dairy cream on postprandial serum TG concentrations were examined in normolipidemic young men. In a second experiment, the serum TG responses to meals containing primarily saturated, monounsaturated or polyunsaturated fats were compared.

METHODS:

1) Subjects:

24 male medical students aged between 20 and 25 volunteered for this study. All were moderately active (1 to 2 hours of exercise per week) nonsmokers and none used any medication known to affect lipoprotein metabolism. Glucose intolerance was excluded by oral glucose tolerance test (115). All had fasting serum TG concentrations below 200mg/dL, and serum cholesterol concentrations below 250mg/dL, on two occasions prior to the study. With one exception, all were non-obese (% body fat as determined from skin fold measurements (91)). The remaining subject was moderately obese (26% body fat).

2) Oral fat tolerance tests:

i) Effects of increasing meal fat content:

The effects of increasing quantities of dairy cream were examined in 12 subjects. Standard pretest meals consisting of four (machine-sliced) slices of white bread, 40g peanut butter, 20g jam, 250mL sweetened orange juice and 500mL whole milk, provided by the investigators were eaten at 6.00 pm on the evening prior to testing. No food or drink (excepting water) was consumed thereafter, until the commencement of testing at 8.00 the following morning.

Oral fat tolerance testing was performed as described in Chapter 1, using meals comprising 100mL (meal 1), 200mL (meal 2), and 300mL (meal 3) cream. The sequence in which the test meals were administered was randomized. The cream used in this experiment was purchased in a single batch and stored at -20 degrees C until use. One hundred mL of cream contained 40g fat. Analysis of the TG fraction of the cream by gas chromatography indicated a fatty acid composition of 11% C14:0, 30% C16:0, 14% C18:0 and 27% C18:1.

ii) Effects of TG-fatty acid saturation:

The serum TG responses to saturated, monounsaturated and polyunsaturated fats were evaluated in 12 men. Saturated fat (meal 4) was given in the form of 100mL light cream (20g fat) and 20g fractionated, hydrogenated palm oil (Morpalm, Marine Oil Refiners, Cape Town). Palmitate (C16:0) and stearate (C18:0) comprised 43% and 36% respectively of the TG-fatty acids in this meal. Olive oil (81% C18:1, meal 5) and sunflower oil (72% C18:2, meal 6) were used as sources of monounsaturated and polyunsaturated fats respectively. These oils were emulsified in 60mL water with glycerol monostearate (2g) and cholesterol (0.5g). Each meal contained 40g fat.

To determine whether the serum TG responses to sunflower oil could be increased by additional fat loading, the subjects were given an additional meal (meal 7) comprising 80g sunflower oil, 4g glycerol monostearate and 1g cholesterol.

3) Analyses:

Serum cholesterol and TG concentrations were determined in a single batch as described in Chapter 1. The fatty acid composition of the meals was determined by gas chromatography as described in Chapter 1.

4) Statistics:

Alimentary lipemia was calculated from the area under the curve described by serum TG concentrations plotted against time (see Chapter 1). Fasting and postprandial serum TG concentrations and postprandial lipemia were compared using the paired t-test. The P values were corrected according to Bonferroni's procedure.

RESULTS:**i) Effects of increasing meal fat content:**

The test procedures were well tolerated by all subjects, although most felt slight, transient nausea after consuming 300mL cream. One subject had diarrhoea and minimal postprandial lipemia after ingesting 300mL cream. This condition persisted when the test was repeated, and these data were therefore excluded from subsequent analyses. Mean fasting serum TG and cholesterol concentrations, postprandial lipemia and maximum postprandial serum TG concentrations are shown in Table 5. Mean fasting serum TG concentration was similar on each test day ($P > 0.25$), but mean postprandial lipemia and the mean maximum postprandial serum TG concentration increased in direct proportion with the dose of fat administered.

Table 5. Fasting serum cholesterol (FTC) and triglyceride (FTG) concentrations, postprandial lipemia (PPL) and maximum postprandial serum triglyceride concentrations (MTG) in 12 normolipidemic men fed 3 doses of dairy cream.

	Meal 1 (40g fat)	Meal 2 (80g fat)	Meal 3 (120g fat)
FTC (mg/dL)	190 \pm 28	190 \pm 34	184 \pm 23
FTG (mg/dL)	92 \pm 37	96 \pm 61	87 \pm 23
PPL (mg/dL ⁻¹ .8hr)	251 \pm 129	503 \pm 233	712 \pm 281
MTG (mg/dL)	59 \pm 27	115 \pm 47	143 \pm 38

The curves described by mean serum TG concentrations plotted against time are shown in Figure 8.

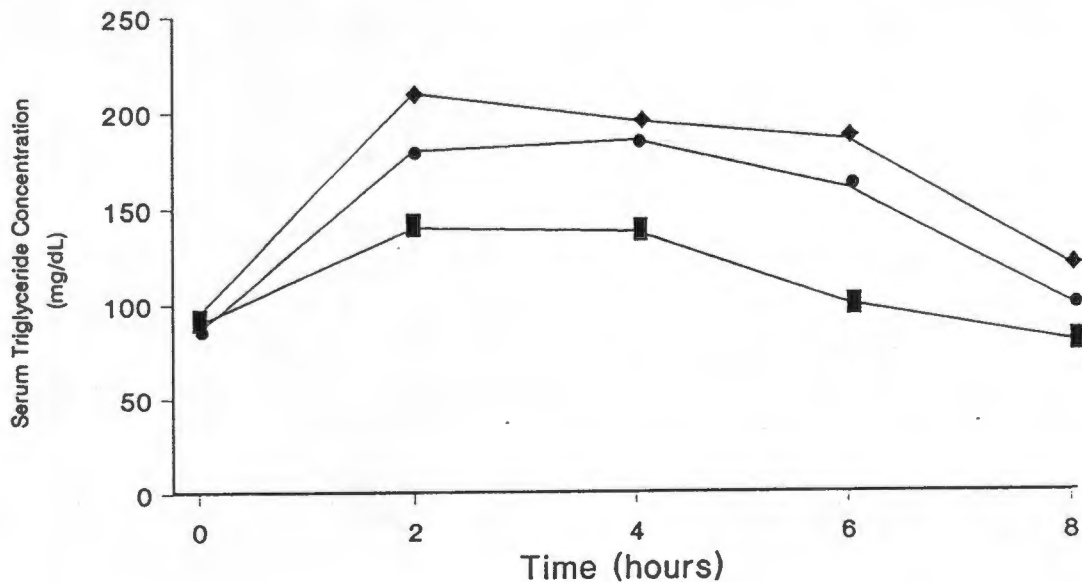


Figure 8. Serum triglyceride responses to 40g (■), 80g (●), and 120g (◆) fat ingestion in 12 normolipidemic men.

After each meal, serum TG concentrations increased rapidly, and maximum (or near maximum) levels were usually achieved within 2 hours. Mean serum TG had returned to baseline (fasting) concentration within 8 hours after meals 1 and 2 but not after meal 3. Extrapolation of the individual curves to baseline at 10 hours increased the mean postprandial lipemia after meal 3 from 712 to 742mg.dL⁻¹.8hr.

ii) Effects of TG-fatty acid saturation:

The test procedures were well tolerated by all subjects and none reported nausea or diarrhoea after any of the tests. Mean fasting TG concentrations were similar before each meal. Mean postprandial lipemia increased with increasing TG-fatty acid saturation (see Table 6). The mean postprandial lipemia was significantly lower after polyunsaturated than after saturated fat.

Table 6. Serum triglyceride responses of 12 normolipidemic men to meals containing 40g saturated (SAT), monounsaturated (MUF), and polyunsaturated (PUF) fat.

	SAT	MUF	PUF
FTG	86±41	85±30	83±32
PPL	161±86	130±98	84±34*
PPI	53±28	56±34	41±23

Abbreviations and units: FTG = fasting triglyceride concentration (mg/dL), PPL = postprandial lipemia (mg/dL⁻¹.7hr), PPI = peak postprandial triglyceride increment above fasting triglyceride concentration
*P < 0.01 compared with SAT (paired t-test)

On average, peak postprandial serum TG concentrations occurred between 2.5 and 3.5 hours after saturated and polyunsaturated fat meals, and at 3.5 hours after the monounsaturated fat meal. Mean serum TG concentrations measured 7 hours after saturated and monounsaturated fats were similar to the fasting values. Seven hours after the 40g polyunsaturated fat meal, mean serum TG concentrations were 30% lower than the fasting value ($P < 0.025$).

In 2 subjects, serum TG concentrations were still increasing 7 hours after the meal containing 80g sunflower oil. These data were therefore excluded from further analysis. In the remaining 10 subjects, serum TG concentrations were maximally increased 4 hours after the meal. Mean postprandial lipemia was 169±122mg.dL⁻¹.7hr in these men. The corresponding value for these 10 subjects after 40g sunflower oil was 83±39mg.dL⁻¹.7hr.

DISCUSSION:

The results of this study indicate that the changes in serum TG concentration which follow the consumption of a fatty meal vary linearly with the fat content of the meal. This relationship was observed over a range of fat intakes typically consumed by men following Western diets. The range of fat loads within which a direct dose-response occurs is somewhat greater than was previously believed. Kingsbury et al (111) suggested that test meals containing more than 60-70g fat did not produce proportionate increases in plasma lipid concentrations, although no data were provided to support this hypothesis. It is possible that this discrepancy is due (at least in part) to differences in the palatability of the test meal. In the present study, the fat load was presented in a palatable form, and although most subjects reported some discomfort after consuming meal 3 (containing approximately 120g fat), this was invariably transient. The appearance of dietary fat in blood (as judged from changes in serum TG concentration) was not delayed at any of the dosages tested, indicating that the onset of fat absorption occurred at a similar time after each test meal. In preliminary studies, however, changes in serum TG concentrations following the ingestion of an equal quantity of fat presented in a less palatable form (olive oil mixed with skimmed milk) were delayed for several hours (J Cohen, unpublished observations).

Although a direct dose-response relationship was observed for both alimentary lipemia (measured as area under the curve) and the maximum postprandial serum TG concentrations after 40g and 80g fat, these responses deviated somewhat from this pattern after consumption of 120g fat. This discrepancy was small for the measurement of total alimentary lipemia (a 2.84 fold increase over meal 1 (40g fat) compared with an expected 3 fold increase) and probably indicates that fat absorption was not completed during the 8 hour measuring period, as serum TG concentrations had not returned to baseline after eight hours in some individuals. The lipemia calculated to 8 hours would therefore underestimate total postprandial lipemia. If the curves of those individuals whose serum TG had not yet returned to fasting values at 8 hours were extrapolated to baseline at 10 hours, mean postprandial lipemia was increased to $742\text{mg}\cdot\text{dL}^{-1}\cdot 8\text{hr}$, reflecting excellent agreement with the expected value ($753\text{mg}\cdot\text{dL}^{-1}\cdot 8\text{hr}$):

The discrepancy between observed and expected values was somewhat greater for the maximum postprandial serum TG concentration (a 2.42 fold increase over meal 1 instead of an expected 3 fold increase). The most likely explanation for this discrepancy is that the maximum rate of fat absorption was reached at a dose intermediate between meals 2 and 3. Beyond this point, additional fat loading would not increase the rate of influx of TG into the circulation, and thus the peak postprandial serum TG concentration would not be further elevated.

It might be argued that malabsorption of fat occurred at the highest fat load used in this study, resulting in a lower peak serum TG concentration than would otherwise be expected. This possibility must be considered unlikely, as previous studies suggest that fat absorption is very efficient, even after large fat meals (85). Patsch et al (75), reported that very small amounts of fat were recovered in the faeces during the 72 hours following meals containing 350mL heavy cream.

Acute fat feeding and the regulation of postprandial lipemia:

If fat ingestion stimulates the fractional rate of plasma TG clearance then incremental fat loads would be expected to produce progressively smaller increases in postprandial lipemia. The finding that incremental fat loads produce directly proportional increases in postprandial lipemia therefore suggests that the hormonal factors elicited by acute fat feeding do not expedite the removal of TG from the circulation. This observation is consistent with the finding that adipose tissue LPL activity is not stimulated by acute fat feeding (49). Therefore, while the ingestion of fat increases the plasma concentration of hormones which appear to increase adipose tissue activity *in vitro*, it appears reasonable to conclude

that the postprandial serum TG excursion is not actively damped by fat-induced increases in TG clearing capacity.

The linear relationship between fat load and plasma TG concentration also indicates that passive augmentation of the fractional rate of chylomicron clearance due to dose related changes in chylomicron size and structure does not play an important role in the regulation of postprandial plasma TG concentrations. Therefore the postulate that large fat meals lead to the formation of large chylomicrons (39) which appear to be more efficiently cleared from the circulation in the rat (38) is probably of little significance in man.

Postprandial saturation of the TG-removal mechanism(s):

While it is now generally accepted that chylomicron and VLDL-TG are removed from the circulation by a common pathway, there is still some controversy concerning the saturability of this mechanism, and the importance of "saturation kinetics" in various forms of hypertriglyceridemia (34). Grundy and Mok (37) found that the infusion of a fat emulsion into the duodenum at a rate of 200mg/kg/hour, which presumably caused an equivalent increase in the rate of TG influx into the circulation, resulted in relatively small changes in plasma TG concentrations. With continued infusion, a new steady state plasma TG concentration was achieved. These data indicate that the removal pathway of plasma TG is not saturated under these conditions. In the present study, saturation of the TG removal mechanism would have been indicated by higher aggregate postprandial serum TG concentrations after meal 3 than would be predicted in terms of a simple linear dose-response. Clearly, this did not occur.

Therefore, although the relative rates of TG influx into, and efflux from the circulation were not independently quantitated in this study, the linear relationship observed between dietary fat load and serum TG concentration supports the hypothesis that, under physiological conditions, the serum TG removal pathway is not saturated in normolipidemic man.

Although several lines of evidence suggest that chylomicron-TG clearance is not saturated under physiological conditions in normal men, it cannot be assumed that the relation between chylomicron-TG flux and concentration is entirely first order. Recent studies using non-linear kinetic analysis (160) of TG disappearance data in the dog indicate that better predictions of intravenous TG clearance can be obtained from models which include an area of mixed order, in which both zero order and first order processes operate simultaneously. This concept is attractive because it allows for the possibility of competition for clearance sites between chylomicrons and VLDL as proposed by Brunzell et al (36). In these studies (160) plasma TG concentrations during the infusions were elevated some 60-fold above the normal fasting concentration. Since feeding seldom increases the fasting plasma TG concentration more than 2 or 3-fold in man, the physiological relevance of these results is debatable.

Effects of TG-fatty acid saturation on postprandial lipemia:

The results of the present study suggest that postprandial lipemia is significantly affected by the degree of saturation of fat in the test meal. Meals comprising largely saturated fat caused significantly greater increments in postprandial serum TG concentrations than did meals comprising polyunsaturated fat. These findings may reflect malabsorption of polyunsaturated fat. This possibility seems unlikely, as several studies have indicated that corn oil (in which linoleic acid is the primary TG fatty acid) is almost completely absorbed in normal volunteers, even in doses several times greater than those employed in the present study (116). To evaluate the possibility of malabsorption in the present study, the subjects were given an additional meal containing 80g sunflower oil. If the low postprandial serum TG concentrations measured after 40g sunflower oil were due to malabsorption, then it seems likely that increasing doses of sunflower oil would yield little or no further increase in postprandial plasma TG concentrations. Accordingly, the observation that doubling the dose of sunflower oil lead to a

proportionate increase in postprandial lipemia suggests that the low postprandial lipemia observed after 40g sunflower oil is not due to diminished absorption.

An alternative explanation for the diminished postprandial lipemia after sunflower oil ingestion is that chylomicron TG clearance is faster after the ingestion of polyunsaturated fat than after saturated fat. This process may be mediated in several ways:

- 1) Sunflower oil may increase the activity of the TG clearance system(s). Support for this concept is sparse, however, and a previous study has indicated that the ingestion of 30g corn oil does not increase adipose tissue LPL activity (49). Furthermore, the direct dose response relationship observed in the present study between sunflower oil and plasma TG concentrations suggests that stimulation of the clearance mechanism does not increase with increasing doses of polyunsaturated TG.

- 2) The ingestion of polyunsaturated fats may generate chylomicrons which are cleared more readily from the circulation, resulting in relatively low postprandial lipemia. On the basis of *in vivo* fat tolerance studies using salmon oil (114), and the *in vitro* lipolytic studies of Engelberg (112) in which TG-rich lipoproteins from subjects fed fish oil appeared to provide better substrate for LPL than did TG-rich lipoproteins from subjects on uncontrolled diets, Goodnight et al (117) have suggested that chylomicrons containing omega-3 fatty acids may be more readily cleared from the circulation than are those containing saturated fats.

Studies in animals suggest that the low postprandial lipemia after polyunsaturated fat feeding observed in the present study and in the study of Harris and Connor (114) is not related to putative alterations in chylomicron structure induced by polyunsaturated fat ingestion, however. In rats and dogs, the clearance of intravenously infused homologous lymph chylomicron- TG was similar, regardless of whether the donor animals had been fed cream or corn oil (118).

Furthermore, in apparent contradiction of his *in vitro* findings, and in contrast to the findings of the present study, Engelberg (112) cited unpublished observations which suggested that postprandial lipemia in man was not influenced by fat saturation. Therefore in the absence of *in vivo* evidence that the effects of TG saturation on postprandial serum TG concentrations reflect structural differences in the chylomicrons, the validity of this hypothesis remains open to question.

- 3) A third possibility is that hepatic VLDL release is diminished by polyunsaturated fat ingestion. Diminished VLDL release would decrease plasma TG concentration directly, by decreasing the plasma concentration of endogenous TG, and possibly also indirectly, by decreasing the plasma pool of endogenous plasma TG-rich lipoproteins which may compete with chylomicrons for a common clearance pathway. This hypothesis would explain the present finding that serum TG concentrations were reduced below the fasting value 7 hours after sunflower oil ingestion, when hepatic VLDL would be expected to provide the major source of plasma TG.

In summary, the present experiments illustrate the significant effects both of the amount and the type of ingested fat on postprandial serum TG concentrations. For a given type of TG, postprandial lipemia rises in direct proportion to the quantity of TG in the meal. These data suggest that fat ingestion does not lead to an increase in the fractional rate of fat clearance. If similar amounts of fat are ingested, postprandial lipemia will be lower after polyunsaturated than after saturated fats. This difference does not appear to be due to differences in TG absorption. From the experimental evidence available to date, however, it is not possible to determine which of the mechanisms described above is responsible for the effects of TG-fatty acid saturation on postprandial lipemia.

CHAPTER 4

THE EFFECTS OF PROTEIN INGESTION ON POSTPRANDIAL LIPEMIA AND CHYLOMICRON-TRIGLYCERIDE CLEARANCE

CHAPTER 4: THE EFFECTS OF PROTEIN INGESTION ON POSTPRANDIAL LIPEMIA AND CHYLOMICRON-TRIGLYCERIDE CLEARANCE

INTRODUCTION:

Previous studies have indicated that postprandial lipemia may be significantly influenced by the fat (see Chapter 3) and carbohydrate (see Chapter 5) content of a test meal. The influence of dietary protein on the degree of postprandial lipemia evoked by a standard fat meal has received little study, however. Sullivan (119) reported that serum TG concentrations measured 3 hours after the ingestion of a fatty meal may be increased by the addition of protein to the test meal. The results of this study are difficult to interpret, as only 4 of the 6 subjects in the study exhibited increased postprandial lipemia after protein ingestion. Accordingly, the effects of protein ingestion on postprandial lipemia have remained unclear.

The present study was undertaken to examine the effects of acute protein ingestion on postprandial lipemia and on intravenous fat tolerance.

METHODS:

1) Subjects:

Fifteen normotriglyceridemic medical students (12 men and 3 women, mean age 20 ± 2 years, mean body weight 67 ± 12 kg) were recruited for this study. None of the subjects smoked, and none used any medication known to affect lipoprotein metabolism.

2) Procedures:

i) Oral fat tolerance tests:

Each subject performed 2 oral fat tolerance tests in random order. On one occasion, the test meal consisted of 100mL cream, 3g chocolate flavouring, and 300mL water (meal 1). For the second test, 25g of a commercial protein powder (Casilan) containing 93% sodium caseinate was added to the meal (meal 2). Blood samples were drawn into plain tubes just before, and at 2, 3, 4, 6 and 7 hours after the meal. The serum TG concentration of each sample was determined enzymatically (see Chapter 1).

To examine the effects of a larger protein load, 3 subjects performed an additional test in which 33g protein was added to the cream meal.

ii) Intravenous fat tolerance tests:

Intravenous fat tolerance testing was performed in 10 men as described in Chapter 1. Each subject performed a baseline test in the morning following an overnight fast. On completion of the test, a solution comprising 25g Casilan in 300mL water was consumed, and a second test was performed 3 hours later.

3) Analysis:

Postprandial lipemia and the rate of disappearance of Intralipid (k_2) was calculated as described in Chapter 1. The mean values for postprandial lipemia and k_2 determined before and after protein ingestion were compared using Wilcoxon's signed rank test for paired data.

RESULTS:**1) Oral fat tolerance:**

Mean fasting serum TG concentration was similar before meal 1 (61 ± 17 mg/dL) and meal 2 (62 ± 22 mg/dL). The shape of the curves of serum TG concentration plotted against time were similar after both meals (see Figure 9). Mean postprandial lipemia was slightly, but not significantly higher after meal 2 (213 ± 112 mg.dl⁻¹.7hr) than after meal 1 (190 ± 101 mg.dl⁻¹.7hr, $P > 0.025$). The mean maximum serum TG increment above the fasting value was 55 ± 28 mg/dL after meal 1 and 57 ± 29 mg/dL after meal 2.

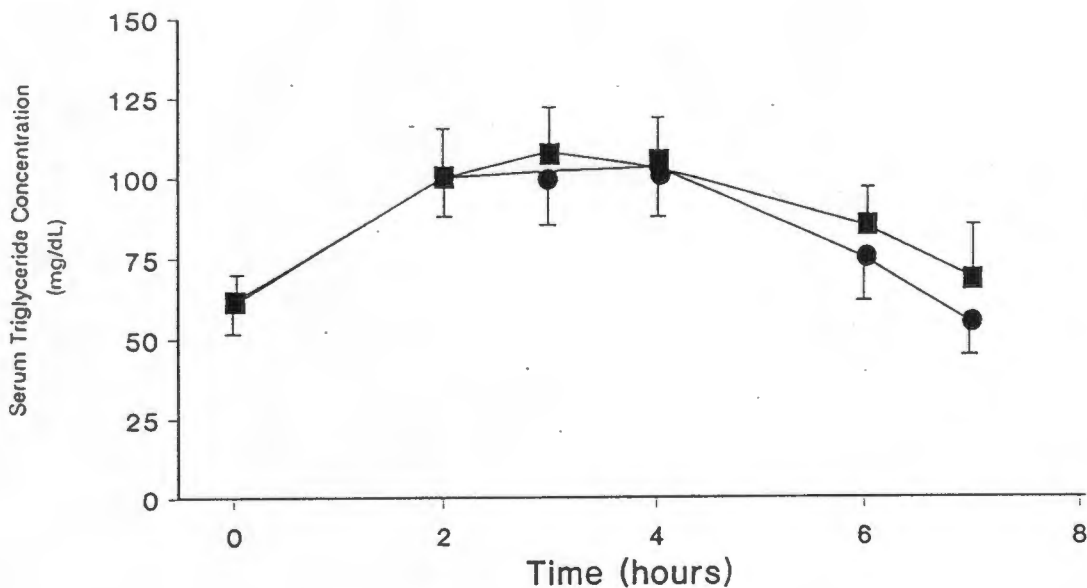


Figure 9. Mean serum triglyceride concentrations of 15 normolipidemic subjects after meals containing 23g protein and 40g fat (■) and 40g fat alone (●).

In the three subjects who received 33g protein, postprandial lipemia was 63, 278, and 160 mg.dl⁻¹.7hr after cream alone. The corresponding values after the ingestion protein and cream were 73, 235, and 151 mg.dl⁻¹.7hr.

2) Intravenous fat tolerance:

The decay curve of plasma Intralipid concentration plotted against time was adequately fitted by a single exponential (mean $r = 0.98$). The rate of disappearance of Intralipid was similar before ($6.4 \pm 1.4\%$ /minute) and after ($6.1 \pm 1.5\%$ /minute) protein ingestion ($P > 0.25$).

DISCUSSION:

The results of this study indicate that acute protein ingestion does not significantly influence the degree of lipemia elicited by a standard fat meal. These findings are supported by the observation that, in the present study, the ingestion of 23g protein did not significantly influence the rate of disappearance of an intravenous bolus of Intralipid, an artificial fat emulsion which models the intravascular decay kinetics of chylomicrons (35). Only one previous study has examined the effects of dietary protein on postprandial lipemia. Sullivan (119) reported that protein ingestion augmented postprandial lipemia in 4 out of 6 subjects. In the present study, postprandial lipemia was slightly augmented by protein ingestion in 8 out of 15 subjects. In the other 7 subjects, postprandial lipemia was slightly lower after meals containing protein and fat than after meals containing fat alone. Therefore, while the different protocols used in the 2 studies preclude more direct comparison, the results of these studies appear compatible.

It is possible that the doses of protein used in this study were too small to significantly affect postprandial lipemia. To evaluate this hypothesis, three subjects were challenged with a larger dose (33g) of protein. The larger dose clearly did not have a greater effect than did the smaller dose used in the study. Therefore it seems likely that the amount of protein typically consumed in a single mixed meal does not significantly affect the magnitude of lipemia induced by that meal.

A previous study has suggested that intravenous glucagon administration may diminish postprandial lipemia (120). Since protein ingestion stimulates endogenous glucagon release (121), it is possible that dietary protein may modulate postprandial lipemia by increasing plasma glucagon concentrations. The results of the present study suggest that, under physiological conditions, hormonal factors (including glucagon) which are released in response to protein ingestion do not play a significant role in the regulation of postprandial lipemia.

CHAPTER 5

THE EFFECTS OF CARBOHYDRATES AND INSULIN ON POSTPRANDIAL LIPEMIA AND CHYLOMICRON CLEARANCE

CHAPTER 5: THE EFFECTS OF CARBOHYDRATES AND INSULIN ON POSTPRANDIAL LIPEMIA AND CHYLOMICRON CLEARANCE

INTRODUCTION:

Although the relationship between dietary carbohydrate and serum TG metabolism has been extensively investigated, few studies have examined the role of acute carbohydrate ingestion in regulating the plasma TG responses to mixed meals. In early studies, the ingestion of large quantities of carbohydrates (119), particularly glucose (122), appeared to reduce the magnitude of lipemia evoked by a fat-containing meal. Since the magnitude of postprandial lipemia is believed to be determined largely by the rate of chylomicron-TG clearance (see reference 30 and Chapter 2), some investigators (123,124) have proposed that changes in the serum TG pool size which follow the ingestion of a mixed meal are actively damped by an acute increase in the capacity of the serum TG removal mechanism(s). Studies in the rat have indicated that acute insulin administration increased the activity of adipose tissue LPL (see 47 for review), the rate limiting enzyme for plasma TG clearance, therefore it was proposed that insulin released in response to glucose ingestion increased adipose tissue LPL activity and hence chylomicron-TG clearance.

The results of several studies appear to support this hypothesis. Mann et al (124) reported lower postprandial plasma TG concentrations after meals containing glucose and fat than after sucrose and fat, and an inverse correlation was found between postprandial plasma insulin and TG concentrations. The ingestion of fructose, which elicits little increase in plasma insulin concentrations, may cause increased rather than decreased postprandial plasma TG concentrations (123). Nilsson-Ehle (49), reported a direct correlation between plasma insulin concentrations and adipose tissue LPL activity after glucose ingestion. Carbohydrate ingestion increases adipose-tissue LPL activity in normal but not in diabetic men (52).

In more recent studies, however, the importance of acute postprandial changes in plasma insulin concentrations in the regulation of postprandial lipemia and plasma TG removal has been questioned. Nicholls et al (125) reported that 50g glucose ingestion did not diminish postprandial lipemia following meals containing 62g fat. Furthermore, increases in adipose tissue LPL activity may only become significant several hours after insulin administration (50), and this effect is significantly blunted by fat ingestion (53). In addition, while several studies have indicated that the administration of glucose alone causes increased adipose tissue LPL activity (49,126), it appears that the ingestion of mixed meals has little effect on the activity of this enzyme in man (33,52).

Although the acute effects of insulin on adipose-tissue LPL activity have been extensively documented (see 48 for review), the effects of glucose ingestion on chylomicron-TG clearance have not been systematically studied, and the reasons for the disparate findings of previous investigations into the effects of glucose ingestion on postprandial lipemia are not known.

The purpose of the present study was to investigate the role of carbohydrate ingestion and acute postprandial changes in plasma insulin concentrations in the regulation of postprandial lipemia. Some studies (123,124) have indicated qualitative differences in the postprandial TG responses to different carbohydrates, but further comparison between the effects of these carbohydrates is hampered by the different doses of carbohydrate and fat used, and by the different blood sampling protocols employed in these studies. No study has compared the effects of each of these carbohydrates examined under similar experimental conditions. In the present study, the effects of glucose, fructose and sucrose on the magnitude of lipemia evoked by a standard fat load have been compared using a randomized, longitudinal experimental design. To clarify the relationship between glucose ingestion and postprandial plasma TG metabolism in normolipidemic subjects a similar experimental design was employed to compare the effects of two different doses of glucose on postprandial lipemia. The possible effects of glucose

ingestion on TG absorption were evaluated using the intravenous fat tolerance test and a duodenal fat perfusion.

To determine whether the putative hypotriglyceridemic effect of glucose is more pronounced in insulin-sensitive individuals, we examined the effects of glucose ingestion on postprandial lipemia in highly trained male athletes, a group characterized by extreme sensitivity to the action of insulin (127). The possible influence of glucose ingestion on LPL-independent TG removal was investigated in 3 men with type I hyperlipoproteinemia due to severely defective LPL activity.

Only one study has examined the effect of acute insulin administration on postprandial serum TG concentrations in man. Krut et al (128) found that the intravenous infusion of insulin in 10 percent glucose solution reduced postprandial lipemia (measured as serum turbidity) and chylomicron TG concentrations, but that the infusion of insulin alone did not. In the present study, the effect of acute postprandial hyperinsulinemia on concomitant serum TG metabolism was evaluated. Since the administration of insulin to healthy subjects may result in hypoglycemia, which in turn provokes the release of counter-regulatory hormones such as adrenaline, glucagon and growth hormone, the effect of insulin alone (ie. without exogenous glucose) on the serum TG responses to a standardized fat meal and on the rate of removal (k₂) of an intravenously administered TG emulsion was examined in young, normotriglyceridemic type I diabetics.

METHODS:

1) Subjects:

i) Sedentary subjects:

Seventy-six medical students (57 men and 19 women) gave informed consent for this study. The subjects were apparently healthy, nonobese (91), normolipidemic, nonsmokers. None performed regular vigorous exercise and none used oral contraception or any other medication known to affect lipid metabolism.

ii) Athletic subjects:

Twelve men who performed at least one hour of vigorous exercise per day, and who competed regularly in running or in triathlon events were recruited on the assumption that they would have increased sensitivity to the glucoregulatory action of insulin. This contention is supported by the observation that the integrated plasma insulin ($80 \pm 39 \text{mU/l.2hr}$) and glucose ($4.0 \pm 3.2 \text{mmol/l.2hr}$) responses to 75g oral glucose were markedly lower in these men than in a group of 12 sedentary men of similar age and weight ($234 \pm 175 \text{mU/l.2hr}$ and $8.5 \pm 6.3 \text{mmol/l.2hr}$ respectively).

iii) Type I hyperlipoproteinemic subjects:

Three men with Type I hyperlipoproteinemia due to familial LPL deficiency were recruited from the Lipid Clinic at Groote Schuur Hospital. The clinical characteristics of these patients have been reported previously (129). Although detailed dietary assessment was not attempted in this study, these subjects reportedly practised little or no dietary restriction, and all had marked chylomicronemia after an overnight fast.

iv) Type I diabetic subjects:

Twenty insulin-dependent diabetics (11 males and 9 females) who did not smoke or drink any alcohol, and who had been treated with insulin for a minimum of 3 years were recruited for the study (see Table 5 for clinical data). All of the subjects were classified as type I diabetic according to the criteria of the National Diabetes Data Group (130). Diabetic control, assessed from measurements of glycosylated haemoglobin, was considered fair to good (see Table 5). Informed consent was obtained from all subjects. Where the subjects were under 18 years of age, parental consent was also obtained.

2) Study design:

i) Comparison of glucose, fructose and sucrose:

Twenty-one sedentary subjects (12 women and 9 men, see Table 6 for clinical data) were randomly allocated to 7 treatment sequences arranged as three 7x7 latin squares (131). The tests were performed at 3-day intervals. At 7 pm on the evening before each test, each subject received a standard meal comprising 4 (machine sliced) slices of white bread, 40g peanut butter, 20g jam, 500mL whole milk and 250mL unsweetened apple juice. Thereafter, no food or drink (excepting water) was consumed until the commencement of the test procedure on the following day.

Each subject reported to the laboratory at 7.30am and a blood sample was drawn without stasis from an antecubital vein. Subjects were seated for at least 10 minutes before, and during phlebotomy. The test meals were then consumed within 10 minutes. Further blood samples were drawn at 2, 4, 6 and 7

hours postprandial. Blood samples were allowed to clot and serum was collected by centrifugation and stored at -20 degrees C.

A stock meal comprising 100mL dairy cream (40g fat), 1 level teaspoon chocolate flavouring and 300mL water was consumed during tests 1 to 6. The ingredients were stirred together to form a readily drinkable liquid. For test 1, the stock meal was consumed without addition of carbohydrate. For test 2, 50g glucose was added to the stock meal. Additional blood samples were drawn from 9 subjects at 0.5, 1, and 1.5 hours postprandial. These samples were taken into tubes containing potassium oxalate and sodium fluoride (for glucose determination) and EDTA (for insulin assay). For test 3, the subjects consumed the stock meal. Thereafter, 500mL of a 10% glucose solution was infused in 45 minutes via a 22 gauge catheter placed in a cubital vein. For test 4, 50g fructose was added to the stock meal, while for test 5, 50g sucrose was added. For test 6, 100g sucrose was added to the stock meal. For test 7, intravenous fat tolerance was determined as described in Chapter 1.

ii) Further investigation into the effects of glucose on postprandial lipemia:

a) Oral fat tolerance:

Eighteen subjects (11 men and 7 women, see Table 7 for clinical data) were randomly allocated to 3 treatment sequences arranged as six 3x3 latin squares (131). Oral fat tolerance tests were performed at 3 day intervals as described in Chapter 1. A stock meal comprising 100mL cream (40g fat), 3g chocolate flavouring and 250mL water was consumed during each test.

For test 1, the stock meal was consumed without addition of glucose. For test 2, 50g glucose was added to the stock meal. For test 3, subjects consumed the stock meal plus 50g glucose. Thirty minutes later, a further 50g glucose was ingested. Blood samples were drawn before, and at 2,3,4,6, and 7 hours after the meals. During tests 2 and 3, blood samples for plasma insulin and glucose determination were taken from 9 subjects before and at 0.5, 1, 1.5 and 2 hours after the meal.

In 8 of these subjects, 2 further tests were performed using the protocol described above. The test meals comprised 100mL cream, 270mL water, 16.6g urea and 30mL lemon concentrate and 100mL cream, 300mL water and 100g starch. The starch was given in two 50g doses as described for meal 3 above.

Oral fat tolerance was also measured in 12 athletes. Each subject was tested twice, with an interval of 3 days between tests. The test order was randomized. For test 1, the subjects consumed the stock meal described above, while for test 2, 50g glucose was added to the stock meal. During test 2, a blood sample for plasma insulin determination was taken 30 minutes after the ingestion of the meal.

b) Intravenous fat tolerance:

Intravenous fat tolerance testing was performed in 17 men, as described in Chapter 1. Each subject was tested 3 times, with an interval of at least 2 days between tests. The tests were performed in random order. One test was performed after an overnight fast (IVFTT1). The other tests were performed 2 hours (IVFTT2) and 4 hours (IVFTT3) after the ingestion of 50g glucose. During IVFTT2, plasma insulin concentrations were measured 30 minutes after glucose ingestion.

c) Intraduodenal fat administration:

Serum TG responses to intraduodenal fat administration were determined as described in Chapter 1 using a fat emulsion comprising 100g olive oil and 7g gum acacia per litre of water infused at a rate of 175mg triglyceride/kg/min. Previous studies have indicated that fat in this form is efficiently absorbed after oral administration (92).

Each subject was tested twice. A baseline test was performed in which the stock emulsion only was given. On another occasion, 200g glucose (20g/hour) was added to the perfusate of 10 subjects (perfusion 2), while the other 10 subjects drank a solution comprising 50g glucose in 400mL water (perfusion 3). The solution was given 3 hours after commencing the perfusion. The test sequence was randomized.

d) Glucose feeding in type I hyperlipoproteinemic men:

Fasting blood samples were drawn at 8.00am from 3 type I hyperlipoproteinemic men. Each drank a solution comprising 50g glucose in 400mL water at 8.05am and again at 8.30am. Further blood samples were taken at 10.00am and at 12.00am.

iii) Effects of insulin administration in Type I diabetics:

a) Oral fat tolerance tests:

Each subject consumed a standardized fat meal (i) 10 minutes after insulin administration (procedure 1), and (ii) 13 hours after insulin administration (procedure 2). Procedures 1 and 2 were performed in randomized order, and were separated by a 7 day interval. In 13 subjects, the test meals comprised 100mL dairy cream (40g fat) diluted with 100mL water. In 7 subjects, the test meals comprised 40mL olive oil in 160mL skimmed milk. Blood samples were collected 10 minutes before the meal and then at 1 hour intervals for 5 hours after the meal. Blood was collected into plain tubes for the measurement of TG; perchloric acid for the measurement of acetoacetate and beta-hydroxybutyrate; EDTA and aprotinin (Trasylol) for the measurement of glucagon and growth hormone; potassium oxalate and sodium fluoride for the measurement of glucose and glycosylated haemoglobin. During the test, the glucose concentrations of the hourly blood samples were measured using an Ames glucometer. Where necessary, hypoglycemia was prevented by the administration of glucose sweets.

b) Intravenous fat tolerance tests:

Intravenous fat tolerance was measured in 9 subjects (i) 2 hours after insulin administration (procedure 3) and (ii) 13 hours after insulin administration (procedure 4).

c) Insulin administration:

During procedures 1 and 3, the subjects received their usual morning dose of insulin by subcutaneous injection (see Table 5). All subjects used combinations of fast-acting (Actrapid) and crystalline (Monotard) insulin preparations. In a preliminary study of 5 of these subjects, plasma free insulin concentrations were measured using the method of Nakagawa (132) before, and at 1, 2, and 3 hours after insulin administration.

3) Analysis of samples:

Serum TG Intralipid-TG, cholesterol, HDL-cholesterol, glucose and insulin concentrations were determined as described in Chapter 1. Samples from the hyperlipoproteinemic subjects were diluted 10 fold with normal saline before assay. Plasma glucagon concentrations were determined by radioimmunoassay with an antibody (V13) which is specific for the C-terminus of glucagon. Beta-hydroxybutyrate (133) and acetoacetate (134) were assayed enzymatically. Glycosylated haemoglobin was assayed by column chromatography (Test Combination 556742, Boehringer Mannheim).

4) Analysis of Data:

i) Postprandial lipemia:

Two indices of postprandial lipemia were calculated from the postprandial serum TG measurements: Total postprandial lipemia was calculated by numerical integration of the area under the curve described by serum TG concentrations plotted against time (Chapter 1). The maximum increment in serum TG concentration was calculated from the difference between the maximum postprandial TG concentration and the fasting serum TG concentration.

ii) Intravenous fat tolerance:

The rate of disappearance of Intralipid (k_2) was determined from the monoexponential decay curve of plasma Intralipid concentrations as described in Chapter 1.

iii) Statistical methods:

a) Analysis of Latin Square design:

These data were compared using analysis of variance (ANOVA) after a normal probability plot of the residuals revealed no evidence of non-normality or serious heteroscedasticity. The blocking variables were SUBJECT and ORDER POSITION OF THE TREATMENT. After the preliminary F-test indicated the variable TREATMENT to be significant, differences between treatments were investigated using the Ryan-Einot-Gabriel-Welsch (REGW) multiple F-test. This test is considered to be more powerful than traditional simultaneous procedures such as Tukey's method (135).

b) Other analyses:

The mean plasma insulin concentrations measured 30 minutes after meal 2 in athletes and in sedentary men were compared using an unpaired t-test. All other parameters were compared using the paired t-test (95). Where the mean values of repeated measures were compared, the level of probability was adjusted using Bonferroni's procedure (135).

The correlation between the insulin response to meals containing glucose and the hypotriglyceridemic effect of glucose (calculated as the difference in postprandial lipemia between meals containing glucose and fat and those containing fat alone) was estimated from the sample correlation coefficient (95).

RESULTS:

In general, the test procedures were well tolerated except for a slight, transient dizziness reported by most subjects 2 hours after the intravenous glucose infusion. No subject was nauseated or had diarrhoea during or after any of the tests.

i) Comparison of glucose, fructose and sucrose:

The ANOVA showed the blocking variable SUBJECT and the variable TREATMENT to be highly significant ($P < 0.0001$). The variable ORDER POSITION OF THE TREATMENT was not significant ($P > 0.6$). Fasting TG concentration fitted as a co-variate was not significant ($P > 0.8$). The REGW procedure showed no significant effect of oral or intravenous glucose administration or of 50g sucrose ingestion on total postprandial lipemia or on the maximum postprandial serum TG increment above the fasting value (see Table 8). The addition of 50g fructose, or of 100g sucrose, to the stock meal resulted in significantly greater postprandial lipemia, and significantly higher maximum serum TG increments than did the ingestion of fat alone, however.

Table 7. Clinical data of the 21 subjects in this study in whom 5 different carbohydrate treatments were compared.

	Men	Women
Age (yr)	20±3	19±1
Weight (kg)	74±7	59±8
Height (cm)	181±8	167±5
% Body fat	13±2	22±2
FTC (mg/dL)	177±21	190±31
HDL-C (mg/dL)	58±10	63±9
HDL2-C (mg/dL)	15±10	21±10

Abbreviations: FTC = fasting total cholesterol, HDL-C = high-density lipoprotein cholesterol, HDL2-C = high-density lipoprotein subfraction 2 cholesterol. Each value represents the mean ± the standard deviation

Table 8. Effects of 5 different carbohydrate treatments on the serum triglyceride responses to a meal containing 40g fat.

Grouping	Treatment	Mean PPL (mg·dL ⁻¹ ·7hr)	Mean PPI (mg/dL)
A	50g Fructose	374±186	94±46
A	100g Sucrose	334±168	85±41
B	A 50g Glucose (IV)	314±149	76±39
B	C 50g Sucrose	243±118	63±28
B	C Stock Meal	214±110	56±29
	C 50g Glucose (Oral)	187±109	50±24

Key: Each value represents the mean ± the standard deviation for 9 males and 12 females. Treatments covered by the same grouping letter are not significantly different (Ryan-Einot-Gabriel-Welsch Multiple F-test).

Abbreviations: PPL = total postprandial lipemia, PPI = maximum postprandial triglyceride increment.

ii) Further investigation into the effects of glucose:

a) Oral fat tolerance:

The mean ages and anthropometric data of the sedentary subjects are given in Table 9.

Table 9. Age and anthropometric data of 18 normolipidemic men and women in whom the effects of 50 and 100g glucose were studied.

	Men (n = 11)	Women (n = 7)
AGE (years)	22±3	19±0.5
HEIGHT (cm)	178±5	166±4
WEIGHT (kg)	72±10	54±3
%BODYFAT	14±3	22±3

Values are means ± standard deviations.

In the sedentary subjects fasting serum TG concentration was similar before each test (see Table 10). Mean postprandial lipemia was lower after the glucose-containing meals than after cream alone, but the responses to glucose were quite variable between individuals and were therefore not statistically significant at the 5% level. In 7 out of 18 subjects, postprandial lipemia was higher after meals containing 50g glucose and cream than after cream alone. The mean maximum TG increase above the fasting value was significantly lower after meals containing glucose and cream than after meals containing cream alone.

Table 10. Plasma insulin and triglyceride responses of sedentary subjects to meals containing different proportions of glucose and fat.

	Meal 1 (40g fat)	Meal 2 (40g fat + 50g glucose)	Meal 3 (40g fat + 100g glucose)
FTG	80±28	76±31	74±28
PPL	171±104	130±106	105±76
PTG	124±39	104±40a	102±41a
TG2	124±39	98±45a	83±43a
FI	9±3	10±5	9±2
IR	27±5	231±112	445±243

All triglyceride data are means ± standard deviations of 18 subjects. Insulin data are means ± standard deviations of 9 subjects for meals 2 and 3 and 3 subjects for Meal 1.

Abbreviations and units: FTG = fasting serum triglyceride concentration (mg/dL), PPL = postprandial lipemia (mg.dL⁻¹.7hr), PTG = peak serum triglyceride concentration (mg/dL), TG2 = serum triglyceride concentration 2 hours after the meal (mg/dL), FI = fasting plasma insulin concentration (mU/L), IR = integrated insulin response (mU.L⁻¹.2hr)

a = P.05 calculated using Ryan-Einot-Gabriel-Welsch multiple F test.

The mean maximum increment in serum TG concentration occurred 2 hours after cream alone, 4 hours after cream + 50g glucose, and 6 hours after cream + 100g glucose (see Figure 10). The integrated plasma insulin concentrations were not significantly correlated with the effect of glucose on postprandial lipemia ($r=0.38$, $P>0.1$).

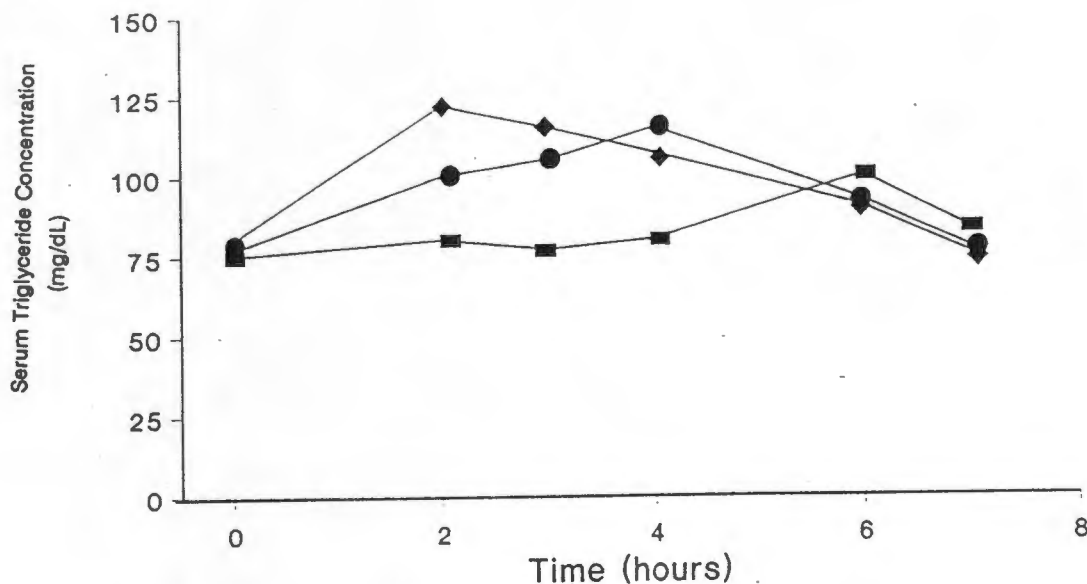


Figure 10. Mean serum triglyceride concentrations after meals containing 40g fat (◆), 40g fat + 50g glucose (●), and 40g fat + 100g glucose in 18 sedentary, normolipidemic subjects (11 men and 7 women). * = $P < 0.05$ (Ryan-Einot-Gabriel-Welsch test).

Fasting serum TG concentrations and postprandial lipemia measured before meals containing urea and cream or starch and cream were not significantly different from the corresponding values measured during meals containing cream alone (see Table 11). The mean serum TG concentration measured 2 hours after the meal, and the mean maximum increase in serum TG concentration (above the fasting value) were lower after meals containing urea and cream than after cream alone.

Table 11. Serum triglyceride responses to meals containing cream alone (CRE), urea + cream (URE), and starch + cream (STA).

	CRE	URE	STA
FTG	72±28	78±18	80±28
TG(2hr)	123±23	103±27a	119±31
PPL	168±108	133±71	159±46
PPI(max)	58±28	49±22	50±21

Abbreviations and units: FTG = fasting triglyceride concentration (mg/dL), TG(2hr) = triglyceride concentration 2 hours after the meal (mg/dL), PPL = postprandial lipemia ($\text{mg}\cdot\text{dL}^{-1}\cdot\text{7hr}$)

a = $P < 0.05$

Peak serum TG concentrations occurred 4 hours after meals containing urea and cream (Figure 11).

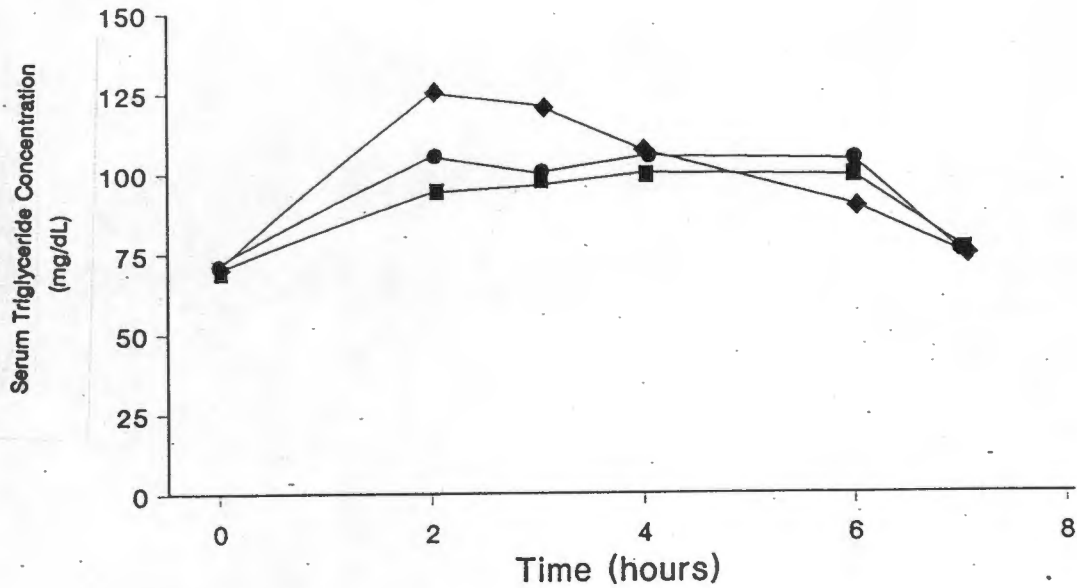


Figure 11. Mean serum triglyceride concentrations of 8 normolipidemic subjects after the ingestion of 40g fat (◆), 40g fat + 50g glucose (■), and 40g fat + 16.6g urea (●).

The addition of 100g starch to the stock meal did not significantly affect any of these parameters (see Figure 12).

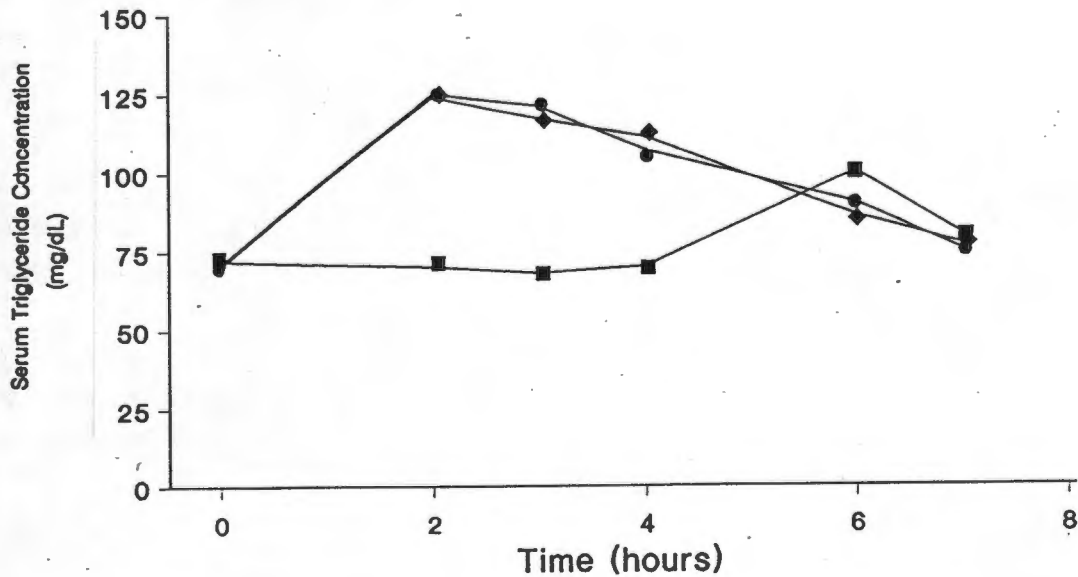


Figure 12. Mean serum triglyceride concentrations of 8 normolipidemic subjects after the ingestion of 40g fat (●), 40g fat + 100g glucose (■), and 40g fat + 100g starch (◆).

In the athletes, the mean fasting serum TG concentration was 62 ± 28 mg/dL before cream ingestion, and 68 ± 23 mg/dL before ingestion of cream + glucose ($P > 0.5$). Mean postprandial lipemia was slightly but not significantly lower after meals containing cream + glucose (108 ± 84 mg.dL⁻¹.7hr) than after meals containing cream alone (139 ± 64 mg.dL⁻¹.7hr, $P > 0.1$).

The mean plasma insulin concentration measured 30 minutes after cream + glucose was significantly lower in athletes than in sedentary men (27 ± 8 versus 56 ± 28 mU/L, $P < 0.001$).

b) Intravenous fat tolerance:

The mean fractional removal rate (k_2) of Intralipid measured 2 hours ($4.5 \pm 1.4\%/min$) or 4 hours ($4.6 \pm 1.5\%/min$) after glucose ingestion did not differ significantly from the rate determined under fasting conditions ($5.0 \pm 1.3\%/min$, $P > 0.05$, see Figure 13). Mean plasma insulin concentrations increased from 5 ± 2 mU/l in the fasted state to 39 ± 20 mU/l 30 minutes after glucose ingestion.

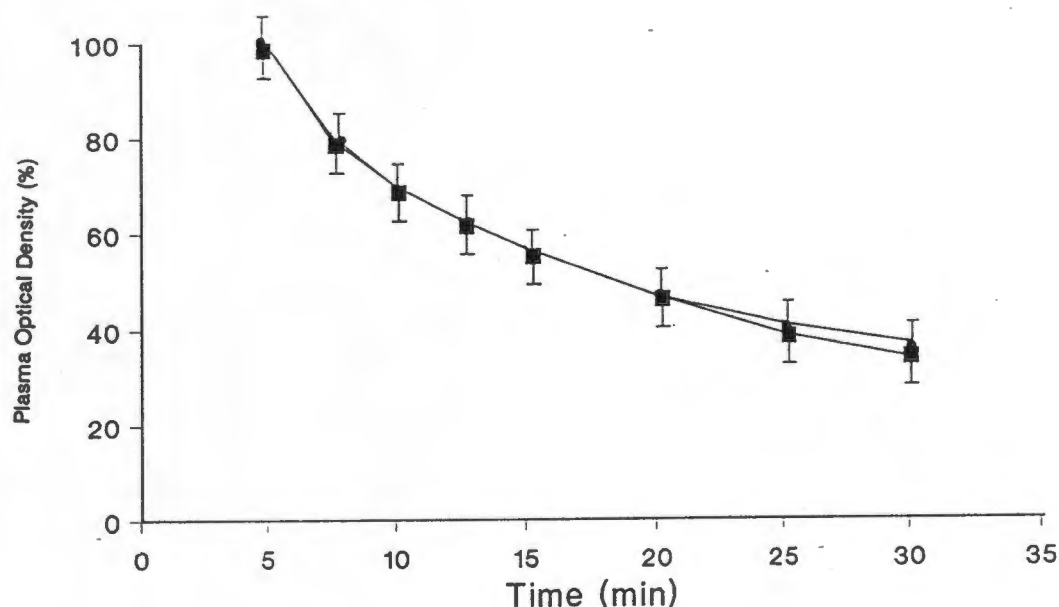


Figure 13. Mean serum optical density after Intralipid injection in 17 normolipidemic men before (■), and 2 hours after (●) 50g glucose ingestion.

c) Chylomicron-TG clearance

During the baseline duodenal perfusion tests (in which no glucose was given) plasma TG concentrations remained constant between 5 and 10 hours after commencement of the infusion (see Figure 14). Mean variation around the steady state value was 13%. Glucose administration, whether orally or by duodenal perfusion, resulted in a gradual decline in plasma TG concentrations during the 5 hour measuring period. The mean plasma TG concentrations of the hourly samples drawn during the baseline study were compared with those drawn at the corresponding times during perfusions 2 and 3. Mean plasma TG concentrations were slightly lower during perfusions 2 and 3 than during the baseline perfusion, but these differences only became significant after 9 hours during perfusion 2 and after 8 hours during perfusion 3.

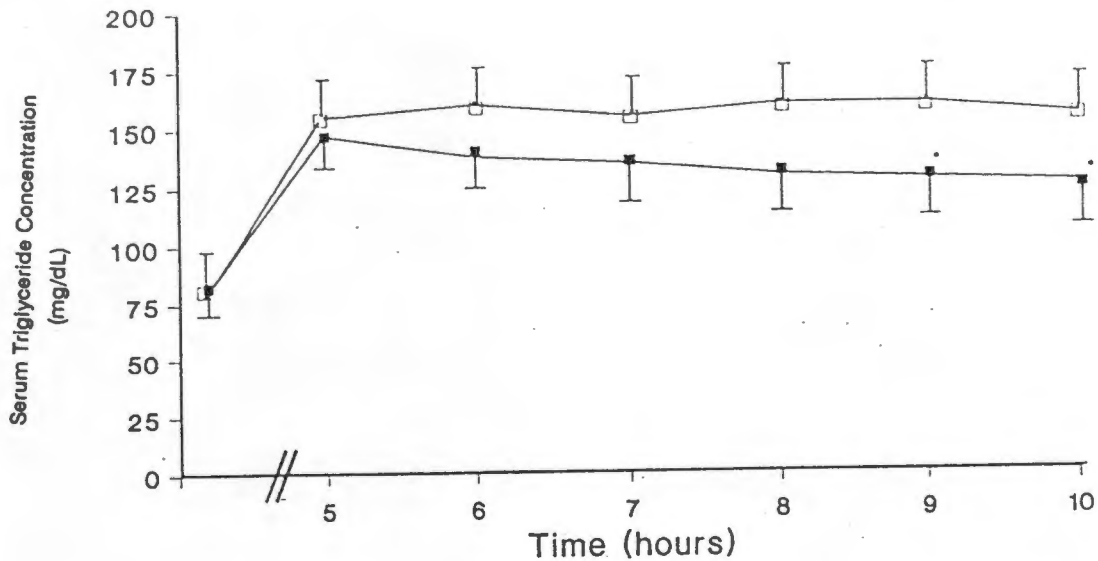


Figure 14. Mean serum triglyceride concentration of 10 men during duodenal perfusion with 10% olive oil (\square), and 10% olive oil + 20g glucose/hour. * = $P < 0.05$ (paired t-test).

d) Type I hyperlipoproteinemic men:

All samples drawn from the type I hyperlipoproteinemic men were grossly lipemic, and a thick, creamy layer formed in samples allowed to stand overnight. Serum TG concentrations decreased slightly during the course of the test (see Table 12).

Table 12. Fasting and postprandial serum triglyceride concentrations in 3 men with type I hyperlipoproteinemia.

	FTG	TG2	TG4
1	2347	2253	2385
2	4600	4300	4400
3	2972	2877	2536

Abbreviations and units: FTG = fasting triglyceride concentration (mg/dl), TG2 = triglyceride concentration 2 hours after glucose ingestion (mg/dL), TG4 = triglyceride concentration 4 hours after glucose ingestion (mg/dL).

iii) Effects of insulin administration in Type I diabetics:

Ages and anthropometric data of the diabetic subjects are given in Table 13.

	Males (n=11)	Females (n=9)
Height (cm)	173±4	157±7
Weight (kg)	67±8	62±7
% Bodyfat*	14±2	30±4
Age (yr)	18±3	19±2
Duration	6±3	8±3
Treatment (Units/Day)	74±24	46±8
HbA1 (%)**	12±2	11±2
SerumCholesterol (mg/dL)	200±27	208±24
HDL-Cholesterol (mg/dL)	64±6	65±8

* Estimated from skinfold thickness
 ** Normal reference range of this laboratory is 5.5-8.5%

a) Oral fat tolerance:

Serum TG responses to olive oil were similar to those measured after cream feeding, therefore the data from these studies were pooled. The mean values of the parameters measured in these studies are given in Table 14.

	Procedure 1	Procedure 2
GLU	15±7	15±5
BHB	468±369	460±572
AA	194±122	109±90
IRG	89±27	97±33
HGH	4±4	5±5
FTG	71±33	68±31
PPL	2.91±2.02	2.46±1.77

Abbreviations and units: GLU = glucose (mmol/L), BHB = beta- hydroxybutyrate (micromol/L), AA = aceto-acetate (micromol/L), IRG = glucagon (ng/L), HGH = growth hormone (µg/L), FTG = fasting triglyceride (mg/dL), PPL = postprandial lipemia (mg.dL⁻¹.5hr).

Glucose and ketone body concentrations:

All subjects were hyperglycemic at the start of each test (see Table 12). Fasting ketone body concentrations were higher than would be expected for a fasted, non-diabetic population (136) but no subject was ketoacidotic, nor did any subject become ketoacidotic during the course of any of the test procedures. After insulin administration, plasma glucose and beta-hydroxybutyrate concentrations fell

sharply in all subjects (see Figures 15 & 16), generally reaching a nadir within 1 to 3 hours. No subject ingested more than 7g glucose, and none complained of hypoglycemic symptoms. In the studies in which no insulin was given, mean glucose and beta-hydroxybutyrate concentrations rose gradually throughout the test.

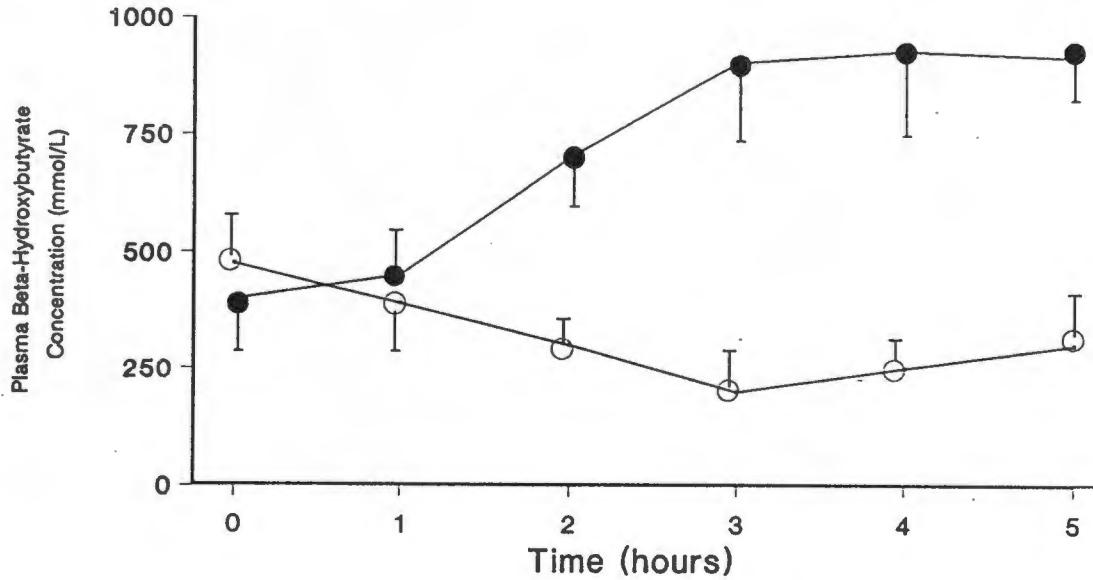


Figure 15. Blood Beta-Hydroxybutyrate responses to fat ingestion in diabetic patients with (○) and without (●) concomitant insulin administration.

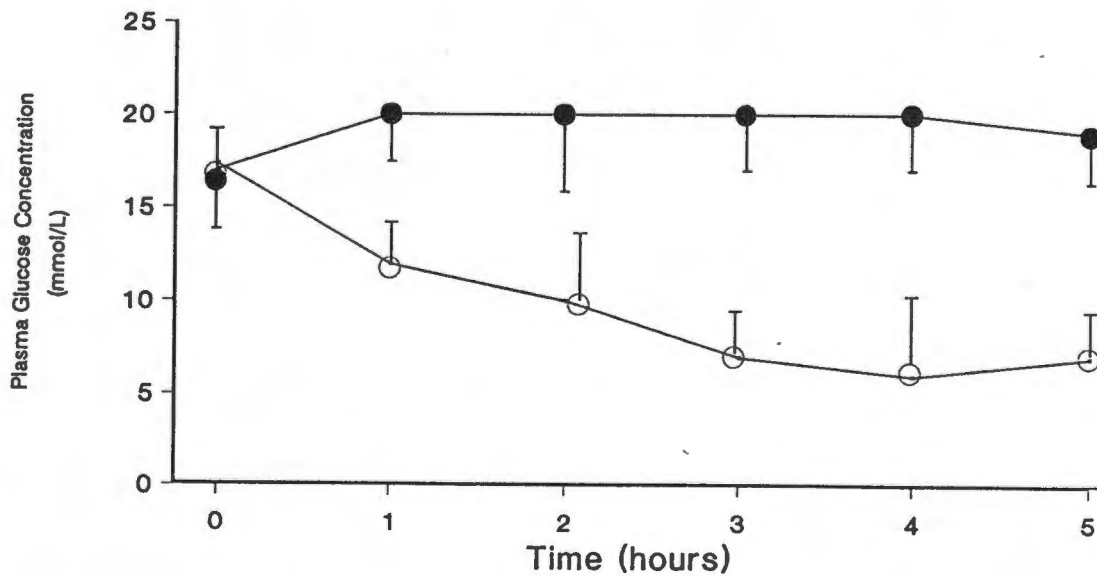


Figure 16. Blood glucose responses to fat ingestion in diabetic patients with (○) and without (●) concomitant insulin administration.

Glucagon and growth hormone concentrations:

In all subjects, fasting plasma glucagon concentrations were within the normal reference range (ng/L) of our laboratory (see Table 12). During procedure 1, mean plasma glucagon concentration decreased (average decline = 41%), generally reaching a nadir within 3 hours following insulin administration. During procedure 2, mean plasma glucagon concentration increased slightly (average increase = 34%) during the first three hours of the test, but decreased towards fasting values thereafter.

The mean fasting plasma growth hormone concentration was within the normal reference range (microgram/L) although 3 subjects had moderately elevated (less than 2-fold) fasting concentrations of this hormone. Plasma growth hormone concentrations did not vary consistently during either of the test procedures.

Serum TG concentrations:

The mean fasting serum TG concentration and the mean magnitude of postprandial lipemia was not significantly different between procedures 1 and 2 (see Figure 17 and Table 12). This result was obtained when males and females were considered separately and when the data from both sexes were pooled.

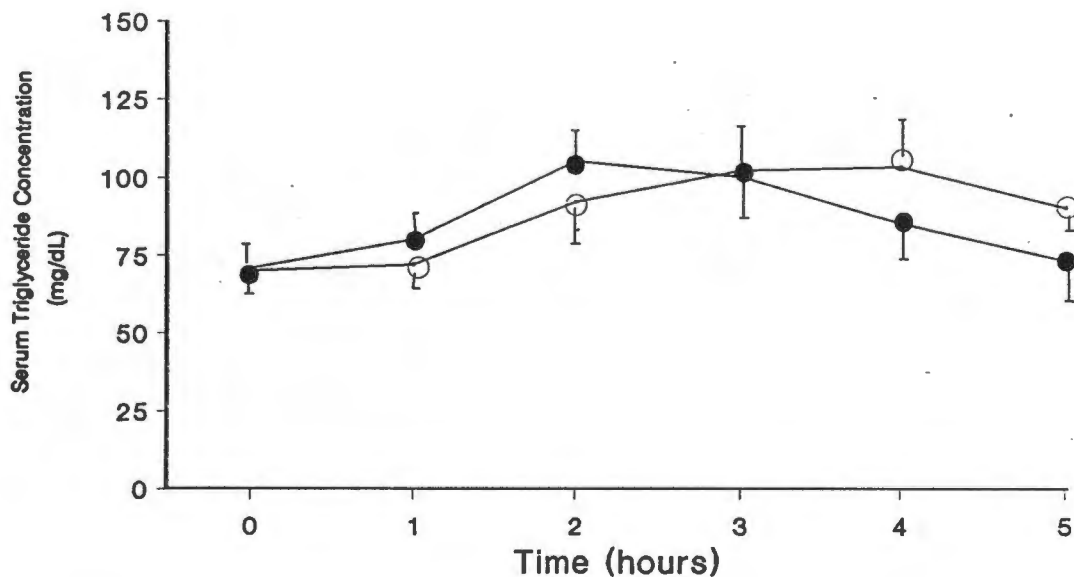


Figure 17. Serum triglyceride responses to fat ingestion in diabetic patients with (○) and without (●) concomitant insulin administration.

b) Intravenous fat tolerance:

Mean k_2 for TG removal was slightly but not significantly lower after procedure 3 ($5.3 \pm 5\%/min$) than after procedure 4 ($6.7 \pm 7\%/min$) (see Figure 18).

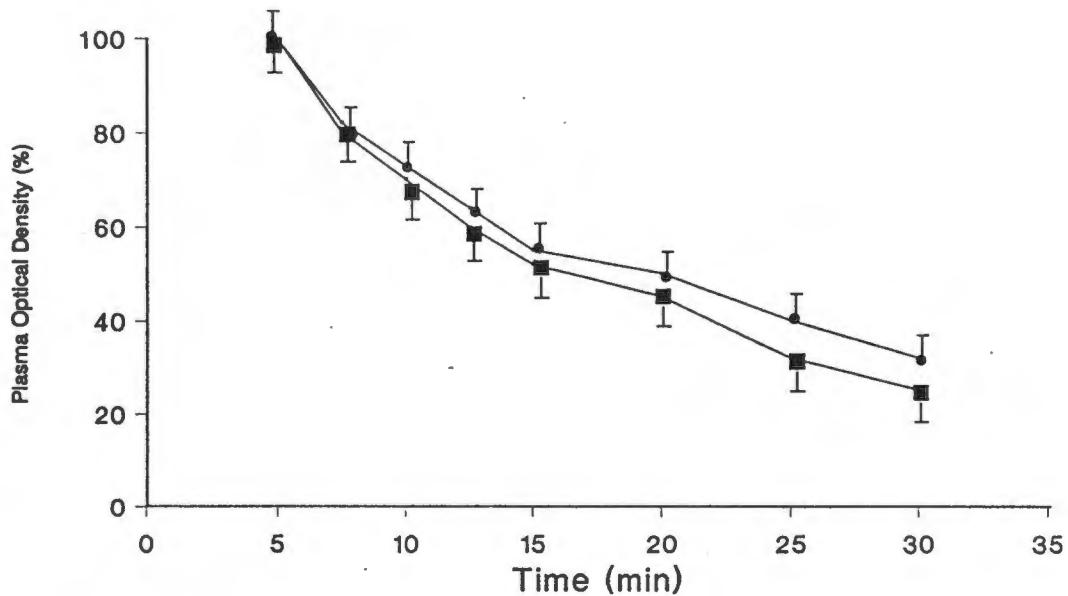


Figure 18. Plasma optical density after Intralipid administration in diabetic patients with (●) and without (■) concomitant insulin administration.

c) Plasma free insulin:

Before insulin administration, the mean plasma free insulin concentration of 5 diabetic patients was $11 \pm 7 mU/L$. In our laboratory, the normal reference range for fasting, non-diabetic men is 2-20 mU/L. Within 1 hour after insulin injection, plasma free insulin concentrations were substantially increased in all subjects (mean = $33 \pm 8 mU/L$). Mean plasma free insulin concentrations rose progressively during the 3 hour measuring period, reaching a peak concentration of $41 \pm 10 mU/L$ 3 hours after insulin injection.

DISCUSSION

The results of this study indicate that the serum TG responses to a mixed meal may vary significantly according to the amount and type of carbohydrate present in the meal. In the normolipidemic individuals who comprised the present study sample, the effects of carbohydrate ingestion appeared to be independent of fasting serum TG concentrations.

i) Fructose and sucrose administration:

In the present study, postprandial lipemia was significantly increased by the addition of 50g fructose, or of 100g sucrose to the stock meal. The addition of 50g sucrose to the stock meal did not augment postprandial serum TG concentrations. A similar effect of fructose has been described previously (123). Two previous studies have examined the effects of sucrose on postprandial lipemia. Mann et al (124) found that meals containing glucose and fat evoked less postprandial lipemia than did meals containing sucrose and fat. Since no control data from meals comprising fat alone were reported, it is not clear whether this difference was due to a hypotriglyceridemic effect of glucose or to a hypertriglyceridemic effect of sucrose. Sullivan et al (137) reported that the ingestion of 100g sucrose did not significantly affect mean postprandial serum TG concentration measured 4 hours after the ingestion of 100g peanut butter. In the present study, mean serum TG concentration measured 4 hours after the meal was 124 ± 46 mg/dL after the ingestion of cream alone and 132 ± 42 mg/dL ($P < 0.2$) after the ingestion of cream and 100g sucrose.

Accordingly, if the serum TG concentration measured 4 hours after the meal was considered to represent postprandial lipemia then our data would be consistent with those of Sullivan et al (137). In the present study, mean serum TG concentrations were higher at 6 hours (88 ± 33 mg/dL vs 136 ± 62 mg/dL, $P < 0.001$), and at 7 hours (77 ± 23 mg/dL vs 93 ± 30 mg/dL) after the ingestion of 100g sucrose and cream than after the ingestion of cream alone. Therefore the increased postprandial lipemia caused by sucrose ingestion reflects a late rise (more than 4 hours after the meal) in serum TG concentrations.

Although the hypertriglyceridemic effect of sucrose is usually ascribed to the fructose component, Thompson et al (138) have reported that postprandial plasma TG concentrations are higher after chronic sucrose ingestion than after the ingestion of equivalent quantities of glucose and fructose, the monosaccharide components of sucrose. The role of this disaccharide effect of sucrose in the present study cannot be directly evaluated as the effects of glucose and fructose fed simultaneously as monomers were not studied. Since glucose ingestion did not appear to affect the magnitude of postprandial lipemia, and since the increment in postprandial lipemia induced by the ingestion of 100g sucrose was quantitatively similar to that induced by 50g fructose, it seems reasonable to propose that the effect of sucrose observed in the present study was due to the fructose component.

Increased lipemia may have been caused by reduced clearance of TG or by increased TG influx into blood. While it is possible that fructose and sucrose may have reduced TG clearance, there is little evidence to support this hypothesis. Acute impairment of the TG removal system seems unlikely, as fructose appears to stimulate adipose-tissue LPL (54). An alternative, and more likely explanation is that fructose may have increased the influx of TG into blood. An increase in TG influx would presumably comprise an increased endogenous TG component, since dietary TG is almost completely absorbed in normal subjects (85).

ii) Glucose administration:

The results of the comparative study indicate that the ingestion of 50g glucose does not significantly diminish postprandial lipemia. These data suggest that acute postprandial changes in plasma insulin

concentrations do not regulate postprandial lipemia. This hypothesis is supported by the further observation that the intravenous administration of 50g glucose caused a moderate increase rather than a decrease in postprandial serum TG concentrations.

To evaluate this hypothesis further, additional oral fat tolerance tests were performed. The results of these studies indicate that glucose ingestion does influence postprandial serum TG concentrations, but that these effects are largely due to changes in TG absorption, rather than TG clearance. Glucose ingestion (50 and 100g) significantly reduced the peak postprandial serum TG concentration and the serum TG concentrations measured 2 hours after the meals.

Total postprandial lipemia was not significantly reduced by glucose ingestion, despite substantial postprandial increments in plasma insulin concentrations. While the mean postprandial lipemia was lower after both glucose-containing meals than after cream alone, a significant proportion of the subjects had higher postprandial lipemia after ingesting glucose and cream than after cream alone. Although it might be argued that a more consistent result would have been obtained using a larger sample size, a similar proportion (7 out of 21) of the subjects in the comparative study did not exhibit diminished postprandial lipemia in response to glucose ingestion. Accordingly, the present data support and extend the findings of a previous study (125) in which postprandial lipemia was not diminished by the ingestion of 50g glucose or by the intravenous administration of 25g glucose.

The results of the present study also help to clarify the disparity between previous investigations into the effects of glucose ingestion on postprandial lipemia. Albrink et al (120) reported that the ingestion of large quantities (up to 250g) of glucose abolished the postprandial lipemia induced by a fatty meal. This conclusion was based on the comparison of plasma TG concentrations measured 3 hours after meals containing glucose and fat, or fat alone. Since it was assumed that serum TG concentrations were maximally elevated 3 hours postprandially, and that glucose ingestion did not affect the rate of TG absorption, the finding that glucose ingestion diminished or abolished the 3-hour rise in plasma TG concentrations was considered to indicate increased clearance of plasma TG.

In the present study, peak serum TG concentrations were reached 2 hours after the ingestion of the meal 1 (40g fat only), and the mean serum TG concentrations were significantly lower 2 hours after meal 2 (50g glucose + 40g fat) and meal 3 (100g glucose + 40g fat) than after meal 1. Therefore if serum TG concentrations measured at this time point were used as an index of postprandial lipemia then the present findings would be consistent with those of Albrink et al (120). Peak serum TG concentrations were reached 4 hours after meal 2 and 6 hours after meal 3, however. These data suggest that the effects of glucose on serum TG concentrations measured 2 hours (in the present study) or 3 hours (122) after fat ingestion are the result of delayed TG absorption, rather than increased TG clearance.

This hypothesis is supported by the observation that the rate of clearance of intravenously administered Intralipid was not increased at 2 hours or at 4 hours after glucose ingestion.

Three caveats need to be considered here:

i) Intralipid kinetics may not reflect the intravascular behaviour of chylomicrons and VLDL, and may be independent of LPL activity. This possibility seems unlikely, as previous studies have indicated that Intralipid particles have clearance properties similar to those of chylomicrons (35,139) and VLDL (140), and the rate of disappearance of an intravenous bolus of Intralipid appears to be related to LPL activity (141).

ii) The intravenous fat tolerance test may not be sufficiently sensitive to detect glucose-induced increases in TG clearing capacity. In a previous study, however, small, heparin-induced changes in the rate of plasma TG clearance which reduced endogenous serum TG concentrations by as little as 12%

were clearly detected by the intravenous fat tolerance test (see Chapter 2). Furthermore, this test has indicated differences in TG clearance between normolipidemic men and women (142), and between athletes and sedentary men (143). Increased TG clearance after moderate intensity acute exercise has also been demonstrated using this method (90). Therefore it seems likely that an increase in TG clearing capacity of sufficient magnitude to abolish postprandial lipemia would also result in a measurable increase in the fractional clearance rate of Intralipid.

iii) The rate of clearance of chylomicron-TG is partly determined by chylomicron structure (38,144), and it is possible that chylomicrons synthesised after the concomitant ingestion of glucose and fat may be more susceptible to enzymic degradation, or to hepatic uptake, than those synthesised after the ingestion of fat alone. Since the intravenous fat tolerance test involves the administration of a standard fat emulsion, changes in TG clearance which occur as a consequence of altered chylomicron structure would not be detected by this method. To evaluate the importance of putative glucose-induced changes in chylomicron structure, we measured the effects of constant intraduodenal glucose administration (20g/hour) on plasma-TG and chylomicron-TG concentrations during duodenal perfusion with a fat emulsion. Since glucose administration in this manner did not significantly diminish plasma or chylomicron-TG concentrations for at least 8 hours, it seems unlikely that glucose-induced alterations in chylomicron structure can account for the effects of glucose ingestion on the serum TG concentrations measured 2 to 3 hours after the ingestion of a fat meal.

To investigate the possibility that glucose ingestion augments chylomicron-TG clearance by a mechanism(s) independent of LPL, we measured the serum TG responses to 100g glucose ingestion in 3 men with familial LPL deficiency. These men had florid chylomicronemia on fasting, and no exogenous fat was administered during the test. In each case, serum TG concentrations were slightly reduced after glucose ingestion but the average decrease did not exceed 6 percent. Accordingly, it seems unlikely that the early effects of glucose ingestion on postprandial lipemia reflect an increase in LPL-independent chylomicron-TG removal.

Given these considerations, it seems probable that glucose ingestion does not significantly increase the rate of chylomicron-TG clearance during the early postprandial phase. Accordingly, the effects of glucose ingestion on the serum TG concentrations measured during this period presumably reflect a decrease in the rate of TG absorption. Previous investigators (125) have suggested that glucose ingestion may slow TG absorption by delaying gastric emptying. To examine this hypothesis, we eliminated variation due to changes in gastric emptying rate by administering fat intraduodenally. Under these conditions, oral glucose administration did not significantly diminish postprandial plasma TG concentrations during the early phase of the perfusion (for at least 4 hours after glucose ingestion). This finding indicates that the early effects of glucose ingestion on postprandial lipemia can be largely attributed to glucose-induced changes in the rate of gastric emptying.

To elucidate the mechanism whereby glucose delays the release of fat from the stomach, the relative importance of the osmotic and metabolic effects of glucose was examined. In the present study, the osmotic effects of glucose were excluded by substituting an equivalent quantity of starch for glucose in the test meal. Our data indicate that the ingestion of 100g starch does not influence postprandial serum TG concentrations. Since starch is digested to glucose before being absorbed from the small intestine, it seems likely that these carbohydrates would have similar metabolic effects after oral administration. Therefore the observation that starch ingestion does not duplicate the effects of glucose on postprandial serum TG concentrations suggests that these effects are related to the osmotic properties of glucose rather than to a metabolic effect. This finding is consistent with the observation that 50g glucose given intravenously did not reduce peak postprandial serum TG concentrations, total postprandial lipemia or the serum TG concentration measured 2 hours after the meal.

If this hypothesis is true then it should be possible to reproduce the effects of glucose on postprandial serum TG concentrations by increasing the osmolality of the stock meal with a metabolically inert

substance. In the present study, the addition of urea to the stock meal (in a dose osmotically equivalent to 50g glucose) caused a significant decrease in the peak postprandial serum TG concentration and in the serum TG concentration measured 2 hours after the meal. The peak postprandial serum TG concentration was delayed by urea ingestion, but total postprandial lipemia was not significantly reduced. These data provide further support for the contention that glucose delays the gastric emptying of fat by increasing the osmolality of the test meal rather than by a metabolic effect.

Glucose administration (both by oral and duodenal route) resulted in significantly diminished serum TG concentrations during the latter part of the duodenal perfusion. This observation could reflect a gradual, progressive increase in TG clearance, a progressive decrease in the rate of TG absorption, or a combination of both processes. The physiological significance of this finding is not clear, since, at the corresponding times after oral administration, serum TG concentrations had returned to near fasting levels. Accordingly, further investigation of this phenomenon was not undertaken.

Effects of insulin on postprandial lipemia:

In the present study, the effects of glucose ingestion on postprandial lipemia and on plasma insulin concentrations were not significantly correlated. These results are at variance with those of a previous study (124), in which postprandial lipemia was found to be inversely related to postprandial plasma insulin concentrations. Those authors proposed that in subjects with high postprandial plasma insulin concentrations, adipose-tissue LPL activity, and thus chylomicron-TG clearance would be greatly increased.

This hypothesis is unlikely to be correct for the following reasons:

i) In the present study, acute increments in plasma insulin concentrations did not result in increased clearance of Intralipid, or decreased chylomicron-TG concentrations during duodenal fat perfusion.

ii) Previous studies have indicated that adipose-tissue LPL activity shows little (33) or no (52) response to the ingestion of a mixed meal. Furthermore, Sadur et al (53) have found that fat ingestion abolishes the insulin-induced increment in adipose-tissue LPL activity.

iii) Adipose-tissue LPL activity is not significantly increased for at least 3 hours after insulin administration (50).

More recently, Bazelmans et al (145) reported that the decrease in plasma TG concentrations which occurs following insulin administration appears to be directly related to insulin sensitivity. Accordingly, it is possible that the postprandial hypotriglyceridemic effects of glucose may be more apparent in insulin-sensitive individuals. In the present study, glucose ingestion did not significantly reduce postprandial lipemia in endurance-trained athletes, a group characterized by extreme sensitivity to the glucoregulatory action of insulin (127).

On the basis of these findings, it seems unlikely that acute postprandial changes in plasma insulin concentrations play a significant role in the regulation of chylomicron-TG clearance or of postprandial lipemia. To further clarify the effects of acute hyperinsulinemia on postprandial TG metabolism, the effects of exogenous insulin administration were studied in young type I diabetic subjects. This model was chosen in order to avoid the confounding variables of exogenous glucose and endogenous counter-regulatory hormones which are of necessity associated with insulin administration in non-diabetic men. The well-known actions of insulin on glucose and lipid metabolism were clearly apparent as shown by the prompt decreases in blood glucose and ketone body concentrations which followed insulin administration. Measurements of plasma glucose, glucagon and growth hormone concentrations suggested that euglycemia was maintained without increased counter-regulatory hormone ac-

tion. Neither the rate of exogenous TG removal, nor the magnitude of postprandial lipemia was affected by insulin administration, however. These findings indicate that acute postprandial hyperinsulinemia does not stimulate chylomicron-TG removal in type I diabetics.

In order to extrapolate from the insulin-dependent diabetic to the non-diabetic state, several important caveats must be considered:

1) In the insulin-treated type I diabetic, insulin is assimilated into the bloodstream from a peripheral injection site and the portal-peripheral insulin gradient which occurs under conditions of endogenous insulin secretion does not arise. To achieve adequate insulinization of the liver, large doses of insulin must be administered, and the peripheral tissues may be exposed to conditions of relative hyperinsulinemia (146). It could be argued, therefore, that the insulin-sensitive components of the TG removal system may always be maximally stimulated in insulin-treated diabetics, and that insensitivity to acute fluctuations in serum insulin concentrations would be expected in these subjects. This possibility seems unlikely, since adipose tissue LPL activity is usually within the normal range in insulin-treated diabetic patients (141) and LPL activity can be increased by acute insulin administration in these patients (147).

2) Since serum TG concentrations are also determined by the rate of TG influx into the intravascular compartment, the possibility that TG absorption differs under conditions of insulin withdrawal and repletion must also be considered. If TG absorption is delayed or incomplete under conditions of insulin withdrawal then the magnitude of postprandial lipemia measured in the present study might have been an underestimate of the true value. Although TG absorption was not directly measured in this study, impaired absorption seems unlikely for the following reasons:

i) The magnitude of postprandial lipemia measured during insulin withdrawal (procedure 2) was similar to that observed previously in studies of nondiabetic men and women fed 100mL dairy cream under similar conditions to those of the present study (see Chapter 3 and reference 96)

ii) No subject was nauseated or had diarrhoea.

iii) During insulin withdrawal maximum serum TG concentrations were reached within 3 hours following fat feeding. Five hours postprandially, mean serum TG concentration had returned to baseline levels.

Furthermore, the apparent absence of an insulin-mediated effect on postprandial lipemia is supported by the finding that acute insulin administration did not increase the rate of removal of an intravenously injected TG emulsion, a result which cannot be ascribed to differences in gastro-intestinal factors.

3) It is difficult to determine the dose of insulin which, when administered to a type I diabetic patient will be functionally equivalent to the insulin response to a given glucose load in a non-diabetic man, and it might be argued that such an equivalence was not demonstrably attained in these subjects. This question of dose-equivalence may be further confounded by the presence of insulin resistance in type I diabetic patients (148). Since the doses of insulin which are required to maintain good glycemic control in type I diabetic patients tend to produce high peripheral insulin concentrations, each subject was treated with his or her regular insulin dosage on the assumption that this dosage would produce sufficiently increased serum free insulin concentrations to simulate the hyperinsulinemic state which prevails after oral glucose administration in non-diabetic men. This contention is supported by the observation that the mean peak plasma free insulin concentration measured after exogenous insulin administration in 5 of the patients in the present study ($41 \pm 10 \text{mU/l}$) was comparable to the mean peak plasma insulin concentration ($58 \pm 23 \text{mU/l}$) measured previously in non-diabetic subjects after ingestion of 50g glucose (94).

Given these considerations, it seems likely that the results of the present study are also applicable to non-diabetic populations. The finding that exogenous insulin administration failed to stimulate

chylomicron-TG removal in diabetics is consistent with the observation that the administration of glucose (by oral or intravenous route) does not diminish postprandial lipemia in non-diabetic subjects, despite substantial postprandial increments in plasma insulin concentrations. Therefore it seems reasonable to propose that acute physiological changes in postprandial plasma insulin concentrations such as those which might occur after the ingestion of a mixed meal do not significantly affect the rate of removal of chylomicron-TG in normolipidemic man.

CHAPTER 6

THE EFFECTS OF CHRONIC ENDURANCE EXERCISE TRAINING ON POSTPRANDIAL LIPEMIA AND CHYLOMICRON-TRIGLYCERIDE CLEARANCE

CHAPTER 6: THE EFFECTS OF CHRONIC ENDURANCE EXERCISE TRAINING ON POSTPRANDIAL LIPEMIA AND CHYLOMICRON TRIGLYCERIDE CLEARANCE

INTRODUCTION:

Several studies have indicated that exercise training reduces fasting serum TG concentrations in normolipidemic and in hypertriglyceridemic men (reviewed in ref 149). The effects of exercise training on postprandial TG metabolism have not been extensively documented, however. In a longitudinal study of 2 subjects, Patsch et al (75) found that exercise training lowered the serum TG response to a fatty meal (postprandial lipemia). These data suggest that chronic exercise may increase the rate of chylomicron-TG clearance. Furthermore, although chylomicron clearance has not been directly quantitated in endurance athletes, Ericsson et al (143) have shown that the clearance of an intravenously injected fat emulsion is faster in runners than in sedentary men. Chronic exercise may also improve intravenous fat tolerance in hypertriglyceridemic men (150).

Chylomicron-TG clearance and postprandial lipemia have been shown to be directly related to the fasting serum TG concentration (see Chapter 2). This relationship may reflect competition for a common clearance pathway between hepatic- and intestine-derived TG-rich lipoproteins (36). Diminished postprandial lipemia and accelerated chylomicron-TG clearance might therefore be expected to occur in association with fasting hypotriglyceridemia in endurance-trained men.

The purpose of the present study was to evaluate this hypothesis, and to investigate the relationship between fasting and postprandial serum TG concentrations in endurance-trained men. If the reduction in postprandial lipemia associated with chronic exercise is due solely to diminished fasting serum TG pool size, then chylomicron-TG clearance should be similar in men with similar fasting serum TG concentrations, regardless of exercise status. Conversely, if chronic exercise facilitates TG clearance by increasing the capacity of the TG removal system, then chylomicron-TG clearance would tend to be faster in endurance athletes than in sedentary men with similar fasting serum TG concentrations.

METHODS:

1) Subjects:

Twenty nine normolipidemic male students who habitually performed at least one hour of vigorous exercise per day gave written informed consent for this study. All of these men competed regularly in running or triathlon events. Twenty nine control subjects matched for body weight and fasting serum TG concentration were selected from a larger group of sedentary, normolipidemic male medical students. The control subjects performed less than one hour of exercise per week (see Table 13 for clinical data). All subjects followed self-selected diets, and all had been weight stable for at least six months prior to the study. None of the subjects was obese (91), and none smoked or used any medication known to affect lipoprotein metabolism.

2) Procedures:

The endurance trained subjects followed their customary exercise regimens on the day before each test.

a) Oral fat tolerance tests:

Oral fat tolerance was measured in 30 subjects (15 athletes and 15 sedentary men) using a procedure described in Chapter 1. Each subject was tested twice, in order to determine the serum TG responses to both large and small fat loads. On one occasion, the test meal comprised 350mL cream (140g fat) and 5g chocolate flavouring. For the second test, 100mL cream (40g fat) and 5g chocolate flavouring were consumed. Water was added to increase the volume to 350mL.

In 10 of the sedentary subjects, a further oral fat tolerance test (350mL cream) was performed 12 hours after an acute bout of moderate intensity exercise lasting 1 hour. The exercise session comprised 15 minutes treadmill running (10km/hr at 0 incline), 30 minutes ergometer cycling (150W) and 15 minutes rowing. No rest periods were permitted between these modalities.

b) Intravenous fat tolerance tests:

Intravenous fat tolerance was measured in 11 athletes and 11 sedentary men using the procedure described in Chapter 1.

c) Duodenal perfusion studies:

Chylomicron-TG clearance was measured in 16 subjects (8 athletes and 8 sedentary men) using the duodenal perfusion method described in Chapter 1. The perfusate, comprising 100g olive oil and 7g gum acacia per litre of water, was infused at a rate of 175mg fat/kg body weight/hour. Previous studies have indicated that TG ingested in this form is efficiently absorbed (92).

Twenty two subjects (11 athletes and 11 sedentary men) participated in both the oral and in the intravenous test procedures. Eight of these subjects (4 athletes and 4 sedentary men) participated in the oral, intravenous and the duodenal perfusion studies.

3) Analysis of data:

Postprandial lipemia, Intralipid clearance (k_2), and the half life ($T_{1/2}$) of chylomicron-TG measured during the duodenal perfusion studies were calculated as described in Chapter 1. The mean values of all parameters were compared using an unpaired t-test (95).

RESULTS:

The test procedures were well tolerated, although most subjects reported transient nausea after consuming 350mL cream. The mean values of all parameters are shown in Table 15.

Table 15. Ages, body weights, fasting serum TG concentrations and parameters of chylomicron-TG clearance in athletes and sedentary men matched according to fasting serum TG concentration.

a) Oral fat tolerance:								
DOSE	AGE		BWT		FTG		PPL	
	Ath.	Sed.	Ath.	Sed.	Ath.	Sed.	Ath.	Sed.
40g	26±4	22±2	72±6	73±10	65±27	74±18	133±59	232±134a
140g	26±4	22±2	72±6	73±10	66±19	68±9	222±103	543±165a
b) Intravenous fat tolerance:								
	AGE		BWT		FTG		k2	
	Ath.	Sed.	Ath.	Sed.	Ath.	Sed.	Ath.	Sed.
	24±3	22±3	70±8	75±10	73±19	79±14	5.4±1.2	4.3±0.8b
c) Duodenal perfusion studies:								
	AGE		BWT		FTG		t1/2	
	Ath.	Sed.	Ath.	Sed.	Ath.	Sed.	Ath.	Sed.
	23±3	21±1	65±5	70±8	57±14	61±15	3.0±0.8	4.0±1.0c
Values are means±standard deviations.								
P values are a < 0.001, b < 0.01, and c < 0.05								
Abbreviations and units: BWT = body weight (kg), FTG = fasting serum TG concentration (mg/dL), PPL = postprandial lipemia (mg.dL ⁻¹ .8hr), k2 = fractional clearance rate of intralipid (% per minute), t1/2 = chylomicron-TG half life (minutes).								

a) Oral fat tolerance tests:

Postprandial lipemia was significantly lower in athletes than in sedentary men, after both the large and the small fat meals. The shape of curve of serum TG concentrations plotted against time appeared to be similar in athletes and in sedentary men. In both groups, peak serum TG concentrations were generally reached 4 hours after consumption of the large fat meal (see Figure 19), and between 2 and 4 hours after consumption of the small fat meal (see Figure 20).

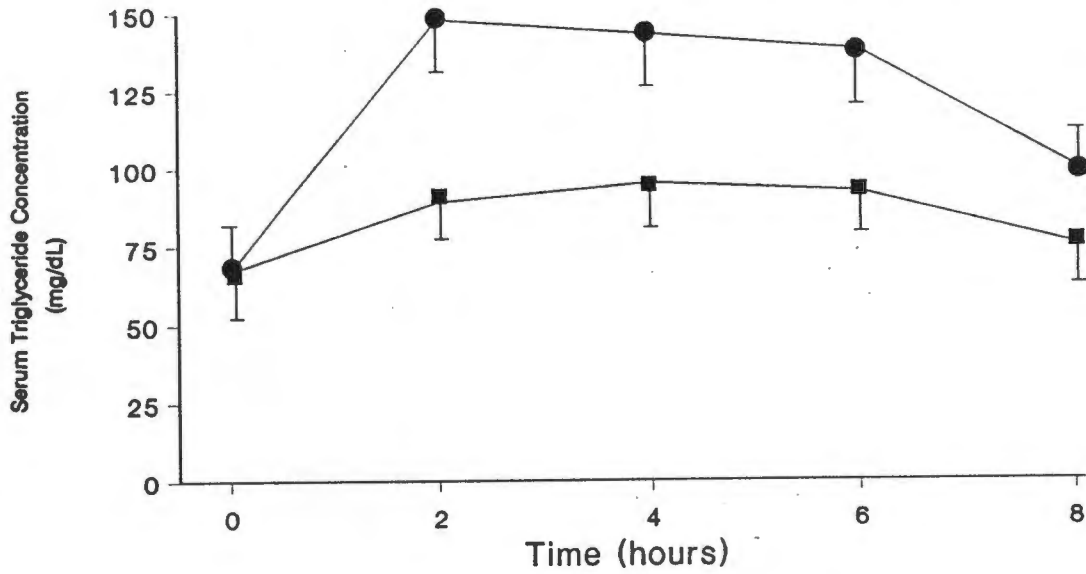


Figure 19. Serum triglyceride responses to 120g fat ingestion in athletes (■) and in sedentary men (●).

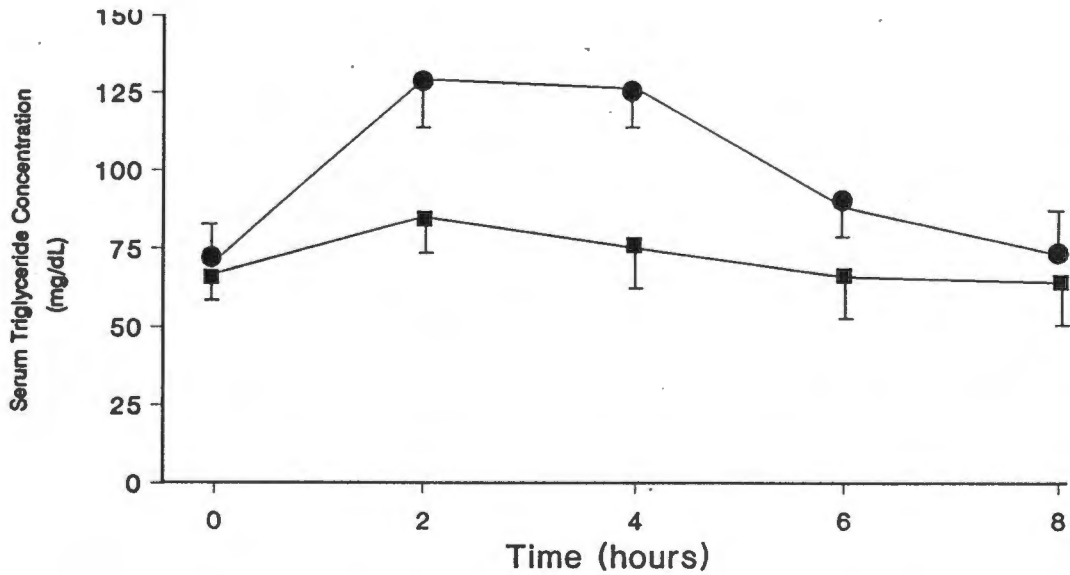


Figure 20. Serum triglyceride responses to 40g fat ingestion in athletes (■) and in sedentary men (●).

Postprandial lipemia was slightly, but not significantly reduced by acute exercise in sedentary men (525 ± 280 vs $482 \text{mg} \cdot \text{dL}^{-1} \cdot 8\text{hr}$, see Figure 21).

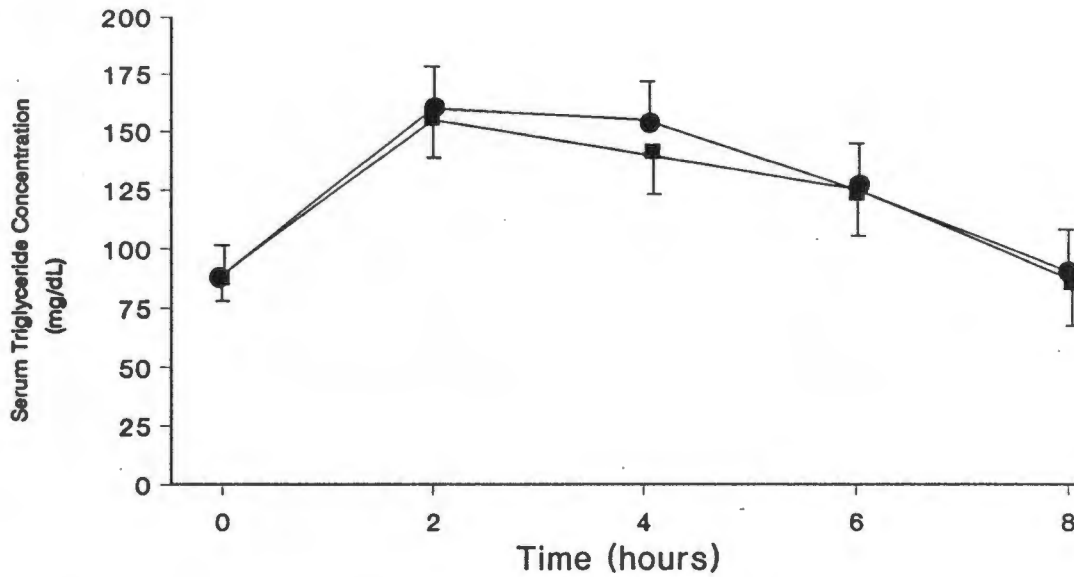


Figure 21. Serum triglyceride responses of sedentary men to 120g fat ingestion before (●) and after (■) exercise.

b) Intravenous fat tolerance test:

Intralipid clearance was significantly faster in athletes than in sedentary men (see Figure 22). In all subjects, the decay curve of Intralipid disappearance was adequately fitted by a single exponential (mean $r = 0.96$).

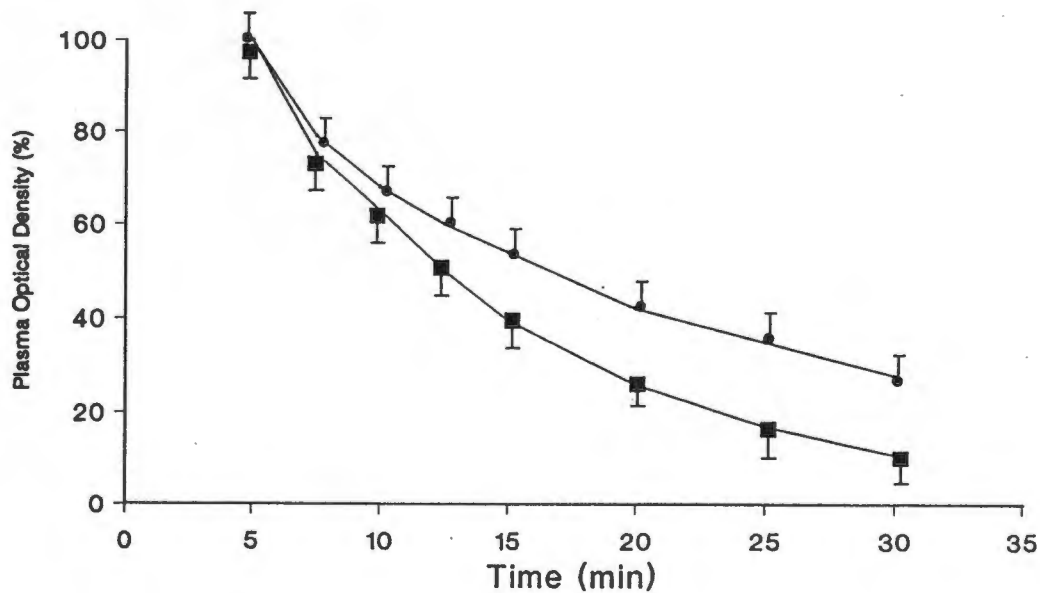


Figure 22. Serum optical density after Intralipid administration in athletes (●) and sedentary men (■).

c) Duodenal perfusion studies:

In all subjects, serum TG concentrations were increased during the duodenal perfusion procedure. The mean half-life of chylomicron-TG was shorter in athletes than in sedentary men (see Figure 23).

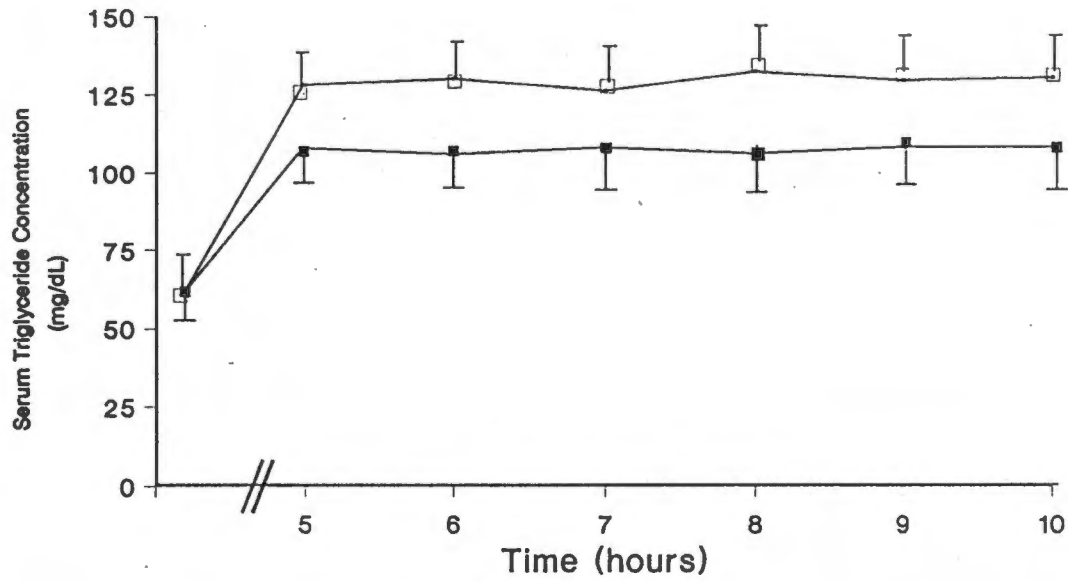


Figure 23. Serum triglyceride responses to duodenal fat perfusion in athletes (■) and in sedentary men (□)

DISCUSSION:

The results of this study indicate that postprandial lipemia is lower in athletes than in sedentary men. The magnitude of postprandial lipemia was diminished in athletes both after large, and after small fat meals. Therefore it seems likely that for any given fat meal, postprandial lipemia will be lower in athletes than in sedentary men. Since men following a Western diet may spend up to three-quarters of each day in a postprandial state, and since the major flux of plasma TG occurs during this period, postprandial plasma TG concentrations probably provide a better index of diurnal plasma TG transport than do fasting concentrations. Accordingly, the findings of this study suggest that chronic exercise will reduce diurnal plasma TG concentrations.

Postprandial lipemia and chylomicron-TG clearance:

In the present study, chylomicron-TG half life determined using the duodenal perfusion method of Grundy and Mok (37) was shorter in athletes than in sedentary men. This finding suggests that exercise may diminish postprandial lipemia by reducing the intravascular residence time of chylomicron-TG.

Two caveats need to be considered here:

1) The duodenal perfusion method is based on the assumption that the rate of influx of chylomicron-TG into the blood plasma is equal to the rate of duodenal perfusion. Therefore it is possible that the short chylomicron-TG half life measured in the athletes reflects impaired TG absorption rather than rapid clearance.

2) The flow rate of the duodenal perfusion was standardized for body weight. Athletes have a greater plasma volume per unit body weight than do sedentary men (151), therefore at a given rate of TG clearance, steady-state chylomicron-TG concentrations would be expected to be lower in athletes than in sedentary men.

Since neither TG absorption nor plasma volume were measured in these subjects, it is possible that the low chylomicron-TG half life of the athletes in this study was an artifact of the measuring technique. To control for these confounding factors, the k_2 of a bolus of artificial chylomicrons (Intralipid) administered intravenously was determined. The results obtained using this method, which is independent of the absorptive processes and of plasma volume, were in agreement with the results of the duodenal perfusion method. These findings therefore provide further support for the hypothesis that chylomicron-TG clearance is greater in athletes than in sedentary men.

Mechanisms of increased chylomicron-TG clearance:

Chylomicron-TG half life may be determined by fasting serum TG concentration, by the composition and nature of the chylomicrons, and by the capacity of the TG clearing system(s). The mean fasting serum TG concentration was lower in the sedentary men in this study than in a randomly selected group of normolipidemic sedentary men described previously (96). The mean values for postprandial lipemia and chylomicron-TG half life were also lower in the present group than in normolipidemic sedentary men in two previous studies (8,37). These findings are in agreement with a previous observation (30) that postprandial lipemia and chylomicron-TG half life are diminished in men with low fasting serum TG concentrations. The low chylomicron-TG half life of athletes may therefore be explained, at least in part, by low fasting serum TG concentrations. To control for variation due to fasting serum TG concentration, the athletes and sedentary men in this study were matched according to fasting serum TG concentration. After this procedure, chylomicron-TG clearance was still significantly lower in the athletes

than in the sedentary men. These results indicate that the effect of exercise on chylomicron-TG half life is not entirely attributable to diminished fasting serum TG concentrations.

To exclude variations due to chylomicron structure, the intravascular kinetics of an artificial fat emulsion (Intralipid) were examined in athletes and in sedentary men. In both groups, the curve of Intralipid disappearance appeared to be mono-exponential, but the fractional clearance rate was significantly greater in the athletes than in the sedentary men. Previous investigators have reported similar effects of chronic exercise on intravenous fat tolerance in cross-sectional (143) and longitudinal (150) studies. The low chylomicron-TG half life in athletes is therefore unlikely to be due to exercise-induced changes in chylomicron structure.

Given these considerations, it seems most probable that the difference between the mean chylomicron-TG half life of the athletes and the sedentary men in the present study is due to a direct effect of exercise on the TG removal system(s).

This hypothesis is consistent with the findings of several previous studies, which have shown increased activity of LPL, the rate-limiting enzyme for plasma TG removal, in postheparin plasma (152), in adipose tissue (153), and in skeletal muscle (154), in endurance trained men.

Effects of acute and chronic exercise:

Previous studies (155,156) have indicated that exercise performed after the ingestion of a fatty meal reduces the magnitude of postprandial lipemia. Therefore, it is possible that low chylomicron-TG half life is a consequence of acute exercise, rather than a metabolic adaptation to exercise training. Some investigators have sought to distinguish acute from chronic exercise effects by testing athletes after brief periods of abstinence from exercise (90,157). Since the metabolic changes associated with exercise adaptation cannot be quantitatively distinguished from those which follow bouts of acute exercise, the duration of the "detraining" period used in those studies was arbitrarily determined. In the present study, the effect of a single bout of exercise (of 1 hour duration) performed 12 hours prior to oral fat tolerance testing was examined in 10 sedentary men. Postprandial lipemia was slightly, but not significantly reduced by acute exercise. On the basis of these results, the athletes in this study were allowed to continue their customary training regimes until 12 hours prior to each test. In addition, these data indicate that the increased rate of chylomicron-TG clearance measured in the athletes in this study cannot be quantitatively induced in sedentary men by exposure to acute exercise.

Self-selection bias:

In cross-sectional comparisons of athletes and sedentary men, it is not possible to distinguish differences attributable to chronic exercise from those which arise as a consequence of self-selection. Williams et al (158), have suggested that men who choose to take up running tend to have low fasting plasma TG concentrations prior to participation in the sport. The low postprandial lipemia and chylomicron-TG half life of the athletes in this study may therefore be a consequence of self selection, rather than an adaptation to chronic exercise. This possibility seems unlikely, however, as the effects of exercise training on fasting serum TG concentration (159), LPL activity (152), postprandial lipemia (75) and intravenous fat tolerance (150) have also been observed in longitudinal studies.

In summary, the following conclusions seem reasonable:

- i) For any given fat load, the magnitude of postprandial lipemia will tend to be lower in athletes than in sedentary men.
- ii) Chronic exercise training reduces postprandial lipemia more effectively than does acute exercise.

iii) The reduction in postprandial lipemia associated with endurance exercise adaptation is due to a decrease in chylomicron-TG half life, rather than to impaired fat absorption.

iv) The low chylomicron-TG half life in athletes may be partly related to reduced competition from a diminished pool of endogenous TG-rich lipoproteins. This mechanism cannot fully account for the low chylomicron-TG half life in these men, however. The effects of exercise on chylomicron-TG half-life are therefore at least partly attributable to increased activity of the TG clearance system(s).

GENERAL CONCLUSIONS

GENERAL CONCLUSIONS

The experiments described in this thesis were designed to examine the possible roles of different nutrients and hormones in regulating plasma TG concentrations during the postprandial period. Although the ingestion of fats and carbohydrates has been shown to elicit the release of hormones which are known to increase the activity of adipose tissue LPL *in vitro*, no evidence for acute postprandial increases in the fractional rate of plasma TG clearance emerged from any of these studies. Incremental fat loads produced directly proportional responses in aggregate postprandial lipemia, indicating the absence of a fat-induced increase in the fraction of plasma TG cleared per unit time. The addition of protein, and of various carbohydrates including starch, glucose, fructose, and sucrose to standard fat meals failed to diminish aggregate postprandial serum TG concentrations. These findings were supported by the observation that neither protein nor glucose ingestion increased the rate of clearance of an artificial fat emulsion from plasma. Therefore it appears that the rate of clearance of TG from the plasma is not actively increased in response to feeding in man. Postprandial TG kinetics in man are thus not entirely consistent with those which would be predicted from *in vitro* observations of adipocyte LPL activity, or from animal models such as the rat, or dog.

Since postprandial plasma TG concentrations appear to be determined by the rate of chylomicron-TG clearance, and since chylomicron-TG clearance does not appear to be actively stimulated by feeding, the massive influx of chylomicron-TG into the circulation which occurs after the ingestion of a fat meal must be accommodated by passive increases in the absolute rate of chylomicron-TG clearance. In this model, the fraction of the plasma chylomicron-TG pool which is removed per unit time remains constant, and the absolute quantity of chylomicron-TG cleared from the plasma pool increases with increasing chylomicron-TG concentration. Support for this contention is provided by the observation that i) aggregate postprandial lipemia increases in direct proportion with increasing dietary fat loads, ii) that the disappearance of an intravenous bolus of Intralipid approximates first order kinetics, and iii) that plasma TG concentrations reach new steady state values during duodenal perfusion. These data imply a large reserve capacity for plasma TG clearance. In terms of the classical enzyme kinetic theory of Michaelis and Menten (see Introduction), the plasma TG clearing reaction operates far below saturation under physiological conditions during the postprandial phase. While it cannot be assumed that the relationship between plasma TG flux and concentration is entirely first order during this period, it appears that the reserve capacity of the clearing mechanism is large enough to prevent significant decreases in the fractional clearance rate of chylomicron-TG after fat feeding.

Given these considerations, it seems likely that the magnitude of postprandial lipemia is largely determined by the amount and type of fat in the meal, by the basal activity of the plasma TG clearance system, and to a lesser extent by the pool size of endogenous TG-rich lipoproteins.

Indications for further work:

The present studies have largely elucidated the role of dietary factors in the acute plasma TG responses to feeding. Several aspects of the intravascular metabolism of diet-derived triglycerides remain unresolved, however. Areas for further work include:

i) The nature of the relationship between endogenous TG-rich lipoproteins and chylomicrons. Although almost all Western men consume mixed diets which contain a substantial proportion of fat, the influence of fat ingestion on the synthesis rate, structure and subsequent intravascular kinetics of hepatic VLDL is unknown. The contribution of diet-derived TG to hepatic VLDL-TG during the postprandial period has not been studied. While it has been argued that chylomicron-TG are hydrolysed in extra-hepatic tissues, the proportion of the resulting fatty acids which are subsequently taken up by the liver is not known. Further studies in which hepatic- and intestine-derived lipoproteins are distinguished using

non-exchangeable markers such as retinyl palmitate and apo B48 and B100 may help to resolve these questions.

ii) The mechanism(s) responsible for the effects of fatty acid chain saturation on postprandial lipemia. This question is particularly interesting in the light of the well-known effects of chronic feeding of polyunsaturated fats on plasma lipoproteins. While the most likely explanations are considered in Chapter 3, there is no definitive *in vivo* evidence for any of these hypotheses. The resolution of this question is limited by the difficulties inherent in determining rates of substrate transport under non steady-state conditions. One approach which may help to circumvent this obstacle would be to measure the turnover of hepatic-derived VLDL during a steady-state duodenal fat perfusion, when serum chylomicron and hepatic-TG concentrations appear to remain relatively constant. By varying the saturation of the fats in the perfusate, the effects of saturated and polyunsaturated fats on hepatic VLDL-TG production and turnover could be assessed.

iii) The mechanism(s) underlying the effects of chronic exercise on postprandial lipemia and chylomicron-TG clearance. This question is also of considerable practical relevance, since endurance exercise appears to confer a degree of protection against coronary artery disease, an effect which may be mediated (at least in part) by alterations in lipoprotein metabolism. While it is generally accepted that lipoprotein lipase activity is increased in endurance athletes, the importance of other factors such as the hepatic response to fat ingestion is completely unknown. The methodology outlined in (ii) above could also be used to elucidate the mechanisms by which endurance exercise ameliorates postprandial lipemia.

REFERENCES

REFERENCES:

1. Man EB, Gildea EF. The effect of the ingestion of a large amount of fat and of a balanced meal on the blood lipids of normal man. *J Biol Chem* 1932;99:66-69
2. Bouchier IAD, Bronte-Stewart B. Alimentary lipemia and ischaemic heart disease. *Lancet* 1961;7173:363-366
3. Brown DF, Heslin AS, Doyle JT. Postprandial lipemia in health and in ischemic heart disease. *New Eng J Med* 1961;264:733-737
4. Simons LA, Dwyer T, Simons J et al. Chylomicrons and chylomicron remnants in coronary artery disease: a case-control study. *Atherosclerosis* 1987;65:181-189
5. Angervall G. On the fat tolerance test. *Acta Med Scand* 1964;Suppl 424:3-84
6. Tall AR. Metabolism of postprandial lipoproteins. *Methods in Enzymol* 1986;129:469-482
7. Rizek RL, Friend B, Page L. Fat in today's food supply - level of use and source. *J Am Oil Chem Soc* 1974;51:244-250
8. Braverman JBS. An introduction to the biochemistry of foods. New York:Elsevier Publishing Co., 1963, p234-251
9. Sheppard AJ, Iverson JL, Weihrauch JL: Composition of selected fats, oils, margarines and butter. In Kuksis A (ed) *Handbook of lipid research*, volume 1, pp341-379. New York, Plenum Press, 1978
10. Friedman HI, Nylund B. Intestinal fat digestion, absorption, and transport. A review. *Am J Clin Nutr* 1980;33:1108-1139
11. Carey MC, Small DM, Bliss CM. Lipid digestion and absorption. *Ann Rev Physiol* 1983;45:651-677
12. Tso P. Gastrointestinal digestion and absorption of lipid. *Adv Lipid Res* 1985;21:143-186
13. Hamosh M, Burns WA. Lipolytic activity of human lingual glands (Ebner). *Lab Invest* 1977;37:603-608
14. Carlson HC, Code CF, Nelson RA. Motor action of the canine gastroduodenal junction: A cineradiographic, pressure and electrical study. *Am J Dig Dis* 1966;11:155-172
15. Kayden JJ, Senior JR, Mattson FH. The monoglyceride pathway of fat absorption in man. *J Clin Invest* 1967;46:1695-1703
16. Redgrave TG. Association of Golgi membranes with lipid droplets (pre-chylomicrons) from within intestinal epithelial cells during absorption of fat. *Aust J Exptl Biol Med Sci* 1971;49:209-224
17. Glickman RM, Green PH, Lees RS, Lux SE, Kilgore A. Immunofluorescence studies of apolipoprotein B in intestinal mucosa: absence in abetalipoproteinemia. *Gastroenterology* 1979;76:288-292
18. Redgrave TG, Dunne KB. Chylomicron formation and composition in unanaesthetised rabbits. *Atherosclerosis* 1975;22:389-400

19. Zilversmit DB. The composition and structure of lymph chylomicrons in dog, rat and man. *J Clin Invest* 1965;44:1610-1622
20. Green PHR, Glickman RM, Saudek CD. Human intestinal lipoproteins: Studies in chyluric patients. *J Clin Invest* 1979;64:233-242
21. Bisgaier CL, Glickman RM. Intestinal synthesis, secretion, and transport of lipoproteins. *Ann Rev Physiol* 1983;45:625-636
22. Kane JP, Hardman DA, Paulus HE. Heterogeneity of apolipoprotein B. Isolation of a new species from human chylomicrons. *Proc Natl Acad Sci* 1980;77:2465-2469
23. Powell LM, Wallis SC, Pease RJ, Edwards YH, Knott TJ, Scott J. A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. *Cell* 1987;50:831-840
24. Imalzumi K, Fainaru M, Havel RJ. Composition of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. *J Lipid Res* 1978;19:712-722
25. Cryer A. Tissue lipoprotein lipase activity and its action in lipoprotein metabolism. *Int J Biochemistry* 1981;13:525-541
26. Havel RJ, Gordon RJ. Idiopathic hyperlipidemia: metabolic studies in an affected family. *J Clin Invest* 1960;39:1777-1790
27. Goldberg IJ, Le N-A, Ginsberg HN, Krauss RM, Lindgren FT. Lipoprotein metabolism during acute inhibition of lipoprotein lipase in the cynomolgus monkey. *J Clin Invest* 1988;81:561-568
28. Nestel PJ, Havel RJ, Bezman A. Sites of initial removal of chylomicron triglyceride fatty acids from the blood. *J Clin Invest* 1962;41:1915-1921
29. Bragdon JH, Gordon RS. Tissue distribution of C¹⁴ after intravenous injection of labeled chylomicrons and unesterified fatty acids in the rat. *J Clin Invest* 1958;37:574
30. Nestel PJ. Relationship between plasma triglycerides and removal of chylomicrons. *J Clin Invest* 1964;43:943-949
31. Patsch JR, Prasad S, Gotto AM, Patsch W. High density lipoprotein₂. Relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase. *J Clin Invest* 1987;80:341-347
32. Taskinen M-R, Kuusi T. High density lipoproteins in postprandial lipemia. Relation to sex and lipoprotein lipase activity. *Atherosclerosis* 1986;59:121-130
33. Lithell H, Boberg J, Hellsing K, Lundqvist G, Vessby B. Lipoprotein-lipase activity in human skeletal muscle and adipose tissue in the fasting and the fed states.
34. Grundy SM, Vega GL. Are plasma triglyceride concentrations explained by saturation kinetics ? In: Berman M, Grundy S M, Howard B V eds. *Lipoprotein kinetics and modeling*. Academic Press, 1982:271-286

35. Hallberg D. Studies on the elimination of exogenous lipids from the blood stream. The kinetics of the elimination of a fat emulsion studied by single injection technique in man. *Acta Physiol Scand* 1965;64:306-313
36. Brunzell JD, Hazzard WR, Porte D, Bierman EL. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J Clin Invest* 1973;52:1578-1585
37. Grundy SM, Mok HY. Chylomicron clearance in normal and hyperlipidemic man. *Metabolism* 1976;25:1225-1239
38. Quarfordt SH, Goodman DS. Heterogeneity in the rate of plasma clearance of chylomicrons of different size. *Biochim Biophys Acta* 1966;116:382-385
39. Lewis B, Chait A, February AW, Mattock M. Functional overlap between "chylomicra" and "very low density lipoproteins" of human plasma during alimentary lipemia. *Atherosclerosis* 1973;17:455-462
40. La Rosa JC, Levy RI, Herbert P, Lux SE, Fredrickson DS. Specific apoprotein activator for lipoprotein lipase. *Biochem Biophys Res Comm* 1970;41:57-62
41. Breckenridge WC, Little JA, Steiner G, Chow A, Poapst M. Hypertriglyceridemia associated with deficiency of apolipoprotein C-II. *N Engl J Med* 1978;298:1265-1272
42. Erkelens DW, Brunzell JD, Bierman EL. Availability of apolipoprotein CII in relation to the maximal removal capacity for an infused triglyceride emulsion in man. *Metabolism* 1979;28:495-501
43. Havel RJ, Kane JP, Kashyap ML. Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. *J Clin Invest* 1973;52:32-38
44. Erkelens DW, Mocking JAJ. The effect of apolipoprotein A on in vitro apolipoprotein C binding and in vivo removal of artificial triacylglycerol-rich particles. *Metabolism* 1985;34:222-226
45. Fielding CJ. Lipoprotein lipase: Evidence for high- and low- affinity enzyme sites. *Biochemistry* 1976;15:879-884
46. Nilsson-Ehle P, Garfinkel AS, Schotz MC. Lipolytic enzymes and plasma lipoprotein metabolism. *Ann Rev Biochem* 1980;49:667- 693
47. Eckel RH. Adipose tissue lipoprotein lipase. In *Lipoprotein lipase*. Borensztajn J (ed.). Evener Publishers Inc. Chicago, 1987
48. Brunzell JD, Schwartz RS, Eckel RH, Goldberg AP. Insulin and adipose tissue lipoprotein lipase activity in humans. *Int J Obesity* 1981;5:685-694.
49. Nilsson-Ehle P, Carlstrom S, Belfrage P. Rapid effects on lipoprotein lipase activity in adipose tissue of humans after carbohydrate and lipid intake. *Scand J clin Lab Invest* 1975;35:373-378
50. Sadur CN, Eckel RH. Insulin stimulation of adipose tissue lipoprotein lipase. Use of the euglycemic clamp technique. *J Clin Invest* 1982;69:1119-1125
51. Taskinen M-R, Nikkila EA. Lipoprotein lipase activity in adipose tissue and skeletal muscle in insulin-deficient human diabetes. *Diabetologia* 1979;17:351-356

52. Pykalisto OJ, Smith PH, Brunzell JD. Determinants of human adipose tissue lipoprotein lipase; effects of diabetes and obesity on basal and diet induced activity. *J Clin Invest* 1975;56:1108-1117
53. Sadur CN, Yost TJ, Eckel RH. Fat feeding decreases insulin responsiveness of adipose tissue lipoprotein lipase. *Metabolism* 1984;33:1043-1047
54. Brunzell JD, Schwartz RS, Bierman EL. Nutritive sweeteners and triglyceride metabolism in humans. *Acta Endocrinol* 1979;91 (suppl 227):17 (abs)
55. Cryer A, Riley SE, Williams ER, Robinson DS. Effect of nutritional status on rat adipose tissue, muscle and postheparin plasma clearing-factor lipase activities: their relationship to triglyceride fatty acid uptake by cells and plasma insulin concentrations. *Clin Sci Mol Med* 1976;50:213-221
56. Borenstajn J. Heart and skeletal muscle lipoprotein lipase. In *Lipoprotein lipase*. Borenstajn J (ed.). Evener Publishers Inc. Chicago, 1987
57. Lithell H, Karlstrom B, Selinus I, Vessby B, Fellstrom B. Is muscle lipoprotein lipase inactivated by ordinary amounts of dietary carbohydrates? *Hum Nutr Clin Nutr* 1985;39C:289-295
58. Eckel RH, Fujimoto WY, Brunzell JD. Gastric inhibitory polypeptide enhanced lipoprotein lipase activity in cultured preadipocytes. *Diabetes* 1979;28:1141-1142
59. Wasada T, McCorkle K, Harris V, Kawal K, Howard B, Unger RH. Effect of gastric inhibitory polypeptide on plasma levels of chylomicron triglycerides in dogs. *J Clin Invest* 1981;68:1106-1107
60. Jorde R, Petterson JE, Burhol PG. Lack of effect of exogenous or endogenous gastric inhibitory polypeptide on the elimination rate of Intralipid in man. *Acta Med Scand* 1984;216:19-23
61. Bourdeaux A, Guidicelli Y, Rebourcet M, Nordmann J, Nordmann R. Influence of some gastrointestinal hormones on adipose tissue lipoprotein lipase activity in vitro: evidence of a stimulating effect of pancreozymin and gastrin. *Biochem Biophys Res Comm* 1980;95:212-219
62. Eisenberg S. High density lipoprotein metabolism. *J Lipid Res* 1984;25:1017-1058
63. Tall AR, Blum C, Forester G, Nelson C. Changes in the distribution and composition of plasma high density lipoproteins after ingestion of fat. *J Biol Chem* 1982;257:198-207
64. Mjos OD, Faergeman O, Hamilton RL, Havel RJ. Characterization of remnants produced during the metabolism of triglyceride-rich lipoproteins of blood plasma and intestinal lymph in the rat. *J Clin Invest* 1975;56:603-615
65. Redgrave TG. Formation of cholesterol ester rich particulate lipid during metabolism of chylomicrons. *J Clin Invest* 1970;49:465-471
66. Mahley RW. Apolipoprotein E and cholesterol metabolism. *Klin Wochenschr* 1983;61:225-232
67. Weisgraber KH, Innerarity TL, Mahley RW. Abnormal lipoprotein receptor-binding activity of the human E apoprotein due to cysteine-arginine interchange at a single site. *J Biol Chem* 1982;257:2818-2821
68. Cortner JA, Costes PM, Le N-A, et al. Kinetics of chylomicron remnant clearance in normal and hyperlipoproteinemic subjects. *J Lipid Res* 1987;28:195-206

69. Kita T, Goldstein JL, Brown MS, Watanabe Y, Hornick CA, Havel RJ. Hepatic uptake of chylomicron remnants in WHHL rabbits: A mechanism genetically distinct from the low density lipoprotein receptor. *Proc Natl Acad Sci* 1982;79:3623-3627
70. Havel RJ. Role of apolipoprotein E in lipoprotein metabolism. *Am Heart J* 1987;113:470-474
71. Hui DY, Innerarity TL, Mahley RW. Lipoprotein binding to canine hepatic receptors. Metabolically distinct apo-E and apo- B,E receptors. *J Biol Chem* 1981;256:5646-5655
72. Windler EE, Greeve JC, Daerr WH, Greten H. The contribution of the hepatic LDL-receptor to the uptake of chylomicron remnants. *Circulation* 1987;76 (suppl IV):iv-39 (abstr.)
73. Wilson DE, Chan I-F, Ball M. Plasma lipoprotein retinoids after vitamin A feeding in normal man: Minimal appearance of retinyl esters among low-density lipoproteins. *Metabolism* 1983;32:514-517
74. Stalenhoef AFH, Malloy M, Kane JP, Havel RJ. Metabolism of apolipoproteins B-48 and B-100 of triglyceride rich lipoproteins in normal and lipoprotein lipase-deficient humans. *Proc Natl Acad Sci* 1984;81:1839-1843
75. Patsch JR, Karlin JB, Scott J, Smith LC, Gotto AM. Inverse relationship between blood levels of high density lipoprotein subfraction 2 and magnitude of postprandial lipemia. *Proc Natl Acad Sci* 1983;80:1449-1453
76. Rossner S. Studies on an intravenous fat tolerance test. Methodological, experimental and clinical experiences with Intralipid. *Acta Med Scand* 1974; suppl (564):1-23
77. Bolzano K, Haslauer F, Krempler F. The influence of starvation on the removal mechanisms of plasma triglycerides in man. *Atherosclerosis* 1979;33:171-180
78. Lambert D, Mejean L, Drouin P, Debry G. Determination of the k₂ lipid clearance coefficient by centrifugation. A comparison with the Gordis test and spectrophotometric measurement. *Clin Chim Acta* 1980;105:59-64
79. Mills GL, Lane PA, Weech PK. A guidebook to lipoprotein technique. In: Burdon RH, van Knippenberg PH, eds. *Laboratory techniques in biochemistry and molecular biology*. Amsterdam: Elsevier, 1984; vol. 14
80. Dole VP, Hamlin JI. Particulate fat in lymph and blood. *Physiol Rev* 1962;42:674-701
81. Gidez LI, Miller GJ, Burstein M, Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. *J Lipid Res* 1982;23:1206-1223
82. Warnick GR, Benderson J, Albers JJ. Dextran sulphate-Mg²⁺ precipitation procedure for quantitation of high-density- lipoprotein cholesterol. *Clin Chem* 1982;28:1379-1388 Selected method.
83. Nestel PJ, Denborough MA, O'Dea J. Disposal of human chylomicrons administered intravenously in ischaemic heart disease and essential hyperlipemia. *Circ Res* 1962;10:786-791
84. Nestel PJ, Barter PJ. Triglyceride clearance during diets rich in carbohydrate or fats. *Am J Clin Nutr* 1973;26:241-245

85. Annegers JH, Boutwell JH, Ivy AC. The effect of dietary fat on fecal fat exchange and subjective symptoms in man. *Gastroenterology* 1948;10:486-495
86. Meng HC, Sasaki H, Thompson SW, et al. Toxicity testing of fat emulsions. I-IV. *Am J Clin Nutr* 1965;16:29,37,43,62
87. Schuberth O. Clinical results of intravenous infusions of fat emulsions. *Nutr Dieta* 1963;5:387
88. Carlson LA. Studies on the fat emulsion Intralipid. I. Association of serum proteins to Intralipid triglyceride particles. *Scand J clin Lab Invest* 1980;40:139-144
89. Boberg J, Carlson LA. Determination of heparin-induced lipoprotein lipase activity in human plasma. *Clin Chim Acta* 1964;10:420
90. Annuzzi G, Jansson E, Kaijser L, Holmquist L, Carlson LA. Increased removal rate of exogenous triglycerides after prolonged exercise in man: Time course and effect of exercise duration. *Metabolism* 1987;36:438-443
91. de Vries HA. *Physiology of exercise for physical education and athletics*. 3rd ed. Iowa: W.C. Brown Co, 1980.
92. Marotta F, Girdwood AH, Young GO, Okeefe SJ, Marks IN. Enteric-coated pancreatic enzyme supplementation. A dose response study. *S Afr J Med* 1987;72:84 (abstr)
93. Cham BE, Knowles BR. A solvent system for delipidation of plasma or serum without protein precipitation. *J Lipid Res* 1976;17:176-181
94. Cohen JC, Schall R. Reassessing the effects of simple carbohydrates on the serum triglyceride responses to fat meals. *Am J Clin Nutr* (in press)
95. Snedecor GW, Cochran WG. *Statistical methods*. Ames, Iowa: Iowa State University Press,
96. Cohen JC, Noakes TD, Benade AJS. Serum triglyceride responses to fatty meals: Effects of meal fat content. *Am J Clin Nutr* 1988 (in press).
97. Tobey TA, Greenfield M, Kraemer F, Reaven GM. Relationship between insulin resistance, insulin secretion, very low density lipoprotein kinetics, and plasma triglyceride levels in normotriglyceridemic man. *Metabolism* 1981;30:165-171
98. Hallberg D. Studies on the elimination of exogenous lipids from the bloodstream. The kinetics of elimination of a fat emulsion studied by continuous infusion technique in man. *Acta Physiol Scand* 1965;64:299-307
99. Rossner S, Larsson-Cohn U, Carlson LA, Boberg J. Effects of an oral contraceptive agent on plasma lipids, plasma lipoproteins, the intravenous fat tolerance and postheparin lipoprotein lipase activity. *Acta Med Scand* 1971;190:301
100. Cohen JC, Benade AJS, Noakes TD. Chylomicron clearance in athletes and in sedentary men. *Am J Clin Nutr* (in press).
101. Huttunen JK, Ehnholm C, Kekki M, Nikkila EA. Post-heparin plasma lipoprotein lipase and hepatic lipase in normal subjects and in patients with hypertriglyceridemia: correlations to sex, age, and various parameters of triglyceride metabolism. *Clin Sci Mol Med* 1976;50:249-260

102. Pagano Mirani-Oostdijk C, Havekes L, Terpstra J, Frolich M, van Gent CM, Jansen H. Diurnal changes in serum triglycerides as related to changes in lipolytic enzymes, (apo)lipoproteins and hormones in normal subjects on a carbohydrate-rich diet. *Eur J Clin Invest* 1983;13:301-309
103. Applebaum-Bowden D, Haffner SM, Wahl PW, Hoover JJ, Warnick GR, Albers JJ, Hazzard WR. Postheparin plasma triglyceride lipases. Relationships with very low density lipoprotein triglyceride and high density lipoprotein2 cholesterol. *Arteriosclerosis* 1985;5:273-282
104. Verschoor L, Chen Y-D, Reaven GM. In search of a relationship between physiologically-induced variations in adipose tissue lipoprotein lipase activity and very low density lipoprotein kinetics in normal rats. *Metabolism* 1982;31:499-503
105. Robinson DS. Lipoprotein lipase - past, present and future. In *Lipoprotein Lipase*. Borenstajn J (ed). Evener Publishers. Chicago, 1987
106. Weintraub MS, Eisenberg S, Breslow JL. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. *J Clin Invest* 1987;80:1571-1577
107. Havel RJ, Eder HA, Bragdon TH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1955;34:1345-1353
108. Redgrave TG, Carlson LA. Changes in the plasma very low density and low density lipoprotein content, composition, and size after a fatty meal in norma- and hypertriglyceridemic man. *J Lipid Res* 1979;20:217-229
109. Robinson DS. The heparin clearing reaction. *Am J Clin Nutr* 1960;8:7-20
110. Moreton JR. Chylomicronemia, fat tolerance, and atherosclerosis. *J Lab Clin Med* 1950;35:373-384
111. Kingsbury KJ, Morgan DM, Shervington PC. The effect of test feeds on the plasma lipids. *Lancet* 1960:1045-1049
112. Engelberg H. Mechanisms involved in the reduction of serum triglycerides in man upon adding unsaturated fats to the normal diet. *Metabolism* 1966;15:796-807
113. Kashyap ML, Barnhart, RL, Srivastava LS, et al. Alimentary lipemia: Plasma high-density lipoproteins and apolipoproteins CII and CIII in healthy subjects. *Am J Clin Nutr* 1983;37:233-243
114. Harris WS, Connor WE. The effects of salmon oil upon plasma lipids, lipoproteins, and triglyceride clearance. *Trans Assoc Am Physicians* 1980;43:148-153
115. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 1979;28:1039-57
116. Kasper H. Faecal fat excretion, diarrhoea, and subjective complaints with highly dosed oral fat intake. *Digestion* 1970;3:321-330
117. Goodnight SH, Harris WS, Connor WE, Illingworth DE. Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. *Arteriosclerosis* 1982;2:87
118. Nestel PJ, Scow RO. Metabolism of chylomicrons of differing triglyceride composition. *J Lipid Res* 1964;5:46-51

119. Sullivan JF. The effect of protein ingestion on alimentary lipemia. *Amer J Med Sci* 1962;243:770-774.
120. Albrink MJ, Fitzgerald JR, Man EB. Effect of glucagon on alimentary lipemia. *Proc Soc Exp Biol Med* 1957;95:778-780
121. Unger RH, Aguilar-Parada E, Eisentraut AM. Pancreatic glucagon secretion in normal and diabetic subjects. *American Journal of Medical Science* 1969;257:415-419
122. Albrink MJ, Fitzgerald JR, Man EB. Reduction of alimentary lipemia by glucose. *Metabolism* 1958;7:162-171.
123. Nikkila EA, Pelkonen R. Enhancement of alimentary hyperglyceridemia by fructose and glycerol in man. *Proc Soc Exp Biol Med* 1966;123:91-94.
124. Mann JI, Truswell AS, Pimstone BI. The different effects of oral sucrose and glucose on alimentary lipemia. *Clin Sci* 1971;41:123-129.
125. Nicholls DP, Cohen H. Effect of oral glucose on alimentary lipaemia in normal man. *Ir J Med Sci* 1985;154:348-353
126. Goldberg AP, Sherrard DJ, Haas LB, Brunzell JD. Effect of high carbohydrate feeding with dextrose or sucrose on adipose tissue lipoprotein lipase activity and plasma triglyceride levels in hypertriglyceridemic patients. *Am J Clin Nutr* 1979;32:1628- 1635
127. Heath GW, Gavin JR, Hinderliter JM, Hagberg JM, Bloomfield SA, Holloszy JO. Effects of exercise and lack of exercise on glucose tolerance and insulin sensitivity. *J Appl Physiol* 1983;55:512-517
128. Krut LH, Barsky BF. Effect of enhanced glucose utilisation on postprandial lipaemia in ischaemic heart-disease. *Lancet* 1964:1136-1138
129. Berger GMB. Clearance defects in primary chylomicronemia:A study of tissue lipoprotein lipase activities. *Metabolism* 1986;35:1054-1061
130. National Diabetes Data Group. Classification and diagnosis of Diabetes Mellitus and other categories of glucose intolerance. *Diabetes* 1979;28:1039-1057
131. Nester J, Wasserman W. Applied linear statistical models. London: Irwin-Dorsey, 1974.
132. Nakagawa S, Nakayama H, Sasaki T, et al. A simple method for the determination of serum free insulin levels in insulin-treated patients. *Diabetes* 1973;22:590-600
133. Williamson DH, Mellanby J. D(-)-3-Hydroxybutyrate in: *Methods of enzymatic analysis* (2nd ed);Bergmeyer HU Ed, Academic Press, New York (1974), pp.1836-1839.
134. Mellanby J, Williamson DH.: Acetoacetate in: *Methods of enzymatic analysis* (2nd ed);Bergmeyer HU Ed, Academic Press, New York (1974), pp.1840-1843.
135. SAS users guide: 5th Ed. Cary: SAS Institute Inc.
136. Robinson AM, Williamson DH. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol Rev* 1980;60:143-187

137. Sullivan JF, Heaney RP, Suchy N. Determination of the endogenous component of alimentary lipemia. *J Clin Endocrinol Metab* 1970;31:640-646
138. Thompson RG, Hayford JT, Hendrix JA. Triglyceride concentrations: The disaccharide effect. *Science* 1979;206:838-839
139. Carlson LA, Hallberg D. Studies on the elimination of exogenous lipids from the blood stream. The kinetics of elimination of a fat emulsion and of chylomicrons in the dog after single injection. *Acta Physiol Scand* 1963;59:52-61
140. Nicoll A, Sigurdsson G, Marsh A, Lewis B. Intravenous fat tolerance. Correlations with very low density lipoprotein apoprotein B kinetics in man. *Atherosclerosis* 1977;20:17-22
141. Nikkila EA, Huttunen JK, Ehnholm C. Postheparin plasma lipoprotein lipase and hepatic lipase in diabetes mellitus. Relationship to plasma triglyceride metabolism. *Diabetes* 1977;26:11-21
142. Tollin C, Ericsson M, Johnson O, Backman C. Clearance of triglycerides from the circulation and its relationship to serum lipoproteins: influence of age and sex. *Scand J Clin Lab Invest* 1985;45:679-684
143. Ericsson M, Johnson O, Tollin C, Furberg B, Backman C, Angquist K. Serum lipoproteins, apolipoproteins and intravenous fat tolerance in young athletes. *Scand J Rehab Med* 1982;14:209-212
144. Clark S, Derksen A. Phosphatidylcholine composition of emulsions influences triacylglycerol lipolysis and clearance from plasma. *Biochim Biophys Acta* 1987;920:37-46
145. Bazelmans J, Nestel PJ, Nolan C. Insulin-induced glucose utilization influences triglyceride metabolism. *Clin Sci* 1983;64:511-516
146. Munkgaard Rasmussen S, Heding LG, Parbst E, Volund A. Serum IRI in insulin-treated diabetics during a 24-hour period. *Diabetologia* 1975;11:151-158
147. Taskinen M-R, Nikkila EA, Nousiainen R, Gordin A. Lipoprotein lipase activity in adipose tissue and skeletal muscle of human diabetics during insulin deprivation and restoration. *Scand J Clin Lab Invest* 1981;41:263-268
148. Yki-Jarvinen H, Kiviluoto T, Taskinen M-R. Insulin resistance is a prominent feature of patients with pancreatogenic diabetes. *Metabolism* 1986;35:718-727
149. Dufaux B, Assman G, Hollman W. Plasma lipoproteins and physical activity - A review. *Int J Sports Med* 1982;3:123-136
150. Wirth A, Diehm C, Hanel W, Welte J, Vogel I. Training-induced changes in serum lipids, fat tolerance, and adipose tissue metabolism in patients with hypertriglyceridemia. *Atherosclerosis* 1985;54:263-271
151. Brotherhood J, Brozovic B, Pugh LGC. Haematological status of middle- and long-distance runners. *Clin Sci Mol Med* 1975;48:139-145

152. Peltonen P, Marniemi J, Hietanen E, et al. Changes in serum lipids, lipoproteins, and heparin releasable lipolytic enzymes during moderate physical training in man: A longitudinal study. *Metabolism* 1981;30:518-526
153. Nikkila EA, Taskinen M-R, Rehunen S, Harkonen M. Lipoprotein lipase activity in adipose tissue and skeletal muscle of runners - Relation to serum lipoproteins. *Metabolism* 1978;27:1661-1671
154. Svedenhag J, Lithell H, Juhlin-Dannfelt A, Henriksson J. Increase in skeletal muscle lipoprotein lipase following endurance training in man. *Atherosclerosis* 1983;49:203-207
155. Schlierf G, Dinsenbacher A, Kather H, Kohlmeier M, Haberbosch W. Mitigation of alimentary lipemia by postprandial exercise - phenomena and mechanisms. *Metabolism* 1987;36:726-730
156. Nikkila EA, Konttinen A. Effect of physical activity on postprandial levels of fats in serum. *Lancet* 1962;1151-1154
157. Kantor MA, Cullinane EM, Sady SP, Herbert PN, Thompson PD. Exercise acutely increases high density lipoprotein-cholesterol and lipoprotein lipase activity in trained and untrained men. *Metabolism* 1987;36:188-192
158. Williams PT, Wood PD, Haskell WL, et al. The effects of running mileage and duration on plasma lipoprotein levels. *J Amer Med Soc* 1982;247:2674-2679
159. Danner SA, Wieling W, Havekes L, Leuven JG, et al. Effect of physical exercise on blood lipids and adipose tissue composition in young healthy men. *Atherosclerosis* 1984;53:83-90
160. Cotter R, Martis L, Cosmas F et al. Nonlinear kinetic analysis of the elimination of lipid emulsion administered intravenously in dogs. *J Parent Ent Nutr* 1983;7:244-250