FACTORS AFFECTING ENERGY EXPENDITURE AND THE EFFICIENCY OF FUEL UTILIZATION: FEEDING AND EXERCISE MODELS

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DEDICATION

For Michael, my husband,

for Ian,

and

for my family

"Knowledge is one of the wondrous gifts of God. It is incumbent upon everyone to acquire it..."

(Baha'u'llah- Tablets of Baha'u'llah)

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DECLARATION

I, Estelle Victoria Lambert, declare that this thesis is my own unaided work, both in concept and execution, and that apart from the normal guidance from my supervisor, I have received no assistance except as stated.

Except where stated, neither the substance nor any part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree in this University or any other University.

FACTORS AFFECTING ENERGY EXPENDITURE AND THE EFFICIENCY OF FUEL UTILIZATION: FEEDING AND EXERCISE MODELS

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Abstract

The first aim of this dissertation was to monitor both rat and human responses to short-term perturbations in energy balance brought about through food energy restriction and refeeding, exercise training and the cessation of exercise training or surgical lipectomy. The second aim of this dissertation was to identify factors which might explain differences in food energy intake in weight-matched, weight-stable "large and small eaters". The final aim of this dissertation was to identify factors which might explain differences in resting energy expenditure in a large sample of weight-stable men and women, including exercising and non-exercising persons, and including persons who may be regarded as "restrained eaters".

In the first study, Long-Evans rats underwent pre-weaning litter size manipulation and were exposed to either a standard

chow diet or a diet comparatively high in fat during the initial 18 weeks following weaning. Additionally, some of the rats were housed as pairs, and others were housed singly during the post-weaning period. Metabolic efficiency was quantified by calculating feeding efficiency, changes in body size, and body fat accretion. In this study, there was no persistent effect of pre-weaning nutrition on post-weaning growth and feeding efficiency.

In the second study of this dissertation, Long-Evans rats, which were habituated to spontaneous running activity in specially designed wheel cages trained for 8 weeks, after which randomly-selected rats were placed in ordinary cages without wheels for 2 weeks. The metabolic responses to short-term detraining were quantified by measuring feeding efficiency, body mass, body fat accretion, and changes in adipose tissue lipogenic enzyme activity in trained, detrained and untrained rats. Rats which had stopped training demonstrated an increased feeding effiency, a two- to three-fold increase in adipose tissue lipogenic activity, and increased fat cell size and fat pad mass, when compared to trained or sedentary counterparts.

The metabolic responses to stopping training were studied further in human athletes. Resting and glucose-stimulated

energy expenditure and body composition were measured before and one and two weeks after stopping training in highly-trained triathletes (>14 hours per week training),and moderately-trained runners (6-10 hours per week training) and were compared to untrained control subjects. Resting energy expenditure was higher in the highly-trained triathletes when compared to control subjects, and was attenuated progressively in the two weeks following the termination of training.

In the next study of this dissertation, free-living, high-mass adults underwent moderate food energy restriction with or without exercise training for 12 weeks, or very-low energy dieting for 4 weeks. All subjects were asked to undergo voluntary refeeding after food restriction. The refeeding period was 3-4 weeks in duration.

Resting and glucose-induced energy expenditure, and body composition were measured before and after voluntary food energy restriction, food energy restriction and exercise training, or very-low energy dieting. Changes in resting energy expenditure relative to changes in fat-free mass were not different across treatment groups, after food energy restriction and refeeding.

The metabolic response to energy deficit was further investigated by characterizing the metabolic rate and thermic response to feeding in women before and after undergoing surgical lipectomy.

In order to address the second aim of this dissertation, resting energy expenditure, and the thermic response to feeding and exercise were compared in matched, weight-stable, body-composition-stable women, who reported very large differences in food energy intake. "Small" eaters had a slight, but significantly lower thermic response to a mixed meal.

The final aim of this dissertation was addressed by studying the relationship between indices of body size, food intake, age and gender on resting energy expenditure in a large and diverse population of men and women, which included exercising and sedentary persons, lean and obese persons and "large and small" eaters.

Conclusions: (i) Short-term perturbations in energy balance following food energy restriction are reversible. (ii) Stopping training in previously-trained rats and humans results in a different metabolic sequelae than is seen

following refeeding after food energy restriction. (iii) The nature of the metabolic responses to short-term perturbations in energy balance are determined, in part, by whether or not the organism is growing, or previously in energy balance. (iv) There is indirect evidence that fat cell size, and not adipose tissue mass, may be the involved effector in regulating the metabolic response to energy deficit. (v) The now well-established relationship between indices of body size, including fat-free mass, and resting energy expenditure was confirmed, however, the limitations for standardizing resting energy expenditure for FFM were identified. (vi) This study provided evidence that the metabolic "activity" of fat-free mass ($kJ \cdot kg^{-1}$ FFM) per unit of fat-free mass decreased with increasing fat-free mass and was higher in exercising persons than in non-exercising persons. (vi) "Weight stability" may describe an organism in energy balance, however, this information does not provide insight into energy "flux" in this organism. The effects of differences in "energy flux" in organisms and metabolic responses to perturbations in energy balance has not yet been elucidated.

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LIST OF PUBLICATIONS

Sections of the work reported in this thesis have been published in the following journal articles;

Lambert, E. V., Hudson, D. A., Bloch, C. E., Koeslag, J. K. The metabolic response to localized surgical fat-removal in non-obese women. *Aesthetic Plastic Surgery* 1991; 15: 105-110.

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CHAPTER 1

INTRODUCTION TO AND SCOPE OF THE THESIS

"Jack Spratt could eat no fat; his wife could eat no lean...between the two, they licked the platter clean"

(John Clarke, 1639).

The vision conjured by this children's nursery rhyme effectively summarizes the great individual variability in food energy required to maintain a state of energy balance, or a constancy of body mass and body energy stores. In addition, one is reminded of the plasticity of energy balance, and the apparent adaptability of an individual's food energy requirement when one examines the physiology of pregnancy and lactation, or the remarkable ability of the body to recover from nutritional stress in the form of starvation.

For example, it has been estimated that the total energy cost of a full-term pregnancy exceeds 250 MJ above that required for energy balance in a non-pregnant, non-lactating woman (FAO/WHO, 1973, Singh et al., 1989). Conversely, individuals have survived for over 300 days of complete starvation, which amounts to an estimated cumulative energy deficit of over 3000 MJ (Barnard et al., 1969).

Conditions such as "obesity" are often mistakenly interpreted as a state of energy imbalance. However, energy balance describes a dynamic relationship. Specifically, energy (E) is the capacity of a system to perform work and energy balance is

the relationship between ingested energy and energy expended as work, dissipated as heat, excreted or stored.

$$E_{in} = E_{out} \pm E_{stored}$$

Thus, a weight-stable, body composition-stable, "obese" person is as likely to be in energy balance as his or her "lean" counterpart.

For descriptive purposes, energy balance may be partitioned into various components. Gross food energy is considered to be the total ingested food energy intake. A certain amount of this energy is not available to the body *in vivo* and is lost in the urine and faeces. These losses are estimated to account for less than 10% of the overall food energy intake in freely-eating individuals. The remaining food energy is referred to as metabolizable food energy intake. The *in vivo* "fate" of this energy is either mechanical or metabolic work, heat production, or energy storage.

In most freely-eating individuals, resting energy expenditure, or the energy output after an overnight fast, at rest and in a thermoneutral environment, when averaged over 24 hours, accounts for between 60 and 70% of their daily energy output or about 6 MJ per day (Ravussin et al., 1986). However, in starvation and underfeeding, this value can be as low as 3 MJ per day (Keys et al., 1950). Conversely, during deliberate

overfeeding, resting energy expenditure has been shown to rise to over 8 MJ per day (Alpert, 1990). The minimal obligatory component of resting metabolic rate is therefore apparently very small.

Approximately 10-15% of total daily energy expenditure is estimated to be accounted for by the thermic effect of feeding (TEF). There is presumably an obligatory component of TEF which is associated with digestion, absorption, storage and net re-synthesis of food as endogenous fuel (Horton, 1983). There is also apparently a facultative component of TEF which results in an increase in heat production but with no net synthesis or work. This portion of the thermic effect of feeding appears to be dependent on insulin release, and is regulated via the sympathetic nervous system, through a permissive effect of thyroid hormones (Danforth, 1983).

The thermic effect of exercise or the heat increment of exercise may account for as little as 15% of daily energy expenditure or as much as 50% of the daily energy expenditure in highly-trained persons. Humans have a mechanical efficiency of only approximately 25%. (Astrand and Rodahl, 1977). Thus, only 25% of the extra energy which is expended above resting energy expenditure during exercise is translated into mechanical work output, while the remainder is lost as heat.

There is also believed to be a further component of energy expenditure which is referred to as adaptive thermogenesis (~ 10% of daily energy expenditure) which is variable and depends on chronic exposure to stimuli such as undernutrition or cold (Horton, 1983). This is, however, a confusing term, since undernutrition can lower resting energy expenditure to less than 3 MJ per day in individuals undergoing semi-starvation (Keys et al., 1950). Thus, one may consider "adaptive thermogenesis" to be any energy expenditure above the previously described "minimum obligatory resting energy expenditure" which is not explained by the TEF or TEE. By this definition, most well-nourished, freely-eating persons would have an "adaptive thermogenesis" of approximately 4 MJ per day.

Energy balance is maintained in most species with a metabolisable energy intake which is 1.3-1.5 times higher than the post-absorptive, resting metabolic rate (Prentice et al., 1985, Stock and Rothwell, 1982). Any increase in metabolisable energy intake over this value would be expected to result in 1) increased energy storage or accumulation and 2) a decrease in overall metabolic efficiency (Keesey, 1989, Sims et al., 1973, Stock and Rothwell, 1982). The net non-heat energy available from each kJ of ingested energy may, therefore, be expected to decrease as the "plane of nutrition" increases or as energy balance becomes more positive. Thus, metabolic efficiency may be defined as the variable which

allows an individual to remain in energy balance on an energy intake above or below predicted energy requirements.

Homeostatic control of an individual's energy balance is remarkably consistent. If an individual were to have a constant 0.3% error in energy intake relative to energy expenditure, they may be expected to gain nearly 15 kg in 30 years after accounting for the increased energy cost of a gain in mass. Despite this, there is evidence for large individual variation in the energy intake without a corresponding variation in weight loss and weight gain in persons of similar size and body composition (George et al., 1991, Edholm et al., 1977, Morgan et al., 1982).

It is well established that if energy balance is perturbed through fasting or food restriction, there is an attenuation of energy expenditure (de Boer et al., 1986, Thompson and Blanton, 1987, van Dale and Saris, 1989). Changes in energy expenditure in response to undernutrition may be accounted for by a reduced TEF, and a smaller energy requirement for a reduced body mass and fat-free mass. Whether the degree of undernutrition influences the subsequent change in fat-free mass or the magnitude of change in resting energy expenditure or both, has not been established (Elliot et al., 1989, Forbes et al., 198, Foster et al., 1992, Klesges et al., 1992, Heshka et al., 1990, Hill et al., 1989, Wadden et al., 1990).

Some studies suggest that the energy requirement of "reduced obese" individuals remains attenuated when compared to weight-matched controls who have not lost any weight (Bray, 1969, Elliot et al., 1989, Geissler et al., 1987, Heshka et al., 1990, Leibel and Hirsch, 1984). This has led to the suggestion that a lower-than-predicted energy expenditure for a specific body size or fat-free mass may be a marker for subsequent weight gain (Ravussin et al., 1988). Conversely, large individual differences in energy required for body mass maintenance may reflect an adaptive response to chronic "underfeeding" or "overfeeding". How rapidly and the extent to which this occurs probably depends on the interaction between genetic characteristics and environmental factors (Bouchard et al., 1990).

Exercise training and food energy restriction possibly provide a similar metabolic challenge to energy balance in weight-stable, body composition-stable individuals. Exercise training, however, may increase the quantity of fat-free mass, relative to the fat mass (Ballor et al., 1988, Hagan et al., 1985, Pollock et al., 1975). Indeed, exercise training may influence the metabolic activity or the "quality" of the fat-free mass, and has been associated in some studies with an increase in resting metabolic rate, relative to untrained persons (Poehlman et al., 1988, Tremblay et al., 1986). Conversely, there is some evidence that exercise training results in a blunting of the facultative component of the TEF,

as a result of downregulation of the sympathetic nervous system (LeBlanc et al., 1986, Poehlman et al., 1988, Thompson and Blanton, 1987, Tremblay et al., 1983).

Finally, there are a number of studies which have addressed the physiological consequences of "recovery" from a deliberate energy deficit, as in refeeding following food energy restriction or the cessation of exercise training following a period of training. Several studies suggest that metabolic adaptations which occur as a result of food energy restriction, such as a reduction in resting metabolic rate and enhanced insulin sensitivity, persist with refeeding and favour efficiency of food energy utilization, with conseqent fat and muscle accretion (Boyle et al., 1981, Eckel and Yost, 1987, Hill et al., 1984, Quig et al., 1983, Schwartz and Brunzell, 1978, Yost and Eckel, 1988).

It is not clear whether a similar mechanism is operative following the cessation of exercise training. In previous studies of detrained rats and hamsters, food energy intake increased following the cessation of training, making it difficult to separate the effects of detraining and refeeding (Applegate et al., 1984, Applegate et al., 1987, Dohm et al., 1977, Sandretto and Tsai, 1988).

Thus, there exists a paradigm in which organisms respond to short-term perturbations in energy balance, and these

responses result in measurable changes in (i) body energy stores or (ii) components of energy expenditure. For example, individuals in energy balance may perturb this balance by restricting their food intake, or increasing their level of physical activity. In both instances, these treatments presumably result in a negative energy balance and subsequent loss of body energy stores. However, the overall impact of these short-term interventions on resting energy expenditure and the maintenance of body energy stores are almost certainly complicated by the variable effects of intervention on individual body composition, and by pre-existing factors which may influence the range and extent of each individual's response to intervention.

Thus, the questions which remain to be answered are (i) whether the metabolic responses to perturbations in energy balance may be explained simply on the basis of changes in body mass, fat-free mass and food intake, and (ii) whether there is evidence for changes in metabolic efficiency during refeeding following food energy restriction or with exercise training and the cessation of exercise training.

The aims of this thesis were firstly, to compare and characterize the metabolic sequelae following short-term perturbations in energy balance. These perturbations include: the restriction of food energy intake and refeeding, as well as training and the cessation of exercise training. The first

model chosen was a rat model, in which pre- and post-weaning nutritional manipulations were compared. Metabolic efficiency was quantified by calculating feeding efficiency, changes in body size, and body fat accretion. This particular model was chosen to address the currently accepted paradigm that pre-weaning undernutrition predicts post-weaning growth and subsequent development.

The second model involved variable food restriction and refeeding, in free-living, high-mass adults. In this model, the effects of the degree of food energy restriction were compared, as well as the effects of the addition of exercise training during food energy restriction, and the metabolic response to refeeding. Resting energy expenditure, the thermic effect of glucose and of epinephrine-infusion, and changes in body composition were measured.

The effects of exercise training and the cessation of exercise training were compared in rats, which were habituated to spontaneous running activity in specially designed wheel cages. After training for 8 weeks, the rats were placed in ordinary immobile cages for 2 weeks. The metabolic responses to short-term detraining were quantified by measuring feeding efficiency, body mass, body fat accretion, and changes in adipose tissue lipogenic enzyme activity in trained and detrained rats, and compared to untrained controls. This study was different from all previous detraining studies, as

spontaneous food energy intake remained unchanged throughout the detraining period.

In humans, this question was addressed by a study which compared the resting energy expenditure and thermic response to glucose feeding in trained vs untrained persons, matched for fat-free body mass and age. Two groups of trained subjects stopped training for a period of two weeks, while one group continued to train. All groups were instructed to maintain a constant food energy intake throughout the experimental trial.

The metabolic response to energy deficit was further investigated by characterizing the metabolic rate and thermic response to feeding in women before and after undergoing surgical lipectomy.

These intervention models provide new insight into the short-term regulation of energy balance, and qualitative changes which may explain differences in metabolic efficiency.

The second aim of the thesis was to address factors which may determine differences in energy expenditure in free-living weight-stable persons. The first model chosen was a study of matched, weight-stable, body-composition-stable women, who reported very large differences in food energy intake.

Resting energy expenditure, and the thermic response to feeding and exercise were compared in the two groups.

In the second model, a large and diverse population of men and women, which included exercising and sedentary persons, lean and obese persons and "large and small" eaters, were studied to further examine those factors which have previously been identified as determinants of resting metabolic rate. In this model, specific attention was paid to (i) the influence of the method of expressing metabolic rate on the interpretation of body size determinants of resting metabolic rate and (ii) differences in these relationships in the various subgroups of this population.

Using both rat and human models, the responses to short-term perturbations in energy balance were measured directly and indirectly. These studies were designed to answer the following questions. Firstly, are responses to perturbations in energy balance related to the degree of energy deficit, the stage of development at which energy balance is perturbed, and the mode of energy deficit? Secondly, is the relationship between energy expenditure and indices of metabolic body size consistent across changing energy states?

CHAPTER 2

REVIEW OF THE LITERATURE:
FACTORS AFFECTING ENERGY EXPENDITURE AND THE EFFICIENCY OF FUEL UTILIZATION: FEEDING AND EXERCISE MODELS

2.1 Introduction

In the study of most biological systems, it is presumed on a teleological basis that each organism is designed to resist changes in the preferred state (a process termed homeostasis), and thus, for each perturbation in a system one would expect a response designed to counteract this disturbance. This response would either 1) return the organism to its previous state or 2) allow adaptation to occur. Thus, there is an expected "cause-and-effect" relationship, and by implication, the response to a perturbation in the system would generally be expected to be biologically favourable.

The maintenance of energy balance may be viewed as an integrated biological system. In the introduction to this dissertation, the plasticity and apparent adaptability of this system was described using the examples of pregancy and starvation. At the same time, there is evidence to suggest that energy balance is tightly regulated. Small, consistently positive differences in food energy intake, relative to energy expenditure (less than 0.5%) are likely to result in a gain in mass of between 400 and 500 g in a normal-weight individual over 1 year.

Welle et al. (1992) studied free-living energy expenditure in 38 women weighing between 53 and 118 kg. Using regression analysis, Welle calculated that daily energy expenditure for

weight maintenance was approximately 80 $kJ \cdot d^{-1}$ higher for each 1 kg increment in body mass between 53 and 118 kg. Thus, in a sample of people with different starting masses, increasing food energy intake by approximately 400 $kJ \cdot d^{-1}$ will not result in an indefinite weight gain, but will result in an exponential increase in mass, eventually reaching a "new" level of energy balance after a gain of approximately 5 kg.

Even after developing this model, Welle et al. (1992) conceded that there was great individual variability, and that some of the heavier subjects from their sample actually ate less energy per day than leaner subjects and still maintained their body weight. These observations lend support to the statement by Widdowson and Kennedy (1962) that each person is a "nutritional individuality". There is similar evidence from rat (Lambert and Noakes, 1990) and human (Ravussin et al., 1986) studies, that there is a concomitant degree of "motor individuality" (Parizkova, 1977). For example, Ravussin et al., (1986) estimated that spontaneous physical activity or "fidgeting" accounted for between 4% and 17% of total daily energy expenditure in a respiration chamber, or an energy equivalent of between 580 and 2860 $kJ \cdot d^{-1}$.

It is apparent that the degree to which an organism responds or adapts to perturbations in energy balance is dependent, in part, on intervening factors which may be deliberate, such as prior undernutrition, or factors related to growth, maturation

and ageing, or to genetic or strain differences. For example, Bouchard et al. (1990) studied the metabolic response to long-term, voluntary, overfeeding in 12 pairs of monozygotic twins. While each subject gained weight and body fat during the 100 days of overfeeding, the variability in response between pairs was 3 times greater than that within pairs. These results suggest that genetic factors account for a significant amount of the variability in response to a specific perturbation in energy balance in freely-eating persons.

One's ability to measure a true response or adaptation to a perturbation in energy balance is only as good as the experimental design, and the sensitivity and specificity of one's measurement techniques. For example, Tremblay and co-workers (1992) have described an individual who appeared to be unable to lose weight, despite a reportedly low food energy intake. When this individual was placed in a respiration chamber and given a diet similar in energy to his reported food intake, he lost weight and his estimated total daily energy expenditure was approximately 1.6 times his resting metabolic rate. In this case, the assessment of dietary intake had not been sensitive. Food energy intake measured under free-living conditions is clearly subject to inaccuracies of self-report and covert eating (Lissner et al., 1989).

In another example, Prentice et al., (1986) studied free-living energy balance in comparatively low- and high-mass women using the $^{2}H_{2}{}^{18}O$, stable-isotopic technique for indirect calorimetry. The high-mass women were selected for so-called "post-partum obesity" and had a significantly greater fat-free mass, fat-mass, and percentage body fat than the low-mass controls. There were two striking findings from this study. Firstly, the obese or high-mass women had a higher average energy expenditure over 2 weeks than the lean women, possibly as a result of a greater body size and fat-free mass. However, the obese subjects reported a mean daily food energy intake which was nearly 1.0 MJ lower than their measured, free-living energy expenditure, while the lean women were in apparent energy balance. Assuming that energy extraction from food was not different in the two groups of women, one may again conclude that reported food energy intake was not accurately assessed by the obese women in this study. However, these results could also suggest that the obese women were not in energy balance during the measurement period. The limiting factor would then be the ability of the investigators to measure changing body energy stores, assuming that their measure of body energy stores and food energy content was as precise as their measure of energy expenditure.

Body composition in the study by Prentice and colleagues (1986) was estimated using $^{2}H_{2}O$ (deuterium) dilution for the determination of total body water. The coefficient of

variation for this technique has been reported to range between 1-3% (Roubenoff and Kehayias, 1991). Fat-free mass is then estimated by assuming that the average hydration of lean tissue is 73%. Changes in body composition during the trial were taken into account, and although not clearly stated, energy equivalents for weight loss and weight gain were apparently added to the total energy intake.

Thus, in an attempt to characterize energy balance in relatively low- and high-mass women, Prentice et al. (1986) have made two assumptions. Firstly, they have assumed that the hydration state of fat-free tissue is not different in persons with widely varying body composition. However, Waki et al. (1991) demonstrated that extracellular fluid volume was significantly higher in middle-aged women, with a mean starting mass of 124 kg compared to a "normal-weight" control group of women, with a starting mass of 57 kg. They concluded that body composition measurements based on estimates of fluid volume distribution, which were originally standardized in non-obese subjects, may over- or under-estimate the hydration state of fat-free tissue.

Secondly, they have assumed that the errors associated with the estimated energy equivalents for weight lost or weight gained are neglible and are similar for women with comparatively low- vs high-mass. This assumption may be incorrect. In a recent compilation of 6 different overfeeding

or nutritional recovery experiments, Forbes (1990) examined the relationships between the excess energy consumed over and above maintenance requirements, the energy cost per gram of tissue gained and the starting mass and body composition of the subjects. He found that the energy cost per gram of body mass gain was linearly related to initial body mass, and initial percentage fat. He concluded that the energy cost of body mass gain was greater in larger, fatter persons, and that this was a result of the fact that a greater proportion of the gain in mass in fatter persons could be attributed to a gain of fat mass.

In a final example, the $^{2}H_{2}{}^{18}O$ technique was again employed to assess free-living energy expenditure in women, during the "hungry" agricultural season in the Gambia (Singh et al., 1989). Food energy intake was quantified using the weighed, food inventory technique and validated by trained field workers. Energy equivalents of local foodstuffs had been previously established. However, investigators were unable to reconcile energy expenditure with food energy intake in this study.

After correcting for the energy costs of fat oxidation which resulted from lost body mass, and accounting for all of the possible over- or under-estimations of energy expenditure measured under these environmental conditions, they were left

with nearly a 5.0 MJ unexplained deficit between daily food energy intake and measured energy expenditure in these women.

The assumptions and limitations associated with quantifying energy expenditure using indirect calorimetry include:

1) All O_2 is used to oxidize degradable fuels, and all the resulting CO_2 is assumed to be recovered;
2) Gas exchange is assumed to occur in non-acidotic, steady-state, conditions and that there is no time delay for the evolution of CO_2 from the body's bicarbonate pool;
3) Energy loss from protein in hair, nails, and skin, as well as energy lost from sweat solutes, or the incomplete oxidation of alcohol and methane are assumed to be small;
4) Errors in the assumptions of energy equivalents for weight lost or gained are assumed to be small (McLean and Tobin, 1988).

Another possible source of error was highlighted in an energy balance study by Webb et al. (1980b). In this experiment, investigators compared energy balance in 4 subjects using both direct calorimetry (water-cooled undergarment with insulated clothing) and continuous indirect calorimetry. They found that when subjects were relatively food-restricted and maintained a high level of physical activity, indirect calorimetry underestimated daily energy expenditure, relative to direct calorimetry by as much as 4 MJ per day. This energy

was considered "unmeasured energy", and these findings suggest that the assumption that all of the energy from fuel oxidation is measureable as heat and external work should be examined more closely.

In the following review, both the responses and adaptations to perturbations in energy balance will be discussed and possible mechanisms for these responses will be proposed. The standardization of expression of the responses will also be discussed, with relation to changes in body energy stores.

2.2. Perturbing energy balance: evidence for response and adaptation

2.2.1. "Undernutrition" and refeeding: introduction

In a United Nations, Food and Agricultural Organization (FAO)/ World Bank study, it was estimated that in 1980 between 800 million and 1 billion persons worldwide, had some degree of energy malnutrition. Energy malnutrition has been loosely defined as a daily food energy intake less than 1.2 times the predicted basal metabolic rate in freely-eating persons (Torun and Viteri, 1988).

Somewhat paradoxically, a recent investigation by a United States House of Representatives subcommittee on Health found that 65 million Americans spend over $33 billion per year on diet aids, slimming books and rapid remedies for weight loss (Rosencrans, 1990).

Thus, for many people, undernutrition is a consequence of socioeconomic and sociodemographic factors which are beyond their control. While for others, undernutrition is a consequence of a deliberate attempt to change body energy stores. In this review, several models of undernutrition will be discussed with special reference to the effect of undernutrition and refeeding on energy balance, body energy stores, feeding efficiency, and components of energy expenditure.

2.2.2. **"Undernutrition" and refeeding: animal models for "critical periods" of ontogenesis**

Nearly 70 years ago, the metabolic consequences of food energy restriction during ontogenesis were first described by Parkes (1926). Parkes pioneered the model of "litter size manipulation" in order to overfeed or underfeed mice pups during the suckling period. Pups from small litters are generally heavier and fatter at weaning than pups from large litters (Eisen and Leatherwood, 1978a, Harris 1980a, Johnson

et al., 1973, Knittle et al., 1968, Oscai and McGarr, 1978, Wainright and Francey, 1987a, Wainright et al., 1987b, Winick and Noble, 1966). Using this model, researchers have for the last half-century attempted to characterize the effects of early undernutrition on post-weaning growth and body fat accretion.

Several investigators have since reported that there are "apparent" critical periods of adipocyte proliferation (Brook, 1972, Knittle and Hirsch, 1969). Winick and Noble (1966) found that pups from large litters had a reduced fat cell DNA content and reduced adult body mass, when compared to those from small litters, suggesting a reduced fat cell number. However, in a study by Hirsch and Han (1969), the effects of starvation in the early post-weaning period were entirely reversible, and adipocyte number was restored to that of *ad libitum*-fed control rats after refeeding. These data suggest that the "critical periods" for adipocyte proliferation are modifiable.

Oscai and McGarr (1978) found that rats raised in small litters had a higher voluntary food energy intake during the post-weaning period than those raised in larger litters. But, Wurtman and Miller (1976) observed, anecdotally, that food intake and body mass were reduced immediately after weaning in rats from large litters removed to separate cages. Lewis et al. (1986) found that pre-weaning nutritional manipulation in

baboons had no persistent effect on post-weaning food intake or growth, when animals were housed communally in the post-weaning period.

These findings suggest that the effects of early undernutrition are reversible, and there is also some evidence to suggest that the manifestation of a persistent effect of pre-weaning undernutrition may be related to the method in which the animals are housed.

2.2.3. **Undernutrition and refeeding: human models for changes in energy expenditure relative to changes in body energy stores**

It is universally accepted that food energy restriction following *ad libitum* food intake in previously weight-stable, body composition-stable persons results in a decline in energy expenditure at rest. Indeed, Keys et al. (1950) describes the decline in metabolic rate with undernutrition as a "useful adjustment to altered circumstances". In 1969, Bray studied the metabolic response to food energy restriction and weight loss in previously obese women. He found that the mean resting energy expenditure in these women decreased by a total of 15% over a period of 21 days while ingesting 1.9 MJ of food energy per day. Mean body mass decreased by 4-7%, and Bray pointed out that "the decline in oxygen consumption closely paralleled the slowing of weight loss".

There is, however, controversy as to whether the decline in resting energy expenditure, which has been demonstrated as a consequence of food energy restriction and weight loss, can be entirely explained by the loss of body cell mass (de Boer et al., 1986, Donnelley et al., 1991, Elliot et al., 1989, Foster et al., 1990, Fricker et al., 1991, Geissler et al. 1987, Heshka et al., 1990, Heymsfield et al., 1989, Hill et al., 1989, Luke and Schoeller, 1992, Nelson et al., 1992, Rumpler et al., 1991, Wadden et al., 1990, Webster and Garrow, 1989, Welle et al., 1984). Some studies of subjects undergoing food energy restriction have demonstrated that the ratio of resting energy expenditure to fat-free mass decreases compared to that of non-dieting subjects (Bessard et al., 1983, Elliot et al., 1989, Franssila-Kallunki et al., 1992). Other studies have found that there is no change in this relationship (Davies et al., 1989, Foster et al., 1990, Ravussin et al., 1985, Welle et al., 1984). Indeed, in a study by Ravussin and coworkers (1985), the entire attenuation of daily energy expenditure which was associated with weight loss and food energy restriction was explained by 1) changes in body mass and fat-free mass, 2) changes in the thermic effect of feeding as a result of a decreased food energy intake and 3) a reduced thermic effect of exercise as a result of a reduced body mass. Still others have found that during the dynamic phase of food energy restriction there is no linear relationship between the change in resting energy expenditure and the change in fat-

free mass (Keys et al., 1950, Nelson et al., 1992). Interpretation of these relationships may be limited by the ability of the researcher to describe the changes in the constituents of the fat-free mass component of the body, using conventional anthropometric or densitometry techniques. (Tremblay et al., 1986, Weinsier et al., 1992).

There are several factors which may influence the direction of change of the relationship between resting energy expenditure and fat-free mass. Firstly, if subjects were not in energy balance when they were first tested, then a change in the ratio of resting energy expenditure to fat free mass (REE:FFM) with food energy restriction may be masked. For example, in the study by Welle et al., (1984), subjects lost weight while ingesting the baseline diet, and thus, it is likely that any change in the ratio of REE:FFM would have occurred during that period. This is further supported by the work of Fricker et al. (1991), who described the time course of change in REE:FFM with the induction of a very-low energy diet in middle-aged women with a relatively high body mass. The sharpest decrease in the REE:FFM ratio occurred during the first 3 days after the diet was introduced.

It has been suggested that treatments which result in a disproportionate loss of fat-free mass per unit body mass lost would be likely to result in a greater-than-predicted attenuation of resting metabolic rate with food energy

restriction (Forbes et al., 1987). In addition, there is also evidence that the thermic effect of feeding is also attenuated with food energy restriction (Bessard et al., 1983. Davies et al., 1989). However, if subjects are "refed" for weight stabilization following food energy restriction, the thermic effect of feeding either remains the same or increases (Astrup et al., 1990, Nelson et al., 1992). This clearly depends on the individual researchers' criteria for weight "stabilization".

There is some evidence to suggest that "reduced-obese" individuals have a lower energy expenditure than weight-matched controls who have not undergone food energy restriction (Fricker et al., 1991, Geissler et al., 1987, Heshka et al., 1990, Leibel and Hirsch, 1984, Luke and Schoeller, 1992). These data have led researchers to conclude that food energy restriction resulting in significant loss of body mass, results in a disproportionate decrease in resting energy expenditure; a "hypometabolic state" which prevents further weight loss and leads to rapid correction of the weight loss on even partial refeeding.

Despite this, studies have been unable to detect differences in resting energy expenditure and the heat increment of feeding and exercise in groups of individuals with two-fold differences in reported food energy intake (Rose and Williams, 1961). Indeed, in studies of agricultural workers in

developing countries, the reported food energy intake, validated under supervision by trained field workers, was less than 65% of the FAO recommended requirements in some of the subjects (Ashworth, 1968) although there was no evidence for an adaptive change in resting energy expenditure in these subjects. Similarly, Singh et al. (1989) described energy balance in Gambian women, who demonstrated the ability to perform heavy physical labour with an apparent energy deficit of over 5 $MJ \cdot d^{-1}$.

Part of the problem in interpreting results of studies involving deliberate manipulation of food energy for the purpose of weight loss, is that any post-treatment measurements of energy expenditure do not represent a state of energy balance if weight has not stabilized. Studies which have not included a "refeeding" period or a period of true weight stabilization, are simply reporting an acute effect of food energy restriction on energy expenditure (de Boer et al., 1986, Franssila-Kallunki et al., 1992, Fricker et al., 1991, Geissler et al., 1987, Mathieson et al., 1986, Rumpler et al., 1991, Schutz et al., 1987, Welle et al., 1984).

There is evidence to suggest that the metabolic response to refeeding is as variable as that to food energy restriction. In studies of persons undergoing short-term food energy restriction for weight loss, "partial refeeding" is often used in order to establish reduced-weight maintenance (Astrup et

al., 1990, Bessard et al., 1983, Elliot et al., 1989, Foster et al., 1990, Heshka et a., 1990). However, the energy content of the refeeding diet is rarely equal to that of the pre-intervention diet, even after correction for changes in body mass and body energy stores. This fact alone suggests that 1) these individuals are still partially food energy restricted, or 2) these individuals are "energy thrifty".

There are few studies in which the nutritional efficacy of refeeding in anorexia nervosa patients is characterized. Dempsey et al. (1984) studied 4 anorexic patients receiving total parenteral nutrition for a period of 63 $\pm$ 18 days. Energy expenditure was estimated using continuous heart rate monitoring and was found to equal 1.1 times the resting energy expenditure. The mean starting weight in these patients was 29.9 kg, or 52% of the ideal weight-for-height for this age group. These investigators estimated that the energy cost of an increase in body mass of 1 kg varied from 20-60 MJ.

In an earlier study, Walker et al. (1979) found that the energy cost of weight gain in anorexic patients was variable, positively related to the initial degree of undernutrition, and lower in patients with a previous history of overweight. It is likely that the energy requirements for weight gain in anorexic patients depend largely on the composition of the "new" weight, and variations in fluid balance associated with refeeding.

2.2.4. **Undernutrition and refeeding: "cyclic undernutrition"**

More recently, there has been increased recognition of the deliberate practice of "cyclic undernutrition" or "weight cycling". This technique is commonly used by athletes in order to "make weight" for a competition, and by chronic "dieters". In studies of growing rats, there is some limited evidence that multiple cycles of food energy restriction followed by *ad libitum* refeeding result in an increased feeding efficiency (or the change in mass per kJ ingested food energy), an attenuated rate of weight loss in the second or third cycle, increased body fat accretion, increased dietary fat selection, and hyperinsulinaemia (Brownell et al., 1986, Cleary et al., 1986, Reed et al., 1988). However, in the study by Brownell et al. (1986), weight cycling rats did not have greater adipose tissue lipogenic activity, not were there differences in carcass fat, and fat pad mass, when obese weight-cycling rats were compared to obese controls.

In the few studies involving cyclic undernutrition in humans, lack of control and standardization of the definition of weight cycling have made it difficult to interpret the data. For example, Steen et al. (1988) found that collegiate wrestlers who "weight-cycled" had a significant reduction in resting energy expenditure. However, this effect could have resulted simply from acute food energy restriction, and was

unlikely to have been a chronic "adaptation" to weight cycling. Manore et al. (1991) demonstrated slight, but significant, attenuation of the thermic effect of diet, plus exercise, in dieters compared to weight-stable controls. In an unpublished study (McQuaide et al., in preparation), the investigators controlled for the "phase" of the weight cycle. For example, individuals who practiced weight cycling were selected on the basis of being at their highest weight, or lowest weight, and were compared to weight-stable, weight-matched controls. In this study, there were no differences in resting energy expenditure between weight-stable persons and weight-cyclers and only small differences between these groups in the thermic response to glucose-feeding.

Thus, in humans, it is widely accepted that a period of food energy restriction, followed by *ad libitum* food energy intake will be characterized by an increased efficiency of food energy utilization and positive energy balance. But, there is little evidence to suggest that these effects persist, or become exacerbated with repeated weight cycling.

2.2.5. **"Overfeeding": effects on energy balance and body energy stores**

In the early 1900's, it became clear from research on overfeeding in individual scientists (Gulick et al., 1922,

Norgan and Durnin, 1980) that not all of the excess food energy ingested during overfeeding was deposited as tissue or resulted in increased body energy stores. The efficiency with which excess ingested food energy is stored depends on a number of factors, including: antecedent nutritional status (Rothwell and Stock, 1982, Sims et al., 1973), heredity (Bouchard et al., 1990), the nutrient composition of the diet (Dallosso and James, 1984, Schutz et al., 1985) and the developmental period during which overfeeding occurs (Rothwell and Stock, 1982, Wainright et al., 1987). The "energy wastage" associated with overfeeding, which results in variable increases in body energy stores was termed "luxuskonsumption" by Neumann in 1902 (cited by Ravussin et al., 1985).

It is unclear whether the increase in total daily energy expenditure associated with overfeeding is strictly a result of the increase in body energy stores and an increased thermic effect of feeding directly proportional to the increased food intake, or whether it is attributable to a decrease in the efficiency of food energy utilization or increased levels of physical activity (Ravussin et al., 1985).

In 1955, Passmore and coworkers found that 3 overfed, young men "absorbed" 90% of the excess energy which was ingested from 10-14 days of overfeeding. They reached this conclusion on the basis of changes in body weight, faecal energy and

nitrogen losses. Thus, the small increase in daily energy expenditure was explained by the gain in mass and the increased thermic effect of feeding. These findings were later supported by the work of Ravussin et al. (1985), who overfed 5 young men for 9 days, with an excess of nearly 8.5 $MJ \cdot d^{-1}$. In this study, the investigators estimated that over 75% of the excess energy ingested was stored, with the remainder accounted for by increased basal metabolic rate and thermic effect of feeding and activity.

However, the conclusion that the energy stored is proportional to the excess energy ingested depends on an accurate assessment of the constituents of body energy stores as well as the energy equivalents used for a gain in mass of fat and protein, respectively. For example, in a study by Forbes et al. (1986), 15 students were overfed for a period of 3 weeks and gained between 3.5-5.8 kg. Fifty-one percent of this gain in mass could be accounted for by increased fat-free mass. Forbes et al. (1986) regressed the change in mass against total excess energy consumed in combined data from this study sample and 33 subjects from previous studies, in which weight maintenance was confirmed, at least 100 MJ excess energy was consumed, food intake was monitored, and body composition was assessed. From this regression, they were able to estimate that the average cost of weight gain was 33.7 $kJ \cdot g^{-1}$, and that 38.4% of the gain in mass was accounted for by a change in fat-free mass.

There is indirect evidence that the antecedent nutritional status influences the energy equivalent or efficiency of weight gain. For example, chronically undernourished individuals demonstrated no increase in daily energy expenditure, measured by doubly-labelled water, even after 8 weeks of overfeeding, and a gain in mass ranging from 0.6 kg to 3.8 kg (Riumallo et al., 1989).

Finally, there is strong evidence that the individuals' genotype exerts considerable influence on the efficiency of weight gain in response to overfeeding, and the constituents of the change in mass (Bouchard et al., 1990, Poehlman et al., 1986, Tremblay et al., 1987). In a recent study by Bouchard et al. (1990), 12 sets of monozygotic twins were overfed for 100 days. The largest gain in mass was 13.3 kg in an individual in whom there was little evidence for "luxuskonsumption", whereas, the individual gaining the least weight (4.3 kg) stored only 40% of the excess energy which was ingested. Despite this wide individual variation in feeding efficiency following overfeeding, the between-twin variability in the response to overfeeding was very low. Thus, there is a genotype-dependent influence in feeding efficiency which accounts for a significant amount of the variability in this response in freely-eating persons ingesting a "Western" diet.

2.2.6. **Nutrient composition of the diet: effects on energy and nutrient balance**

It is apparent that the energy expenditure associated with the ingestion of various foodstuffs depends largely on their metabolic fate. Flatt (1978) has estimated that the energy cost for the storage and remobilization of dietary fat is approximately 7% of the ingested fat energy, whereas, the energy cost for protein synthesis and storage is approximately 24% of the ingested energy. The estimated energy cost of *de novo* lipogenesis from carbohydrate ingestion is 23% of the ingested energy, in comparison to the oxidative disposal of carbohydrate, for which there is a net energy gain, and the storage of dietary carbohydrate as glycogen, for which the energy cost is approximately 5% of the ingested energy.

The maintenance of energy balance, by definition, depends on the **average** daily matching of energy intake with energy expenditure, as well as the proportional oxidation of carbohydrate, fat and protein, relative to the contribution of these macronutrients to total energy intake. Flatt (1987) has proposed that carbohydrate intake and oxidation are tightly regulated as a result of the smaller endogenous reservoir of carbohydrate. The opportunity for storage disposal is therefore minimal. However, fluctuations in dietary fat intake theoretically do not result in the same rapid oxidative disposal as with carbohydrate. For this reason, Flatt (1985,

1987) studied the relationship between dietary fat intake, fat balance and changes in energy balance in both humans and rats.

In the first study, healthy young men were fed one of three single meals including: low fat (2 MJ), high fat (long-chain triglycerides, 3.6 MJ), or high fat (medium-chain triglyceride, 3.6 MJ). Post-prandial energy expenditure and substrate balance were measured for 9 hours. As expected, post-prandial energy expenditure was higher following the meals which were higher in energy content. However, the thermic effect of the meals, expressed relative to the energy content ingested were not different between trials. There was no difference in the post-prandial respiratory exchange ratio between the low-fat meal and the high-fat meal (long-chain fatty acid constituents). Thus, under the high fat conditions, fat balance was substantially positive during the 9 hour period following feeding.

In studies on rats ingesting either a low fat (13%) or a high fat (45%) diet, Flatt (1987) found that a ratio between RQ and FQ (food quotient, ratio of CO_2 produced and O_2 consumed from the complete oxidation of the nutrient composition of diet) greater than 1 was associated with positive energy balance, while an RQ:FQ ratio of less than 1 was associated with a negative energy balance. Thus, when rats were oxidizing more fat than they were ingesting, independent of total energy intake, they tended to be in negative energy balance.

Thomas et al. (1992) studied 11 weight-stable low mass persons and 10 weight-stable high-mass persons in a definitive study of substrate balance in response to short-term perturbations in dietary carbohydrate and fat content. Diet composition was adjusted during two, 2-week feeding periods to yield a high-carbohydrate diet (FQ greater than 0.04 units higher than baseline RQ) or a high-fat diet (FQ more than 0.04 units lower than baseline RQ). *Ad libitum* food energy intake was higher across both groups of subjects when ingesting the high-fat diet, compared to the high-carbohydrate trial. There was a strong relationship between 24-hour carbohydrate intake and carbohydrate oxidation in both groups during the high-carbohydrate trial. However, during the high-fat trial, the obese subjects did not demonstrate any relationship between fat intake and fat-oxidation and were apparently in positive fat balance. These findings provide indirect evidence that substrate balance might be implicated in the control of energy balance and may be associated with the development of obesity.

In a similar study by Tremblay et al. (1989), subjects demonstrated significant hyperphagia when FQ was less than 0.85 (high fat intake). The authors concluded that the hyperphagia was related to the amount of ingested carbohydrate which was required to maintain carbohydrate balance in these subjects. Lissner et al. (1987) also demonstrated that a

high fat intake was associated with an increased food energy intake and subsequent gain in body mass.

In a cross-sectional study of 155 middle-aged men, Dreon et al. (1988) provided indirect support for the effect of a high-fat diet on body energy stores. In this sample, body fat was significantly related to reported dietary fat intake, independent of total energy intake.

Thus, in short-term studies of perturbations in energy balance, a high fat intake coupled with a lower rate of fat oxidation results in positive energy and fat balance in both rats and humans.

2.2.7. **Feeding patterns: effects on energy expenditure**

Evidence from animal studies suggests that meal feeding results in a greater efficiency of weight gain than isocaloric "nibbling". In a landmark study by Rothwell and Stock (1979), rats were tube-fed a portion of their daily energy intake (up to 75% as a single bolus) and the remainder of the diet by *ad libitum* eating. The energy intake of the tube-fed rats was between 95% and 100% of that of control, *ad libitum*-fed rats, and yet, body weight gain was nearly double that of controls, over a 20-30 day period. Subsequent studies suggest that the increased efficiency was associated with a decrease in brown

adipose tissue thermogenesis (Rothwell et al., 1984). However, it is also possible that feeding efficiency was influenced by changes in patterns of physical activity and torpor following meal feeding (Djazayery, 1987).

Increased efficiency of weight gain associated with meal feeding when compared to "nibbling" was found in a study by Dallosso et al. (1982) in which men ate either 2 or 6 meals each day for 2 weeks while maintaining a constant level of physical activity. Conversely, in a cross-sectional study of 155 middle-aged men, Dreon et al. (1988) found no relationship between the number of meals eaten each day, the distribution of energy intake within the meals, body composition and body weight.

de Groot et al. (1989) manipulated the food intake pattern, during food energy restriction in 27 women selected for "overweight", in an attempt to attenuate the decline in resting metabolic rate associated with dieting. Each woman was assigned to one of three slimming regimens, including: continuous energy intake (50% of maintenance diet), alternating intake (bread and water, followed on a subsequent day by 100% maintenance diet), and alternating intake (alternating 50% and 100% previous maintenance diet).

Regardless of the dietary pattern, daily energy expenditure decreased proportionately with changes in mass, and fat-free

mass, and was associated with the degree of food energy restriction. Thus, alternating low energy intake with previous food intake does not attenuate the decline in resting metabolic rate as a result of food energy restriction.

Meal feeding in the growing rat results in a marked increase in efficiency of weight gain and a rapid accretion of body weight and body fat. However, the results of meal feeding and alternate days of low or normal food energy intake in humans are equivocal, and may be related to whether or not the subjects were in energy balance prior to the initiation of the trial.

2.2.8. **The effect of a single bout of exercise on energy balance**

A single bout of exercise may vary from a gentle 30-minute walk to an ultra-marathon run of more than 80 km. The energy cost of these exercise bouts, for a man or woman weighing 70 kg, would be approximately 700 kJ and 28,000 kJ, for the walk and run, respectively. Thus, the thermic effect of physical activity may account for as little as 15% of the daily energy expenditure, to as much as 50%, or more, of total daily energy expenditure. Indeed, a recent study has demonstrated that spontaneous physical activity or "fidgeting" (movement in which there is little change of centre of gravity) of

individuals in a respiration chamber may account for between 0.6 and 2.8 $MJ \cdot d^{-1}$ (Ravussin et al., 1986).

There are several mechanisms by which exercise is thought to influence energy balance, apart from the energy expenditure associated with the work bout itself. A single bout of exercise has been shown to result in a persistent elevation of oxygen uptake for up to 12-18 hours post-exercise (Bahr et al., 1987, Bielinski et al., 1985, Maehlum et al., 1986). This excess post-exercise oxygen consumption (EPOC) has been associated with a concomitant increase in circulating catecholamines, an increased core temperature and an increased rate of triglyceride-fatty acid substrate cycling in the post-exercise period (Bahr et al., 1987, Bahr et al., 1990, Bahr et al., 1991). More recently, the EPOC has been dissociated from changes in rectal temperature in the post-exercise period (Lambert et al., 1992).

However, the magnitude and duration of the post-exercise effect on energy expenditure remain controversial. Few studies have been able to demonstrate any significant elevation of oxygen uptake beyond the immediate post-exercise period when the duration of the exercise was less than 60 minutes (Freedman-Akabas et al., 1985, Sedlock et al., 1989). Only exercise at high intensities, lasting more than 60 minutes has been shown to result in any persistent increase in energy expenditure over a 12-hour, post-exercise period. In a

recent study by Gore and Withers (1990), the intensity of exercise (30% vs 70% maximal aerobic capacity) accounted for 5 times more variance in the 8-hour EPOC, than the duration of exercise (20 min, vs 50 min, or 80 min).

The metabolic sequelae following an acute bout of exercise may also indirectly affect the maintenance of energy balance. For example, Heath et al (1983) found that a single bout of exercise, in previously-trained persons, was sufficient to increase target tissue sensitivity to insulin and oral glucose tolerance. Increased tissue sensitivity to insulin may indirectly increase the thermic effect of feeding (Ravussin et al., 1983, Ravussin et al., 1985). In addition, acute exercise increases adipose tissue lipoprotein lipase activity and epinephrine-stimulated lipolysis, thus, increasing the availability of free fatty acids from circulating triglycerides and adipose tissue stores for fat oxidation (Savard et al., 1987).

Segal et al. (1985), studied the interaction between acute exercise and the thermic effect of feeding in weight-matched, men with a low- and high-fat-free mass. In that study, the effects of mixed-meal feeding and acute exercise on the thermic effect of exercise and feeding were additive in the lean subjects. However, there was little effect of mixed-meal feeding on the thermic effect of exercise in the "obese" subjects.

Thus, acute exercise may have both direct and indirect effects on energy balance, which are dependent on the exercise intensity, and may be influenced in comparison between groups by factors such as fat-free mass.

2.2.9. **The effect of exercise training on energy balance**

Exercise training may perturb energy balance directly, by increasing one's daily energy requirement for weight maintenance, or indirectly, through changes in body composition or changes in the type of substrate oxidation.

In any attempt to quantify the effects of exercise training on energy balance, cognizance must be taken of the great "motor individuality" which exists. In both cross-sectional and even some longitudinal studies, the effect of exercise training on the direct and/ or indirect control of energy balance is highly variable. For example, in a study by Lambert and Noakes (1990), groups of rats which were allowed to exercise spontaneously in wheel cages demonstrated as much as a 12.5-fold difference in weekly training distance. Despite this variability in activity, body mass was not different between groups.

In a similar example, Edholm et al. (1970) quantified the weekly energy expenditure and food energy intake in young military recruits undergoing basic training. From this sample, there were examples of individuals expending between an estimated 12.5 and 18.8 MJ per day during the course of 1 week, while ingesting between 8.5 MJ and 27.2 MJ per day. There was no relationship between the food intake and energy expenditure on the same day.

The energy expenditure associated with exercise training may result in a net energy deficit and a change in body energy stores. In the exercising rat model, there are sex-specific differences in body mass and body fat changes with exercise training. In female rats, food energy intake increases during training, resulting in a compensatory maintenance of body mass (Oscai et al., 1973). Conversely, training results in a significant reduction in fat cell size, body fat and body mass in male rats (Booth et al., 1974, Kral et al., 1974, Oscai et al., 1974).

In a novel study, McMurray et al (1985) compared the consequences of energy deficit induced by exercise vs a comparable deficit induced by food energy restriction, in endurance-trained persons. Six endurance-trained men underwent 1 week of baseline stabilization, during which they ingested 150 $kJ \cdot kg^{-1} \cdot d^{-1}$, and were instructed to refrain from

any exercise other than activities of daily living. Following this period, subjects exercised daily at 80% of maximal aerobic capacity for one week, in order to achieve an energy deficit of approximately 4.2 $MJ \cdot d^{-1}$. Food energy intake was held constant. After a two-week "wash-out" period, and another baseline period, subjects were asked to restrict exercise to activities of daily living, while ingesting 85 $kJ \cdot kg^{-1} \cdot d^{-1}$. Total weight loss was significantly greater during the period during which the energy deficit was induced by dieting, compared to the exercise period (2.16 $\pm$ SEM 0.19 kg vs 0.76 kg $\pm$ SEM 0.28 kg). However, nitrogen balance was significantly less negative during the exercise period compared to the period of food energy restriction, suggesting that the differences in weight loss may be explained, in part, by changes in fat-free mass.

Klesges et al. (1991) devised a more indirect way of quantifying the effect of exercise training on energy balance. In this study, 294 men and women completed questionnaires regarding food intake and physical activity (Baecke physical activity scale) once each year for three years. Height and weight were measured at each visit and a multiple regression analysis was used to identify predictors of 2-year changes in body mass. In both men and women, dietary fat intake was a significant longitudinal predictor of subsequent weight gain, and in women, work and leisure activity were significantly associated with a loss of weight over a two-year period.

Conversely, a high initial level of sporting activity predicted a gain in mass over two years in men. Thus, the women demonstrated the expected relationship between energy expenditure in the form of work and leisure activity, and energy balance, whereas the men who were reportedly more active actually gained weight over the two year period. It is not clear whether this discrepancy is a result of some behavioural difference, or simply a result of the inability to account for changes in fat-free mass.

Exercise training in both humans and laboratory rodents has been associated with a reduction in adipocyte size, increased mobilization of endogenous lipid stores, enhanced tissue insulin and catecholamine sensitivity, and biochemical and morphological changes in skeletal muscle (Craig et al., 1983, Krotkiewski et al., 1983).

There is conflicting evidence as to whether regular physical training results in an elevation in resting energy expenditure, which is not simply a residual effect of a previous exercise bout. Tremblay and co-workers (1986) studied a cross-sectional sample of lean and obese, trained and untrained individuals. After regressing absolute resting energy expenditure against fat-free mass, they concluded that for a given unit of fat-free mass, resting energy expenditure was higher in trained persons.

Conversely, Broeder et al. (1992a, 1992b), Sharp et al. (1992) and Schulz et al. (1992) were unable to demonstrate any relationship between training and level of fitness (as measured by maximal oxygen uptake) and resting metabolic rate, expressed per unit fat-free mass. These investigators concluded that resting metabolic rate was not higher in trained persons, when compared to untrained, age-matched controls. However, the convention of expressing resting energy expenditure per unit fat-free mass is based on the assumption that the metabolic activity of fat-free mass, per unit fat-free mass is not different between trained and untrained persons.

There is some evidence that exercise training results in "energy sparing" in response to thermogenic stimuli such as exercise and feeding. For example, exercise training is associated with attenuated catecholamine secretion and oxygen uptake during standard submaximal exercise (Koivisto et al., 1982, Kjaer et al., 1989, Lambert and Noakes, 1990). In studies by Tremblay and coworkers, it has been repeatedly demonstrated that highly-trained runners have a lower thermic effect of glucose and mixed-meal feeding compared to age-matched, untrained controls (Tremblay et al., 1985, Tremblay et al., 1986, Poehlman et al., 1988). In addition, in a recent study by Gilbert et al. (1991), exercise training resulted in an attenuated rise in oxygen uptake to a combined stimulus of exercise and feeding, when compared to untrained

controls. However, in studies by Davis et al., (1983) and Hill et al. (1984), the thermic effect of feeding was enhanced with exercise training. The underlying cause for these differences is not clear. There are no studies which have examined the evolution of this response longitudinally, before and after an exercise training programme. Longitudinal studies would provide additional information, but would likely be confounded by changes in body mass, body fatness and standardization of the measurement period following the last bout of exercise.

2.2.10. **Exercise training and food energy restriction: evidence for attenuation of a decline in resting energy expenditure**

It has also been demonstrated in some studies, that when energy deficit is induced by combining exercise training with food energy restriction, the percentage of mass lost as fat is greater than when compared to food energy restriction alone (Hill et al., 1985). Thus, it has been proposed that exercise training may attenuate the previously-described "hypometabolic" state which results from food energy restriction by conserving fat-free mass and thus, indirectly, increasing the metabolic rate.

Most studies which have compared the effects of food energy restriction to those of exercise training combined with food

energy restriction on resting and total daily energy expenditure in individuals before and after weight reduction have found little difference between treatments (Donnelly et al., 1991, Henson et al., 1987, Heymsfield et al., 1989, Hill et al., 1989, Lennon et al., 1985, Tremblay et al., 1985, Wadden et al., 1990). Results from studies in which exercise training in combination with food energy restriction has been found to attenuate the decline in metabolic rate associated with food energy restriction alone may be influenced, in part, by residual effects from the previous exercise bout (Belko et al., 1987, Donahoe et al., 1984, Mole' et al., 1989). Moreover, in at least one study, exercise training in combination with very-low energy intake exacerbated the decrease in resting metabolic rate (Phinney et al., 1988).

Differences between the results of previous studies may be attributed, in part, to 1) lack of control of the last bout of exercise, 2) variable levels of food energy restriction and adherence, 3) intensity and duration of exercise training, and 4) the initial energy balance status prior to intervention.

2.2.11. **Detraining or the cessation of exercise training: effect on energy balance**

There is much anecdotal evidence in former athletes and sportsmen and women, that the cessation of physical training

perturbs energy balance. Detraining has been shown to result in rapid fat accretion and weight gain in both humans (Parizkova, 1977) and in rats (Applegate et al., 1984, Applegate and Stern, 1987, Arnold and Richard, 1987, Craig et al., 1983, Dohm et al., 1977, Sandretto and Tsai, 1988). However, the increased effciency of weight gain demonstrated with the cessation of training is probably not simply a result of removal of the exercise stimulus. Previously-trained, growing rats which are detrained, gain weight more rapidly than sedentary controls. The mechanism for this effect has not been elucidated.

In addition, in previous animal studies of detraining, there has been a concominant increase in food energy intake, especially in rats genetically predisposed to obesity or those fed a high-fat diet (Applegate et al., 1984, Applegate and Stern, 1987, Arnold and Richard, 1987, Sandretto and Tsai, 1988). This has made it difficult to separate the effects of stopping training from those seen with refeeding or overfeeding.

2.2.12. **Evidence for differences in the response to perturbations in energy balance in rats vs humans**

There is evidence to suggest that the regulation of energy balance and the response to perturbations in energy balance in

rats is different to that of humans. Perhaps the most significant differences in responses to various thermogenic stimuli between rodents and humans may be attributed to brown adipose tissue thermogenesis.

The metabolic activity of brown adipose tissue is largely regulated by norepinephrine secreted by sympathetic nerve endings. Norepinephrine secretion results in breakdown of brown adipose tissue triglyceride stores. The resultant increased fatty acid concentration has been implicated in the control of uncoupling of the proton-conductance mechanism in brown adipose tissue mitochondria. In human adults and infants, norepinephrine infusion has been shown to result in a 30-60% increase in metabolic rate. Conversely, norepinephrine infusion in rats may result in as much as a 300% increase in metabolic rate (Flier and Underhill, 1984).

Differences in the norepinephrine-stimulated response to food intake may be responsible, in part, for differences in dietary-induced thermogenesis in trained rats vs trained humans. Studies by Gleeson et al. (1982), Hill et al. (1983) and MacDonald et al. (1988) found that exercise training in rats resulted in an enhanced thermogenesis in response to feeding. Conversely, studies by Tremblay and coworkers (LeBlanc et al., 1984, Tremblay et al., 1983) have consistently demonstrated a blunted thermogenic response to feeding in exercise-trained humans.

Differences in efficiency of fuel utilization between rats and humans have been demonstrated in the studies of Pullar and Webster (1977) and Forbes et al. (1986). Pullar and Webster (1977) used indirect calorimetry to estimate the cost of protein and fat deposition in growing rats, and found the energy cost of depositing 1 g of protein or 1 g fat was equal to approximately 53 kJ of metabolisable energy per g. Conversely, Forbes et al. (1986) estimated that the average cost of weight gain in "overfeeding" humans was considerably less, 34 kJ metabolisable energy per g tissue.

These studies highlight a few of the methodological problems in extrapolating information on the regulation of energy balance from rat to human studies. Moreover, most rat studies are conducted on rats during a dynamic phase of growth, while most human studies involve individuals who are in a state of relative energy balance.

2.3. **Summary: Energy requirements for maintenance of energy balance and changing energy status**

The significant positive relationship between fat-free mass or body cell mass and resting energy expenditure has been demonstrated in studies of freely-eating, high-mass and low-mass men and women who are weight-stable and considered to be

in energy balance (Astrup et al., 1990, Cunningham et al., 1980, Foster et al., 1990, Keys et al., 1950, Owen et al., 1987, Ravussin et al., 1986, Tzankoff and Norris 1977, Warwick et al., 1988, Welle et al., 1992). Indeed, in a study of nearly two-hundred Pima Indians, over 83% of the variance in resting energy expenditure was explained by differences in fat-free mass alone (Bogardus et al., 1986).

For this reason, attempts have been made to standardize (or "normalize") resting energy expenditure in groups with widely varying body size and body composition, by expressing energy expenditure relative to kg fat-free mass. However, it is only possible to standardize energy expenditure for differences in fat-free mass if 1) this relationship is linear and 2) if the y-intercept of this relationship is not different from zero.

Recent studies clearly demonstrate that these two conditions cannot be met. Ravussin and Bogardus (1989) repeatedly demonstrate the effect of a non-zero y-intercept on the standardization of resting energy expenditure in persons of differing size and body composition. Moreover, a recent study by Weinsier et al. (1992), in which the slopes of the regression between REE and FFM were compared between infants, adolescents and adults, suggests that the relationship between resting energy expenditure and fat-free mass is, in fact, curvilinear. Thus, under conditions where fat-free mass is likely to be changing, the standardization of resting energy

expenditure per unit of fat-free mass may be difficult to interpret.

The adjustment in resting energy expenditure in response to a perturbation in energy balance has been described as a "useful adjustment to altered circumstances" (Keys et al., 1950). Thus, by implication the relationship between resting energy expenditure and body size must change with food energy restriction and weight loss, or there would be no evidence for adaptation. Moreover, it is apparent that not only is body size and metabolic body size reduced with food energy restriction, but the metabolic activity of the body cell mass is also likely to be reduced (Keys et al., 1950, Luke and Schoeller, 1991, Weinsier et al., 1992).

This change in metabolic activity may be related to a change in the constituents of the body cell mass. For example, there is an increase in total body water which is associated with refeeding following severe food energy restriction. Thus, the metabolic rate per unit fat-free mass under these conditions would not reflect "true" metabolic activity. On the other hand, a reduction in muscle mass, core temperature, or thyroid hormone concentrations may also increase metabolic efficiency or decrease daily energy expenditure.

An enhanced efficiency of weight gain has been consistently demonstrated in growing rats during refeeding following food

energy restriction (Boyle et al., 1981). Furthermore, there is selective evidence for that "reduced-obese" humans have an enhanced energy "thriftiness". However, in humans it may be largely a matter of the "chicken and the egg" in separating the genotype-dependent thriftiness from an adaptation to a chronically-low food energy intake.

In a recent theoretical review, Alpert (1990) suggested that it is difficult to predict the amount of food energy required to maintain energy balance or to elicit a gain in body energy stores. He has proposed the following equation to predict the rate of change in body energy stores.

$$Á\ df/dt + ß\ dl/dt = EP - REE(l,\ g) - Š\ (l+f)$$

where: Á = energy density of fat body mass,

ß = energy density of lean body mass,

E = fraction of ingested energy available (less TEF, faecal and urine losses)

P = food energy input,

REE = resting energy expenditure which is a function of FFM (l) and growth (g),

Š = activity coefficient,

(l+f) = total mass

It is the aim of the present dissertation to evaluate these relationships in dynamic models of short-term perturbations of energy balance, and to relate these findings to the existing models. Moreover, this dissertation will provide insight into the nature of the signals which result in both short-term and chronic adaptation to perturbations in energy balance.

CHAPTER 3

THE INTERACTION OF PRE- AND POST-WEANING NUTRITION ON GROWTH, BODY COMPOSITION AND FEEDING EFFICIENCY IN LONG-EVANS RATS

Introduction

Traditionally, pre-weaning litter size manipulation has been used as a model to induce long-term adaptations in the rate of growth and fat deposition in various rat strains (Bassett and Craig, 1988, Eisen and Leatherwood, 1978a, Faust et al., 1980, Harris, 1980a, 1980b, Johnson et al., 1973, Knittle et al., 1968, Oscai and McGarr, 1978, Wainright and Francey, 1987a, Wainright et al., 1987b, Winick and Noble, 1966, Wurtman and Miller, 1976). Results from previous studies suggest that rats raised in small and average litters grow faster during both the pre- and post-weaning periods than do rats raised in large litters (Eisen and Leatherwood, 1978a, Harris, 1980a, Johnson et al., 1973, Knittle et al., 1968, Oscai and McGarr, 1978, Wainright and Francey, 1987a, Wainright et al., 1987b, Winick and Noble, 1966).

There are two apparent mechanisms by which pre-weaning under- or over-nutrition may influence post-weaning growth and body composition. Firstly, there is evidence for the existence of critical periods of adipocyte proliferation, especially in early post-natal life (Hirsch and Knittle, 1969, Ravelli et al., 1976). In rats, it has been observed that 1) infantile undernutrition results in some degree of transient growth retardation, and 2) increasing the number of pups per litter during suckling is associated with a reduced adult body mass and adipose tissue DNA content, suggesting a decreased fat cell number (Winick and Noble, 1966).

Secondly, in some studies, pre-weaning litter size appears to influence post-weaning food intake. The lower food intake during suckling appears, therefore, to be carried over into the rest of the juvenile period. Hence, it has become accepted that pre-weaning litter size manipulation pre-programmes appetite throughout maturation and alters post-weaning growth patterns (Oscai and McGarr, 1978, Winick and Noble, 1966). However, this effect is highly variable, with strain- and gender-specific responses (Bassett and Craig, 1988, Harris, 1980b, Wainright et al., 1987b, Wurtman and Miller, 1976,).

There is also evidence to suggest that animals may compensate for temporary disturbances in food intake during the growth period by increasing feeding efficiency. Hirsch and Han (1969) demonstrated that starvation arrested fat cell replication in rats during the early post-weaning period. However, adipocyte number was restored to that of control animals following *ad libitum* refeeding.

An increased feeding efficiency has also been demonstrated in rats ingesting a high-fat diet in the pre- and post-weaning period (Levin et al., 1983a, Levin et al., 1983b, Levin et al., 1986, Schemmel et al., 1970, Wainright and Francey, 1987a, Zaragoza-Hermans et al., 1984). Adult rats fed a high-fat diet and rats fed a high-fat diet in the early post-weaning period deposit more body fat and are more energetically efficient than chow-fed rats.

Therefore, this study was designed to address the relative importance of both pre- and post- weaning nutritional manipulation on feeding efficiency and changes in body mass and body composition in rats during the initial 5 month period of growth and maturation. This was achieved by pre-weaning litter size manipulation and post-weaning alterations in the nutrient content of the diet. These treatments have the potential for changing both the total energy and the nutrient densities of food intake in ad libitum-fed rats.

This study examined the long-term effects of early relative under- or over-nutrition on subsequent energy requirements or "thriftiness" for growth and fat accretion during *ad libitum* refeeding in Long-Evans rats.

Materials and Methods

Animals and feeding: Twelve prima gravida female Long-Evans rats bred in the animal facility at the University of Cape Town Medical School were each placed with two males from the same facility for the purposes of mating. The females which were impregnated were then separated from the males and housed individually in a controlled thermoneutral environment (20-22°C) with a 12/12 hour light/dark cycle. The study was approved by the Ethics and Research Committee of the University of Cape Town. The pregnant females (dams) were fed standard laboratory "chow" (Epol Rat Cubes, Epol (Pty) Limited, Maitland, South Africa) *ad*

libitum. The composition of the food is given in Table 3.1. No attempt was made to quantify the intake of these rats.

Table 3.1. Composition (% w/w) of Epol Rat Cubes for the standard "chow" diet, as supplied by the manufacturers (Epol (Pty) Ltd., Maitland, , Cape Town, South Africa).

Energy content	: 12.75 MJ DE (digestible energy)·kg^{-1}
Moisture content	: 11.0%
Crude Protein content	: 20.5%
Ash content	: 7.0%
Fat content	: 5.0% (10% pure Sunflower oil)
Carbohydrate content	: 56.5% (52.64% soluble starches and sugars, 3.86% crude fiber such as: cellulose,hemi-cellulose, and lignin)

Dams produced litters with a mean size of 12 pups. Dams were randomly selected to rear "small" (each with 4 pups), "normal" (11-14 pups each) and "large" (17-23 pups each) suckling groups. This was achieved by immediately re-assigning all pups from litters born on the same day to foster mothers to form suckling groups of different sizes. Pups were rolled in the bedding of the new mother before being transferred in order to minimize the risk of rejection. Dead pups were not replaced in litters, and final litter sizes for each group were as follows: small litters, N = 4 pups, normal litters, N = 11-13 pups, and large litters, N = 14-19 pups. Groups ("litters") were weighed every other day for the duration of the suckling period.

Pups were weaned at 23 days and groups of 3-5 pups from the same litter were placed in suspended wire cages. Animals were randomly assigned to one of two feeding groups: standard laboratory chow, or a supplemented mixed-fat diet. This diet has previously been shown to result in diet-induced obesity (Levin et al., 1983, Levin et al., 1983b). Both groups were allowed to feed *ad libitum*. After 2 weeks, the pups were separated and pups from the same litters were randomly assigned to be housed as same-sex pairs, or, in some instances, housed singly. Thus, for the remainder of the feeding period, there were 40 pairs, and 16 singletons (Total n for statistical analyses = 56).

An excess of food was always available for consumption. The composition of each diet treatment is described in Table 3.2. Food consumption was calculated by measuring the difference between weighed portions and the uneaten food over a 48-hour period. This included food remaining in the cages as well as spillage onto wire-mesh screens situated below each cage. Food and faecal matter were separated by hand.

Table 3.2. Composition of control (C) and experimental mixed-fat diet (44% condensed milk, 9% sunflower oil, and 47% chow).

		Control	Mixed-fat
Carbohydrate	(% energy)	62.2	50.2
Protein	(% energy)	24.3	13.2
Fat	(% energy)	13.5	36.6
Energy	($kJ \cdot g^{-1}$)	12.6	15.3

Body mass was measured at post-weaning weeks 1, 2, 4, 6, 8, 10, 12, 14 and 18. Food intake was only measured at 4, 8, 12, and 18 weeks following weaning. Gross feeding efficiency was calculated as the change in mass (mg) per kJ food energy ingested (mg body mass · kJ food energy^{-1}). Thus, feeding efficiency at week 8 is based on the change in mass from weeks 4 to 8, and the measured food energy intake at week 8. Mean body mass, and change in mass were calculated for each pair of rats. Total food intake for each pair housed together was halved and expressed as kJ intake per rat.

Fat pad and carcass fat analyses:

Carcass fat and water content (g · 100 g wet wt $^{-1}$) were measured in randomly selected samples from each group of pups just prior to weaning (total n = 16), and in mature rats from large and small litters after 4 months of dietary manipulation (11 males and 6 females). Compositional analyses were performed as previously described for total carcass fat and water determination (Atkinson et al., 1972, Clark and Tarttelin, 1976, Treadway et al., 1986).

Carcasses were shaved and weighed before being placed in large glass beakers and autoclaved for 2 hours at 100-120°C and 100 kPa. Carcasses were diluted 4:1 (water:carcass, w/w) and ground in a Waring blender to a uniform consistency. Aliquots were then added to pre-weighed evaporating dishes and dried for 24 hours at

70°C. Samples were weighed and dried until a constant weight was achieved, and water content was calculated. Lipid determinations were performed on dry homogenate by extraction with chloroform: methanol:water (1.0:1.0:0.9, v/v) solution. Chloroform-phase aliquots were dried to a constant weight and total carcass lipid content was estimated gravimetrically.

Epididymal fat pad mass was measured in male rats from large and small litters, ingesting either the control diet or the mixed-fat diet, at 18 weeks post-weaning. Interscapular fat mass was measured similarly in male and female rats.

Statistical analyses:

Pre-weaning body mass was determined by weighing all pups belonging to a litter group together. Therefore, comparisons for body mass between litter groups over the pre-weaning period were made using a Mann-Whitney U test. Body composition at weaning was compared using one-way analyses of variance (ANOVA) and where significant F-ratios were found, Tukey's post-ANOVA test was performed to determine differences between group means.

All feeding data were expressed as means $\pm$ standard errors of the mean for 4, 8, 12 and 18 weeks of dietary manipulation. The change in mass and feeding efficiency were calculated for the period between each measurement.

Initial comparisons were made for the effects of gender on body mass, rate of change in body mass, food intake and feeding efficiency, using one-way analyses of variance. Gender groups were then considered separately. Analyses of covariance were used to determine the effects of diet composition and litter size on body mass and food energy intake, covarying for starting mass, the number of rats housed per cage, respectively. Following this, the effects of diet and litter size on the rate of change in body mass and feeding efficiency were compared using two-way analyses of covariance, after covarying for the number of rats housed per cage.

Body composition and fat pad mass were compared using two-way analyses of variance.

Results

Pre-weaning:

There were no differences in body mass during the first week of suckling between groups. However, body mass was significantly greater in pups from small litters than in pups from large litters from day 7 through 23 ($p < 0.025$; Figure 3.1a). The differences in mass between pups from normal vs the two experimental groups (large- and small- litters) were not significant. Small-litter pups had a significantly greater carcass fat content than pups from normal and large litters

($p < 0.0005$; Figure 3.1b). Pre-weaning water content was significantly lower in small litter pups when compared to pups from both large and normal litters ($p < 0.03$).

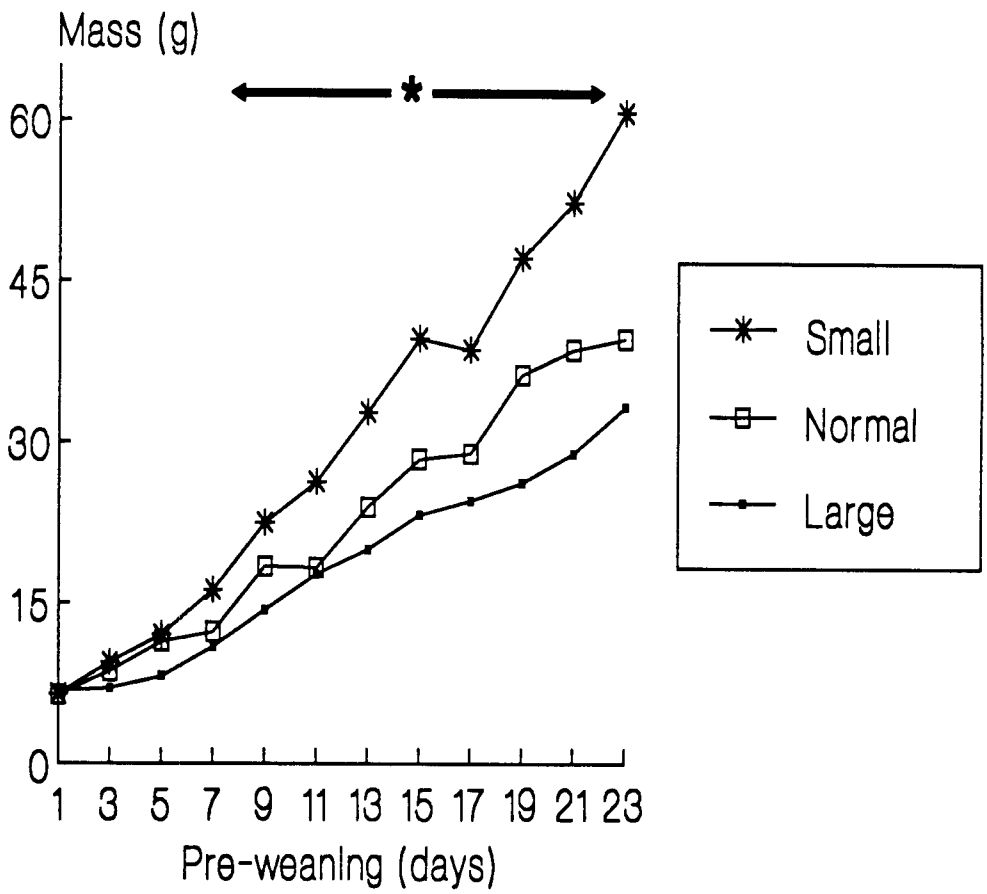

Figure 3.1a. Changes in mean body mass during 3 weeks of suckling in Long-Evans pups which were redistributed to foster dams to form three litter size groups, small (n=4), normal (n=11-13) and large (n=14-19); (* $p < 0.03$ for body mass differences between small vs large litter size groups).

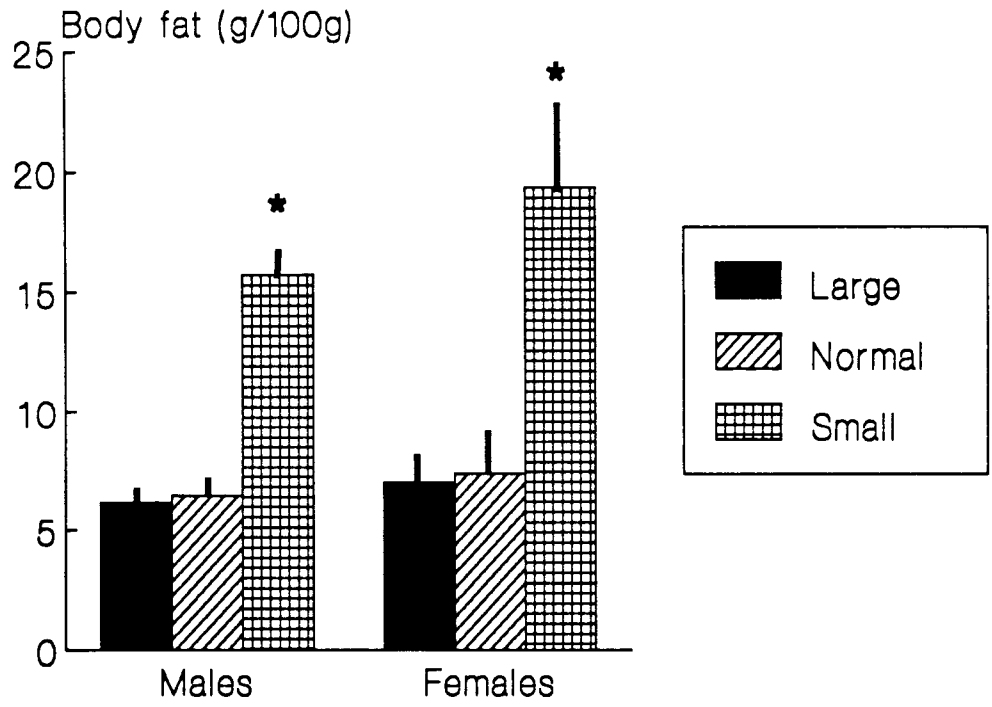

Figure 3.1b. Pre-weaning carcass fat content (g·100g wet wt^{-1}, means ± standard errors of the mean) of pups raised in small, normal or large litter size groups during suckling (* $p < 0.001$ for carcass fat content differences between small vs large and normal litter size groups).

Post-weaning:

Body mass and change in mass per day: There was a significant effect of gender on body mass throughout the post-weaning period. Male rats were consistently larger than female rats ($p < 0.0001$, Figure 3.2). Male rats raised in large litters weighed less than those raised in normal or small litters throughout the 18 weeks of the post-weaning period ($p < 0.001$, Figure 3.2). However,

after covarying for starting mass, the effects of litter size on body mass were eliminated in the latter stages of the post-weaning period (10, 12, 18 weeks post-weaning). Nor was there any effect of litter size on the change in mass per day in male rats from 8 weeks post-weaning (Table 3.3.).

Female rats from large litters had a significantly lower body mass than those from normal and small litters from weeks 2-6 post-weaning ($p < 0.01$). There was no persistent effect of pre-weaning litter size on differences in post-weaning body mass in female rats from 8 weeks post-weaning (Figure 3.2), even after covarying for starting mass. There was no significant effect of litter size on the change in mass per day up to 18 weeks following weaning in female rats (Table 3.3.).

There was a significant overall effect of the nutrient composition of the diet on the body mass of rats at 18 weeks post-weaning; mixed-fat-fed rats were significantly heavier than the chow fed rats ($p < 0.05$, Figure 3.2). Male rats ingesting the mixed-fat diet had a lower body mass at 2 weeks post-weaning when compared to chow-fed counterparts ($p < 0.02$, Figure 3.2).

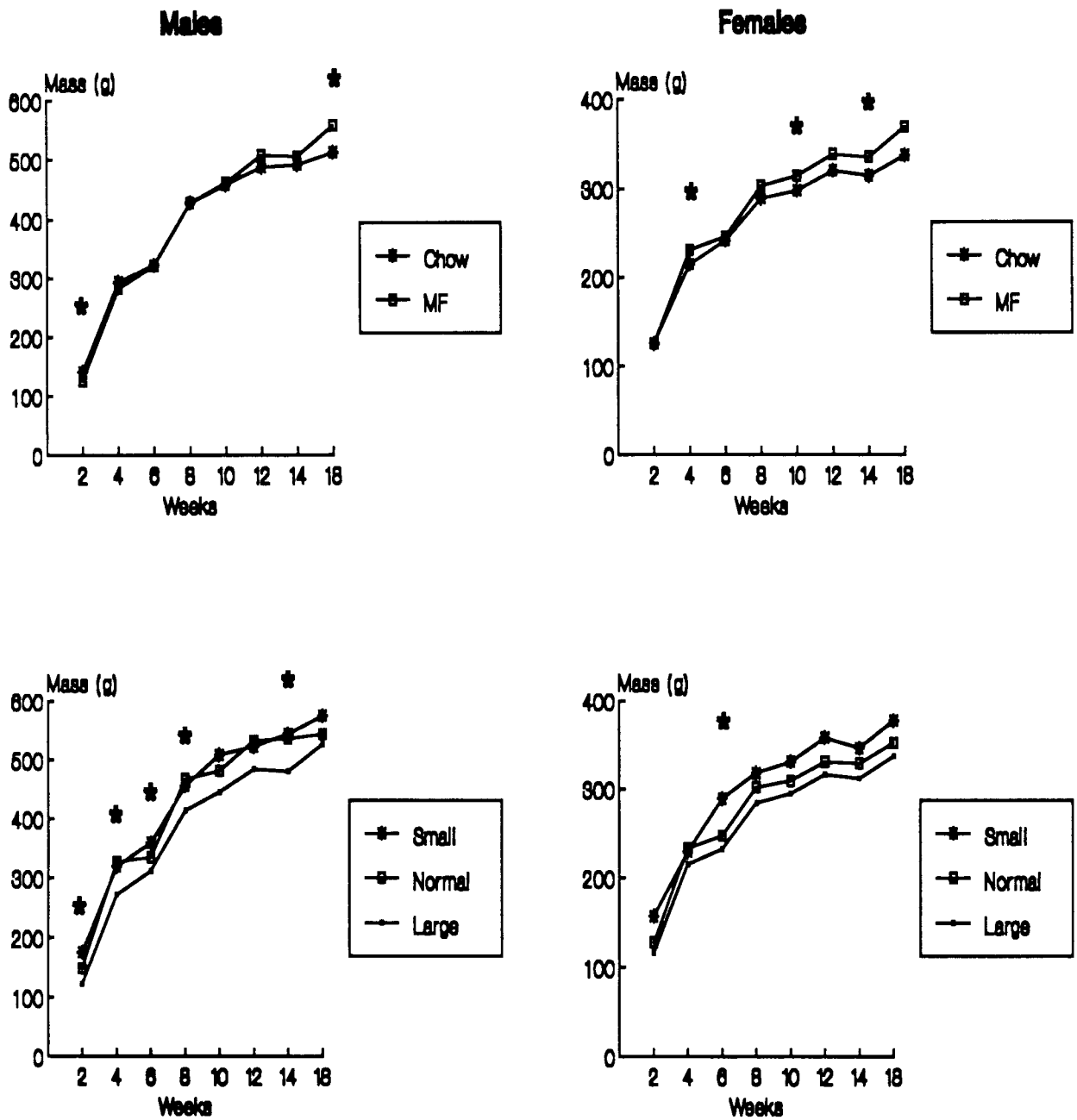

Figure 3.2. Body mass from wks 2-18 post-weaning (g, means $\pm$ SEM). Male rats from large litters weighed significantly less than those from small and normal litters at 2, 4, 6, 8, and 14 wks post-weaning ($p < 0.001$, $p < 0.005$, $p < 0.05$, $p < 0.05$, and $p < 0.001$, respectively). Male rats ingesting chow were heavier at 2 wks, and weighed less than MF-fed rats by 18 wks post-weaning ($p < 0.02$ at 2 wks, $P < 0.005$ at 18 wks). Female rats ingesting the MF diet were heavier than chow-fed rats at 4, 10, and 14 wks post-weaning ($p < 0.005$). There was a significant interaction effect between diet and litter size in male rats at wks 8, 12 and 14 post-weaning ($p < 0.05$). MF rats from large litters compensated, while chow-fed rats from large litters remained lighter.

However, by 18 weeks post-weaning, male rats ingesting the mixed-fat diet were significantly heavier than the chow-fed rats ($p < 0.005$, Figure 3.2). Female rats ingesting the mixed-fat diet were significantly heavier than the chow-fed rats at 4, 10, and 14 weeks post-weaning ($p < 0.05$, Figure 3.2).

There was no effect of the nutrient composition of the diet on the change in mass per day in female rats from 4 weeks post-weaning. Male rats ingesting the mixed-fat diet had a significantly greater change in mass per day than chow-fed rats at 12 weeks following weaning ($p < 0.005$, Table 3.3.).

There was also a significant interaction effect between diet and litter size on body mass of male rats at weeks 8, 12, and 14 post-weaning ($p < 0.05$). Body mass of rats fed the mixed-fat diet was similar, regardless of litter size, however, chow fed rats raised in large litters weighed significantly less than those raised in small and normal-sized litters.

Food intake and feeding efficiency:

Energy intake, expressed absolutely or relative to body mass, was significantly influenced by the number of animals housed in each cage. Animals which were housed singly had a lower overall energy intake than animals housed as pairs (419 ± 19 vs 442 ± 13 $kJ \cdot d^{-1}$ for singletons vs pairs, respectively, $p < 0.05$).

Table 3.3. Change in mass ($g \cdot d^{-1}$) in rats during the post-weaning period: Effects of diet and litter size (means ± SEM).

		Weeks 1-4	Weeks 4-8	Weeks 8-12	Weeks 12-18
		<-------POST-WEANING------>			
Males	**Diet**				
	Chow	6.3	3.8	1.7	1.2
	(n)	(15)	(15)	(15)	(15)
	SEM	0.3	0.2	0.1	0.2
				*	
				*	
	MF	5.9	4.2	2.4	1.6
	(n)	(15)	(15)	(15)	(15)
	SEM	0.2	0.2	0.1	0.3
	Litter				
	Small	6.7	4.3	2.1	1.5
	(n)	(4)	(4)	(4)	(4)
	SEM	0.2	0.2	0.3	0.5
	Normal	7.0	3.9	1.8	1.5
	(n)	(5)	(5)	(5)	(5)
	SEM	0.5	0.6	0.3	0.6
		*			
	Large	5.8	4.0	2.1	1.3
	(n)	(21)	(21)	(21)	(21)
	SEM	0.2	0.1	0.1	0.2
Females	**Diet**				
	Chow	4.2	2.1	0.9	0.8
	(n)	(16)	(16)	(16)	(16)
	SEM	0.1	0.2	0.1	0.1
		*			
	MF	4.7	2.1	1.04	0.9
	(n)	(10)	(10)	(10)	(10)
	SEM	0.2	0.1	0.1	0.1
	Litter				
	Small	4.2	2.8	1.1	0.5
	(n)	(3)	(3)	(3)	(3)
	SEM	0.2	0.9 *	0.4	0.2
	Normal	4.6	2.0	0.8	1.1
	(n)	(9)	(9)	(9)	(9)
	SEM	0.3	0.1	0.1	0.1
	Large	4.2	2.0 *	1.0	0.7
	(n)	(14)	(14)	(14)	(14)
	SEM	0.1	0.2	0.1	0.1

(* $p < 0.05$, ** $p < 0.01$)

Food energy intake ($kJ \cdot d^{-1}$) was also significantly higher in male rats when compared to female rats throughout the post-weaning period ($p < 0.003$). In male rats, there was no effect of pre-weaning litter size on post-weaning food intake, expressed absolutely or relative to body mass.

Nor was there any effect of pre-weaning litter size on post-weaning food energy intake in female rats, when expressed absolutely (Table 3.4a). However, when food energy intake was expressed relative to body mass ($kJ \cdot g^{-1} \cdot d^{-1}$), female rats from large litters ate more than those from small and normal litters in the early post-weaning period (weeks 1-4, $p < 0.002$, Table 3.4b).

The overall effect of the diet composition on food energy intake in both male and female rats was significant. Rats ingesting the mixed-fat diet had a lower overall food energy intake than their chow-fed counterparts (Table 3.4a, $p < 0.05$). From 1-4 and 4-8 weeks post-weaning, male rats ingesting the mixed-fat diet had a lower food energy intake than chow-fed rats ($p < 0.006$, Table 3.4a). However, from weeks 12-18 post-weaning, mixed-fat-fed male rats ingested more total energy per day than chow-fed rats ($p < 0.0001$, Table 3.4a). When energy intake was expressed relative to body mass, this difference was no longer significant (Table 3.4b).

Female rats ingesting the mixed-fat diet had a lower food energy intake than chow-fed rats from weeks 1-8 post-weaning ($p < 0.005$, Table 3.4b, expressed relative to body mass). There was a significant interaction effect between diet composition and litter size in female rats only at 8 weeks following weaning. Female rats from normal litters ingesting the chow diet had the lowest food energy intake while rats from normal litters ingesting the mixed-fat diet had the highest food energy intake ($p < 0.005$).

Feeding efficiency was significantly greater in male rats than in female rats from 4-8 weeks ($p < 0.0005$) and from 8-12 weeks ($p < 0.002$) post-weaning (Table 3.5). Gender had a significant overall effect on feeding efficiency for the post-weaning period ($p < 0.001$).

There was no effect of pre-weaning litter size manipulation on feeding efficiency in male or female rats for the 18 week period following weaning. However, male rats ingesting a mixed-fat diet had significantly higher feeding efficiency than chow-fed rats from 1-4 weeks ($p < 0.05$, Table 3.5), 4-8 weeks ($p < 0.0001$, Table 3.5), and 8-12 weeks ($p < 0.001$, Table 3.5) post-weaning and for the entire post-weaning period ($p < 0.0005$).

Table 3.4a. Energy intake ($MJ \cdot d^{-1}$) during the post-weaning period: Effects of diet and litter size (means ± SEM).

		Weeks 1-4	Weeks 4-8	Weeks 8-12	Weeks 12-18
		<-------POST WEANING------>			
Males	**Diet**				
	Chow	0.55	0.56	0.49	0.46
	(n)	(15)	(15)	(15)	(13)
	SEM	0.03 *	0.03 *	0.03	0.02 *
			*		*
	MF	0.45	0.38	0.43	0.55
	(n)	(15)	(15)	(15)	(14)
	SEM	0.02	0.03	0.03	0.03
	Litter				
	Small	0.52	0.54	0.52	0.52
	(n)	(4)	(4)	(4)	(4)
	SEM	0.04	0.08	0.07	0.05
	Normal	0.49	0.48	0.42	0.42
	(n)	(5)	(5)	(5)	(3)
	SEM	0.03	0.09	0.04	0.10
	Large	0.49	0.45	0.46	0.51
	(n)	(21)	(21)	(21)	(20)
	SEM	0.02	0.03	0.03	0.02
Females	**Diet**				
	Chow	0.41	0.40	0.37	0.40
	(n)	(16)	(16)	(16)	(14)
	SEM	0.02	0.02 *	0.04	0.02
			*		
	MF	0.35	0.32	0.35	0.43
	(n)	(10)	(10)	(10)	(8)
	SEM	0.02	0.02	0.04	0.04
	Litter				
	Small	0.32	0.35	0.35	0.39
	(n)	(3)	(3)	(3)	(3)
	SEM	0.03	0.04	0.04	0.10
	Normal	0.38	0.39	0.36	0.44
	(n)	(9)	(9)	(9)	(8)
	SEM	0.02	0.04	0.03	0.03
	Large	0.41	0.36	0.35	0.40
	(n)	(14)	(14)	(14)	(11)
	SEM	0.02	0.02	0.02	0.02

(* $p < 0.05$, ** $p < 0.005$

Table 3.4b. Energy intake (kJ·g body $wt^{\cdot -1 \cdot d^{-1}}$) after weaning: Effects of diet and litter size (means ± SEM).

		Weeks 1-4	Weeks 4-8	Weeks 8-12	Weeks 12-18
		<-------POST-WEANING------>			
Males	**Diet**				
	Chow	1.9	1.3	1.0	0.9
	(n)	(15)	(15)	(15)	(13)
	SEM	0.1	0.1	0.1	0.1
	MF	1.6	0.9	0.8	1.0
	(n)	(15)	(15)	(15)	(14)
	SEM	0.1	0.1	0.1	0.04
	Litter				
	Small	1.6	1.2	1.0	0.9
	(n)	(4)	(4)	(4)	(4)
	SEM	0.1	0.2	0.1	0.1
	Normal	1.5	1.0	0.8	0.8
	(n)	(5)	(5)	(5)	(3)
	SEM	0.1	0.1	0.04	0.1
	Large	1.8	1.1	1.0	1.0
	(n)	(21)	(21)	(21)	(20)
	SEM	0.1	0.1	0.1	0.04
Females	**Diet**				
	Chow	1.9	1.4	1.2	1.2
	(n)	(16)	(16)	(16)	(14)
	SEM	0.1	0.1	0.1	0.1
	MF	1.5	1.1	1.1	1.2
	(n)	(10)	(10)	(10)	(8)
	SEM	0.1	0.1	0.1	0.1
	Litter				
	Small	1.4	1.1	1.3	1.0
	(n)	(3)	(3)	(3)	(3)
	SEM	0.1	0.2	0.2	0.2
	Normal	1.6	1.3	1.1	1.2
	(n)	(9)	(9)	(9)	(8)
	SEM	0.1	0.1	0.1	0.1
	Large	1.9	1.3	1.1	1.2
	(n)	(14)	(14)	(14)	(11)
	SEM	0.1	0.1	0.1	0.1

(* $p < 0.05$, ** $p < 0.005$)

Female rats ingesting the mixed-fat diet only demonstrated greater feeding efficiency than chow-fed rats during the early post-weaning period (weeks 1-4, $p < 0.005$).

The overall effect of the nutrient content of the diet on feeding efficiency was significant for the entire post-weaning period ($p < 0.0001$). Mixed-fat fed rats had a greater feeding efficiency than chow-fed counterparts.

Body composition and fat pad mass:

Body composition, epididymal fat pad and interscapular fat mass were compared between randomly selected rats from large and small litters in the two diet treatment groups (Table 3.6). The epididymal fat pad mass was larger in all male rats ingesting the mixed--fat diet ($p < 0.008$, Table 3.6). There was no effect of litter size on epididymal fat pad mass. Similarly, interscapular fat mass was significantly higher in the mixed-fat fed rats ($p < 0.008$, Table 3.6). Inter-scapular fat mass was greater in male rats vs female rats ($p < 0.03$, Table 3.6), although there was no significant effect of pre-weaning litter size.

Female rats and rats fed the mixed-fat diet tended to have a higher carcass fat content. However, there were no significant effects of gender, litter size or diet treatment on total carcass fat or water content. Nor were there any significant interaction effects between diet, litter size and gender.

Table 3.5. Feeding efficiency (mg body mass·kJ ingested^{-1}) after weaning: Effects of diet and litter size (means ± SEM).

		Weeks 1-4	Weeks 4-8	Weeks 8-12	Weeks 12-18
		<-------POST-WEANING------>			
Males	**Diet**				
	Chow	11.7	6.9	3.7	2.7
	(n)	(15)	(15)	(15)	(15)
	SEM	0.6 *	0.3 * *	0.3 * *	0.6
	MF	13.5	11.4	6.0	2.9
	(n)	(15)	(15)	(15)	(15)
	SEM	0.5	0.6	0.5	0.5
	Litter				
	Small	13.2	6.9	3.7	2.9
	(n)	(4)	(4)	(4)	(4)
	SEM	1.1	0.3	0.3	0.9
	Normal	14.3	11.4	4.3	3.3
	(n)	(5)	(5)	(5)	(5)
	SEM	0.8	0.9	0.6	1.5
	Large	12.1	9.4	5.1	2.7
	(n)	(21)	(21)	(21)	(21)
	SEM	0.5	0.7	0.5	0.5
Females	**Diet**				
	Chow	10.6	5.8	2.6	1.9
	(n)	(16)	(16)	(16)	(14)
	SEM	0.6 * *	0.9	0.4	0.3
	MF	13.4	6.8	3.3	2.1
	(n)	(10)	(10)	(10)	(8)
	SEM	0.6	0.6	0.6	0.2
	Litter				
	Small	13.7	8.4	2.2	1.4
	(n)	(3)	(3)	(3)	(3)
	SEM	2.0	3.5	0.4	0.5
	Normal	12.3	5.6	2.5	2.4
	(n)	(9)	(9)	(9)	(8)
	SEM	0.8	0.8	0.5	0.3
	Large	10.9	6.0	3.2	1.8
	(n)	(14)	(14)	(14)	(11)
	SEM	0.6	0.7	0.5	0.2

(* $p < 0.05$, ** $p < 0.001$)

Table 3.6. Carcass fat, water content, and fat pad mass in randomly selected rats: main effects of litter size, diet and gender (means ± SEM).

Diet		Chow	Mixed-fat
Total mass	(g)	463.0 ± 40.9 (n=10)	471.4 ± 42.1 (n=10)
Carcass fat	(g%)	25.9 ± 2.1 (n= 9)	30.4 ± 3.8 (n= 8)
Carcass water	(g%)	59.1 ± 2.2 (n= 9)	58.0 ± 2.7 (n= 8)
Epididymal pad mass	(g)	3.58 ± 0.43^{a} (n= 6)	6.32 ± 0.66^{b} (n= 5)
Intrascap mass	(g)	0.61 ± 0.09^{a} (n=10)	0.94 ± 0.65^{b} (n=10)

Litter Size		Large	Small
Total mass	(g)	473.9 ± 35.4 (n=12)	457.1 ± 49.9 (n= 8)
Carcass fat	(g%)	29.8 ± 2.8 (n=11)	24.8 ± 2.9 (n= 6)
Carcass water	(g%)	56.2 ± 2.0 (n=11)	62.9 ± 1.9 (n= 6)
Epididymal pad mass	(g)	4.76 ± 0.85 (n= 7)	4.93 ± 0.58 (n= 4)
Intrascap mass	(g)	0.84 ± 0.09 (n=12)	0.69 ± 0.07 (n= 8)

Gender		Males	Females
Total mass	(g)	572.5 ± 16.7^{a} (n=11)	338.5 ± 10.8^{b} (n= 9)
Carcass fat	(g%)	25.7 ± 2.3 (n=11)	32.2 ± 3.9 (n= 6)
Carcass water	(g%)	60.5 ± 1.9 (n=11)	55.2 ± 2.9 (n= 6)
Intrascap mass	(g)	0.92 ± 0.09^{c} (n=11)	0.61 ± 0.07^{d} (n= 9)

(^{a,b} $p < 0.008$ for standard vs mixed-fat diet, ^{c,d} $p < 0.03$ for males vs females, means which do not share a common superscript are significantly different).

Discussion

This study showed that pre-weaning litter size manipulation had no persistent effect on subsequent body mass, feeding efficiency or the rate of change in body mass of rats, irrespective of gender or diet treatment. This suggests that rats from large and small litters were not "pre-programmed" for differences in feeding efficiency as a result of early nutritional experiences.

Effect of litter size on pre-weaning growth:

These findings cannot be ascribed to a failure in the pre-weaning model. Indeed, in this study, litter size manipulation resulted in a similar augmentation of growth and fat accretion in rats from small litters prior to weaning, as has been demonstrated in previous studies. Pups raised in small litters were significantly larger as well as being fatter than pups raised in large litters, and fatter than normal controls. Most other studies have found that pups from large litters differ from normal controls, and small litter pups do not (Harris, 1980a, Harris 1980b, Oscai and McGarr, 1978, Wainright et al., 1987b, Wurtman and Miller, 1976).

Differences between these data and other studies may be due, in part, to the use of different rat strains, such as the Sprague-Dawley, Wistar and Osborne-Mendel, which are genetically predisposed to obesity (Harris, 1980a, Schemmel et al., 1976).

It is important to note that pups from large litters, even though "relatively undernourished" still grew and remained in positive energy balance throughout the pre-weaning period. Thus, it may not be possible to compare these results to those from studies in which animals were in negative energy balance.

Effect of litter size on post-weaning growth, food intake and feeding efficiency:

Pre-weaning litter size manipulation has gained widespread acceptance as a model for the effects of early nutrition on post-weaning growth and development in various rat and mouse strains. In this study, no persistent effect of pre-weaning litter size on post-weaning food intake, growth or body composition was demonstrated.

Pre-weaning litter size manipulation was thought to alter post-weaning growth by increasing the number of committed fat cells during a developmental window. This hypothesis was based on a finding by Winick and Noble (1966) that adult body mass and fat cell DNA content seemed to be influenced by litter size and not by post-weaning nutrition. Although most studies demonstrate a substantial effect of litter size on mass and carcass fat *at weaning*, this effect has not been consistently reproduced in adulthood (Eisen and Leatherwood, 1978a, Greenwood and Hirsch, 1974). Thus in any given study, sometimes none, or only a subset of the experimental animals show any carry-over effect of pre-

weaning nutrition on post-weaning growth rate (Bassett and Craig, 1988, Eisen and Leatherwood, 1978, Harris, 1980a).

Furthermore, Bassett and Craig (1988) found that when juvenile rats raised in small litters were pair-fed with rats raised in large litter rats during the post-weaning period, the pre-weaning differences in fat cell number were no longer significant. Fat cell number decreased in the rats from small litters when pair-fed to those from large litters, and remained attenuated even after a period of *ad libitum* refeeding. If undernutrition during the period of cell replication was the major factor determining post-weaning growth and fat deposition, it is unlikely that this effect would be masked or altered by post-weaning manipulation. Indeed, the Winick and Noble (1966) model specifically excludes this possibility.

Greenwood and Hirsch (1974) characterized the postnatal development of adipocyte cellularity in the normal rat and found that there were three phases of development: cell proliferation, differentiation and lipid-filling. They measured the incorporation of [^{3}H]-thymidine into adipose tissue of Sprague Dawley rats up to 5 months of age and estimated that new cells continued to be formed until 12-14 weeks of age. Thus, it may be argued that nutritional manipulation in the pre-weaning period and early post-weaning period may alter adipose cellularity and that post-weaning nutritional experiences may be as physiologically important an influence on growth and body composition as pre-weaning litter size manipulation.

Furthermore, Eisen and Leatherwood (1978b) found that food restriction in mice from 4 to 6 weeks of age did not result in any permanent change in fat cell number. Following 10 weeks of *ad libitum* refeeding, fat cell size and number were not different between restricted rats and controls. Thus, the question remains as to whether or not these adaptations are irreversible.

Indeed, there is evidence to suggest that early undernutrition slows the rate of fat cell replication and lengthens the replication period (Kirtland and Gurr, 1978). Thus, it is possible for cell replication to proceed even under conditions of pre-weaning food restriction. This concept is supported by those studies in which early undernutrition during cell replication did not permanently alter adipocyte number after refeeding (Eisen and Leatherwood, 1978a, Haugeback et al., 1974). This suggests that the "developmental window" for fat cell replication may be modified, prolonged or even, as Harris (1980a) postulated, interrupted, depending on a number of factors. These factors may include strain differences (Harris, 1980b), the degree and duration of undernutrition (Meyer and Clawson, 1964), and the age of onset of undernutrition (Winick and Noble, 1966). Indeed, there might not be a "window" at all in the developmental sense of the word.

In fact, rats which are undernourished during a supposedly "vulnerable" period (such as the early post-weaning period) may display "catch-up" growth and more rapid fat deposition than *ad*

libitum-fed controls (Harris, 1980a). A similar, increased metabolic efficiency and fat accretion is demonstrated in adult rats undergoing refeeding following chronic food restriction (Hill et al, 1985). Wainright and Francey (1987a) found that mice from large litters actually gained more weight during the post-weaning period than did mice from small litters, although they remained lighter at 72 days post-weaning than small-litter and medium-litter controls.

One cannot exclude the possibility that "catch-up" growth took place in the present study. Pups from large litters were significantly smaller and leaner than pups from small litters at weaning. However, these effects were no longer evident 14 weeks post-weaning.

In studies which show a positive effect of litter size manipulation on post-weaning growth rate, adult food intake was consistently higher in rats raised in small litters (Bassett and Craig, 1988, Harris, 1980a, Eisen and Leatherwood, 1978, Oscai and McGarr, 1978). In the present study, there was no effect of litter size on food intake at 4, 8, 12, and 18 weeks following weaning. Therefore, differences in post-weaning growth rates or fat deposition would not be expected. The discrepancies between these studies might be related to the manner in which the rats were housed after weaning.

Faust et al. (1980) still found that Osborne-Mendel rats raised in small litters had significantly more fat cells, were bigger

and fatter, than their large litter counterparts, even after 1 year of ad libitum feeding and inspite of being housed 3-5 to a cage. Thus, the effects of early nutrition on post-weaning growth and development are likely to be strain-dependent.

Effects of numbers of rats housed in each cage on food intake:

In this study, rats were housed in small groups of three to five pups per cage for the first two weeks following weaning. Pups from the same litters were then randomly allocated to cages as either same sex pairs or as singletons. This procedure resulted in 80 rats housed as 40 pairs, and 16 rats housed singly. After covarying for litter size, gender and diet group, mean food energy intake was significantly higher in the rats which were housed as pairs (440 ± 14 $kJ \cdot d^{-1}$ and 403 ± 14 $kJ \cdot d^{-1}$, $p<0.05$), for rats housed as pairs or singly, respectively.

When litter size groups were analyzed separately, there was no effect of the number of rats housed per cage on overall food intake in small and normal-litter-size groups. There was, however, an effect in animals from large litters. Thus, the finding that rats housed singly eat less than rats housed communally confirms anecdotal observations by Wurtman and Miller (1976) that food intake decreased in rats separated after weaning. The extent and duration of this effect has not been measured. When pre-weaning nutritional manipulation was studied in communally-housed members of another sociable species, baboons

(Lewis et al., 1986), there was no difference in adult food intake and consequent body size and fat mass. Thus, the effect which has previously been attributed to a critical period of development of fat cell number, or of appetite, may be a consequence of environment and social behaviour.

Effect of gender on growth and feeding efficiency:

Gender had the greatest impact on growth and feeding behaviour. Male rats ate more, grew more rapidly and had higher feeding efficiencies than their female counterparts. These data are similar to the results of the comparative study of Schemmel et al. (1970) in which feeding efficiency in mature female Long-Evans rats was only about 66% of that found in male rats. This gender effect is even more marked in male and female Wistar rats (Bassett and Craig, 1988). Besides these obvious effects of gender, nutrient composition of the diet had the second most significant effect on overall feeding efficiency in rats up to 18 weeks following weaning.

Effects of post-weaning food composition on growth, food intake and feeding efficiency:

Rats fed a mixed-fat diet gained more body mass relative to food intake than did chow-fed rats, despite an overall lower energy intake. The post-weaning nutrient manipulation in this study had a marked effect on rate of growth and feeding efficiency and fat deposition for the period of 18 weeks post-weaning. The changes

in the rate of growth were only seen from 8-12 weeks. Feeding efficiency was, however, greater than chow-fed controls from 1-12 weeks post-weaning.

In many studies involving fat-supplemented *ad libitum* feeding in rats, it is difficult to dissociate the effects of the higher dietary fat content and hyperphagia. Changes in the fat content of the diet can make food more, or less palatable, in addition to changing its energy density, nutrient content and thermogenic properties.

The experimental diet in this study was chosen because it was higher in fat, it was relatively easy to prepare and to feed, and earlier reports indicated that it caused rats to have a greater energy intake than chow-fed controls, resulting in diet-induced obesity (Levin et al, 1983a). Subsequent studies using this diet found that hyperphagia was not consistently reproducible across age and strain, and the diet demonstrated a bimodal effect on food energy intake in adult rats (Levin et al., 1983b, Levin et al., 1986).

Using the criteria described here, we did not find a bi-modal response to the mixed-fat diet with respect to food energy intake after 18 weeks post-weaning, in either the male or female rats. However, the variance for post-weaning weight gain was two-fold higher in male rats ingesting the mixed-fat diet, than chow-fed male rats. In addition, the female rats also did not show a bi-modal response to the diet for food energy intake, and the

variances in weight gain between diet groups were more similar than for male rats.

However, despite a relatively lower food energy intake in some instances, this diet still resulted in an increased deposition of fat, indicating a greater feeding efficiency.

There are three possible reasons for the effects of fat feeding on post-weaning growth and fat deposition. Firstly, fat feeding may cause hyperphagia when compared to feeding standard chow, with marked differences in energy content of the diet and consequent changes in body mass and carcass fat. Secondly, fat feeding may alter dietary-induced thermogenesis and tissue insulin sensitivity with a resultant increased feeding efficiency (Bjorntorp and Sjostrom, 1978).

Finally, fat feeding may cause decreased energy intake and thus, increased feeding efficiency may reflect some genetically-determined or strain-dependent factor controlling the post-weaning rate of growth and development. Schemmel et al. (1970) studied fat-supplemented feeding in 7 rat strains and found that post-weaning rate of growth was determined equally by genotype and environment, though this is presumably highly dependent on the default diet. For example, if the default diet is nutritionally inadequate, then it is likely that environment will play a predominate role determining growth and feeding behaviour, especially in response to an altered feeding regimen. However,

if the default diet is nutritionally adequate, then it is more likely that genotype effect will predominate.

Conclusions:

In the present study, rats fed a mixed-fat diet were not hyperphagic and so differences in post-weaning mass and body fat depots can be attributed only to an increased metabolic efficiency. There were no persistent effects of pre-weaning litter size manipulation on post-weaning growth and development after 8 weeks. From these data, it appears that post-weaning nutritional manipulation may play a more important role in determining adult mass and depot fat in freely-eating rats than early nutritional experiences. This suggests that critical periods for differentiation and growth of adipose tissue may occur even after 2 months of age in Long-Evans rats.

Thus, in this model, there was little evidence to suggest that pre-weaning undernutrition resulted in greater post-weaning metabolic or feeding efficiency, appetite or marked changes in body energy stores. However, it should be noted that animals in this experiment were always in positive energy balance, indicated by the consistent weight gain; therefore, these findings cannot be compared to experimental models of gross undernutrition, in which animals are in negative energy balance, and losing mass or body energy stores.

CHAPTER 4

EXERCISE TRAINING AND THE SHORT-TERM CESSATION OF TRAINING: RATE OF GROWTH, FOOD INTAKE, FEEDING EFFICIENCY, FAT ACCRETION, AND ADIPOSE TISSUE LIPOGENIC ACTIVITY IN LONG-EVANS RATS

Introduction

In the previous chapter litter size manipulation resulted in relative "under"- or "over"-nutrition in suckling rats, and as a result, a marked, but transient augmentation or attenuation of rate of growth and fat accretion. These effects did not persist after 8 weeks of *ad libitum* feeding during the post-weaning period. Thus, during periods of growth (overall positive energy balance) relative food energy restriction resulted in an attenuation in the rate of growth. There was no evidence, however, for long-term adaptation suggesting a persistent "hypometabolic" state or enhanced efficiency of food utilization.

In the study reported in this chapter, growing Long-Evans rats were exposed to a voluntary exercise stimulus which was subsequently removed. This protocol had the potential to perturb energy balance by changing energy requirements for the maintenance of "normal" growth and development in these rats.

The rationale for the present study is that the termination of exercise training (detraining) has been shown to result in rapid fat accretion and weight gain in both humans (Parizkova, 1977) and in rats (Applegate et al., 1984, Applegate and Stern, 1987, Arnold and Richard, 1987, Craig et al., 1983, Dohm et al., 1977, Jen et al., 1992, Lowney et al., 1988,

Sandretto and Tsai, 1988, Walberg et al., 1983). Short-term detraining has been characterized by increased body mass, body fat content and adipocyte size (Booth et al., 1974, Craig et al., 1983), as well as increased activity of the lipogenic enzymes involved in fatty acid synthesis (Dohm et al., 1977, Sandretto and Tsai, 1988) and adipose tissue lipogenesis (Applegate et al., 1984. Applegate and Stern, 1987).

However, detraining has also been associated with increased food intake, especially in rats genetically pre-disposed to obesity (Applegate and Stern, 1987, Applegate et al., 1984, 1984, Dohm et al., 1977). Refeeding following energy restriction in both animal and human models has been shown to result in increased efficiency of weight gain and adipose tissue lipogenic activity (Boyle et al., 1981, Eckel and Yost, 1987, Hill et al., 1985, Quig et al., 1983, Schwartz et al., 1978, Taskinen and Nikkila, 1987). Therefore, during detraining, it is difficult to dissociate a lipogenic response which may occur as a result of an increased food intake, from one which results solely from the removal of the exercise stimulus.

A single bout of exercise is a potent stimulus for increased plasma triglyceride clearance (Anuzzi et al., 1987) and increased adipose tissue lipoprotein lipase activity (Applegate and Stern, 1987, Savard et al., 1987). Adipose tissue lipoprotein lipase (ATLPL) is the enzyme responsible

for hydrolysis of extracellular lipoprotein triglyceride for uptake of fatty acids by adipose tissue. Therefore, this enzyme has a pivotal role in the maintenance of adipose tissue triglyceride stores. In both human and animal models, ATLPL has been shown to be rate-limiting for the deposition of fat, both *in vivo* and *in vitro* (Eckel, 1988).

ATLPL activity is stimulated *in vivo* and *in vitro* by increased concentrations of insulin and glucocorticoids, and is elevated during refeeding following a period of starvation (Eckel, 1988). There is also some evidence to suggest that following an acute bout of exercise, the activity of this enzyme exhibits a "dose-response" relationship with increasing exercise intensity (Savard, et al., 1987), and that the response in a physically-trained animal or human may be different than that exhibited by an untrained control subject (Barakat et al., 1981).

Thus, the present study was designed to address these questions in freely-running, wheel-trained rats by measuring the *in vitro* activity of adipose tissue lipoprotein lipase in trained rats and rats which had stopped training for 1, 2, 3 days or 1 and 2 weeks, respectively. Membrane-bound ATLPL activity was measured at rest and after acute, exhausting exercise. Feeding efficiency, fat pad mass, and adipocyte size were also measured.

Methods

Animal maintenance, exercise training and food intake:

Male Long-Evans rats (~ 310 g) were housed individually in specially designed cages in which they were able to run spontaneously (Campus Industries, Cape Town, South Africa) as described previously (Lambert and Noakes, 1990). Briefly, each freely rotating wheel had a diameter of 36 cm and a width of 11 cm. The running surface consisted of metal gauze (8 x 8 mm) which was large enough to keep the wheels free of food and faecal matter. A dispenser containing food and water was fitted over an opening in the metal plate to which the wheels were attached. A mechanical counter recorded the number of complete revolutions of the wheel as the animals walked or ran. The number of revolutions was noted each day and spontaneous training distance was calculated ($km \cdot wk^{-1}$).

Sedentary control animals were housed individually in suspended, stainless-steel cages of a similar volume to the running wheel cages. Rats which had stopped training were removed and placed individually in suspended stainless-steel cages after the 8 week spontaneous training period. Experimental and control rats were housed in the same room and were exposed to a 12 hour light/dark cycle and a controlled thermoneutral environment (20-22°C) with free access to

standard laboratory chow (Epol Rat Cubes, Epol (Pty) Limited, Maitland, South Africa). The composition of the diet is described in Chapter 3, Table 3.1. Rats living in the wheels trained *ad libitum* for a period of 8 weeks.

Three experiments were undertaken, and where possible, data are combined. The three protocols are described below and graphically illustrated in Figure 4.1.

Experiment 1: Thirty-five rats spontaneously trained for 8 weeks; 24 were subsequently detrained for 1 and 2 weeks (n = 13 at 1 week; n = 11 at 2 weeks). Rats were sacrificed at rest either at week 8, or at the end of week 1 and week 2 of the detraining period.

Experiment 2: Thirty-eight rats spontaneously trained for 8 weeks; 12 and 14 rats were then detrained for 1 and 2 weeks, respectively. Prior to being placed in wheel cages, and again at the end of the 8 week period, rats were exercised to exhaustion on a motor-driven treadmill for the determination of maximal oxygen uptake (VO_2max) and running performance. Rats were sacrificed, immediately following the last VO_2max test and at least two hours after the last feeding.

Experiment 3: Seventeen rats which had trained for 8 weeks were sacrificed in the rested state 24 hours (n=3), 48 hours (n=4) or 72 hours (n=10) after an exhausting bout of exercise.

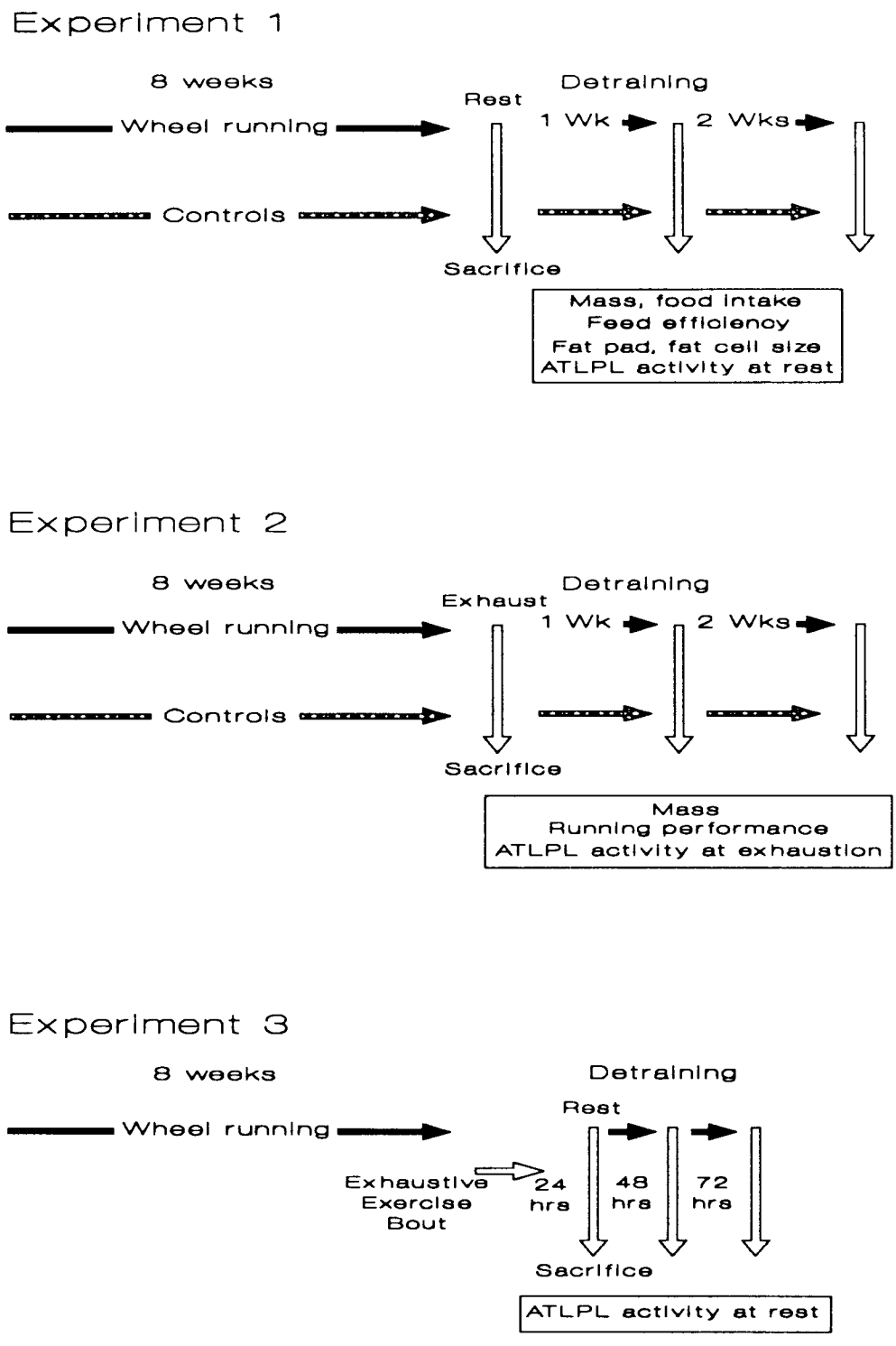

Figure 4.1. Schematic presentation of experimental protocols for Experiments 1-3.

Food intake, change in mass per day, feeding efficiency:

Food consumption was calculated by measuring the difference in mass between weighed portions and uneaten food, including the spillage onto wire-mesh screens situated below each cage. Food and faecal matter were separated and removed. Food intake was measured at weeks 7 and 8 in trained rats and compared to that measured at the end of weeks 1 and 2 of detraining. The food intake and feeding efficiency of five age-matched sedentary control rats were compared to those of the trained and detrained rats. Gross feeding efficiency was calculated as the change in body mass (mg) expressed relative to the food energy ingested (mg body mass·kJ food energy^{-1}).

Total body mass was measured and the change in mass per day ($g \cdot d^{-1}$) was calculated from 0 to 8 weeks, and at the end of 1 and 2 weeks of detraining. Body mass data were combined for experiments 1 and 2 and compared to the data from an age-matched, sedentary, control group (n=20).

Treadmill running performance and maximal oxygen consumption:

After 8 weeks of training, maximal treadmill running performance and VO_2 max were measured as previously described (Lambert and Noakes, 1990). Briefly, rats ran in a bottomless, rectangular, Plexiglas chamber which was suspended over a single-lane, motor-driven treadmill. Ambient air was

drawn through the chamber and exited through a double ceiling/mixing chamber at a rate of 6.5-7.5 $l \cdot min^{-1}$ STPD. Flow rate was calibrated before and after each test using a Tissot spirometer (Warren E. Collins, Inc., Braintree, Mass., USA).

Expired air was passed through copper coils immersed in ice for the purpose of condensing moisture, and directed to an oxygen analyzer (Applied Electrochemistry, S-3A/1) and carbon dioxide analyzer (Applied Electrochemistry CD-3A, Thermox Instruments, Pittsburgh, Pennsylvania) for the determination of O_2 and CO_2 concentration. Analyzers were calibrated prior to each test using a gas of a known concentration and after each test to note if any drift had occurred. Oxygen uptake was calculated each minute using the formula of Consolazio et al. (1963). Running performance was measured as the total time running on the treadmill during the VO_2 max test (Lambert and Noakes, 1990). Running performance and VO_2 max were compared between sedentary control rats (n=15), 8-week, spontaneously-trained rats (n=12), 1- and 2-week detrained rats (n=12 in each group).

Epididymal and interscapular fat pad mass and epididymal adipocyte diameter:

Following decapitation of the rats in *experiment 1*, epididymal fat pads from a randomly-selected sample of rats

from each group were rapidly removed and weighed. Fat samples weighing between 20 and 80 mg were placed in Krebs-Ringer/HEPES buffer, pH 7.4, with 1.5 $mg \cdot ml^{-1}$ collagenase (Type II) and 3 $g \cdot 100\ ml^{-1}$ bovine serum albumin (Fraction V, Rodbell, 1964). Fat samples were incubated at 37°C and shaken gently for 20 minutes. Thereafter, the tissue and medium were filtered through a 250 μm nylon mesh. Fat cells were washed twice with collagenase-free buffer and resuspended in 0.5 ml buffer. Isolated fat cell size was determined using calibrated light microscopy (Bray, 1970). A minimum of 100 adipocytes were sized for each analysis.

Epipidymal fat samples were also frozen in liquid N_2, and stored at -80°C for later enzymatic analyses. The interscapular fat was also removed and weighed.

Adipocyte size and epididymal and interscapular fat mass were compared between age-matched controls and rats which had trained for 8 weeks, vs 1- and 2-week detrained rats.

Heparin-releasable adipose tissue lipoprotein lipase activity (ATLPL activity):

Heparin-releasable ATLPL activity was measured using an adaptation of the methods of Taskinen et al. (1980) and Schotz et al. (1970). ATLPL activity was measured in randomly

selected fat samples from each group of rats under the following conditions: rested vs exhausted, trained vs detrained and controls. In *experiment 3,* ATLPL activity was also compared in 8 week trained, rested rats after 1 day, 2 days and 3 days of stopping training (Figure 4.1.)

Frozen epididymal fat pad samples weighing between 20 and 60 mg were minced, and pre-incubated in Krebs-Ringer, Tris/HCl buffer with 2.5 IU heparin and 1.0% fatty-acid-free bovine serum albumin at pH 8.4, and shaken at 28°C for 45 minutes. After pre-incubation, the adipose tissue was removed from the medium and 0.5 ml of substrate mix were added to the released enzyme.

The substrate mix included: 3.5/5.0 (v/v) Tris/HCl buffer, pH 8.1, with lecithin (0.6 mg/ml), 1.0/5.0 (v/v) 10.0% bovine serum albumin, 0.5/5.0 (v/v) pooled fasting serum, with 5.0 μmoles triolein (Sigma Chemical Company, St. Louis, Missouri) per 0.5 ml, and 0.25 μCi ^{14}C-glycerol trioleate (Amersham International, Buckinghamshire, England). This substrate mix was sonicated on ice for 5 minutes using a cycle of 30 seconds on/10 seconds off. The final mixture had a milky white appearance.

The substrate mix and released enzyme were incubated at 28°C for 2 hours. The contents of each tube were transferred to clean glass vials and the reaction was terminated by the

addition of 2.0 ml of an isopropanol:3M H_2SO_4 solution (40:1, v/v). A portion of the isopropanol:H_2SO_4 mixture was used to rinse each reaction tube.

For lipid extraction, 5.0 ml of N-hexane and 2.0 ml of distilled H_2O were added to each vial. Vials were vortexed and placed horizontally in a mechanical shaker for 30 minutes. The contents of each vial were allowed to settle for 10 minutes. Four ml of the hexane layer were then transferred to graduated, conical, capped test tubes and 2.0 ml of 0.1 M KOH with 1.0 % bovine serum albumin were added. Tubes were placed horizontally in the shaker for an additional 10 minutes to extract the fatty acids into the alkali phase. Contents were allowed to settle for 10 minutes.

After noting the volume of the upper and lower phases, the upper phase was aspirated. An additional 5.0 ml N-hexane were added to complete the free-fatty acid extraction. Tubes were placed in the shaker for 5 minutes, allowed to stand for 10 minutes and the upper phase was aspirated. A 1.5 ml aliquot of the lower phase was placed in scintillation vials and 10 ml of scintillation fluid were added. ^{14}C-oleic acid (Amersham) was added in triplicate to blank tubes in order to measure total free fatty acid recovery following the extraction procedure.

The activity of ATLPL, expressed as nmoles FFA·g $^{-1}$·hr^{-1}, was calculated as follows:

$$(FFA_{dpm} * \text{triglyceride}(\mu\text{moles}) * 10^6) - (\text{total}_{dpm} * \text{tissue mass(mg)})$$

Statistical analyses:

The data are expressed as means and standard errors of the mean. Comparisons between groups for factors measured over time were made using a two-way analysis of variance for repeated measures. A probability level of less than 5.0% was considered statistically significant and a Tukey's post-hoc analysis was used to determine significant differences between means from various groups. Comparisons between groups for factors such as epididymal fat pad mass and ATLPL activity were performed using one-way analyses of variance. Pearson-product-moment correlation coefficients were calculated for training distance vs ATLPL activity.

Results

Performance parameters:

The mean weekly *ad libitum* running distance (km·wk^{-1}) is shown in Table 4.1. All three experimental groups were exposed to a similar mean training stimulus. There was no difference in

VO_2 max between trained rats and those which had stopped training for 1 and 2 weeks. However, trained rats had a longer endurance time to exhaustion than control rats (Table 4.1, $p < 0.005$). Rats which had stopped training for 2 weeks became fatigued more rapidly than 8-week trained rats during the treadmill running test ($p < 0.05$, Table 4.1).

Table 4.1. Spontaneous running, VO_2max, and running performance in rats which had trained for 8 weeks and rats which, after having trained for 8 weeks, stopped training for 1 and 2 weeks compared to sedentary controls (means ± SEM).

Group	Distance ($km \cdot wk^{-1}$)	VO_{2max} ($ml \cdot min^{-1}$)	Runtime (min)**
Controls (n)	-	31.4 ±0.9 (15)	21.5^{c} ±0.7 (15)
Trained (n)	27.4 ±4.3 (23)	32.8 ±0.7 (12)	27.8^{a} ±1.3 (12)
1 Week Detrain (n)	26.3 ±3.7 (25)	34.1 ±1.5 (12)	25.2^{ab} ±1.2 (12)
2 Weeks Detrain (n)	30.3 ±5.3 (23)	35.7 ±0.8 (12)	22.2^{c} ±1.3 (12)

($^{a, c}$ $p < 0.005$ for trained vs controls and 2 wk detrained, $^{b, c}$ $p < 0.02$ for controls vs 1 wk detrained; ** Run time = treadmill run time to exhaustion)

Change in mass, food intake, and feeding efficiency:

Sedentary control rats were significantly heavier than all training groups by the sixth week of training ($p < 0.02$, Figure 4.2). These differences remained significant after cessation of training ($p < 0.05$).

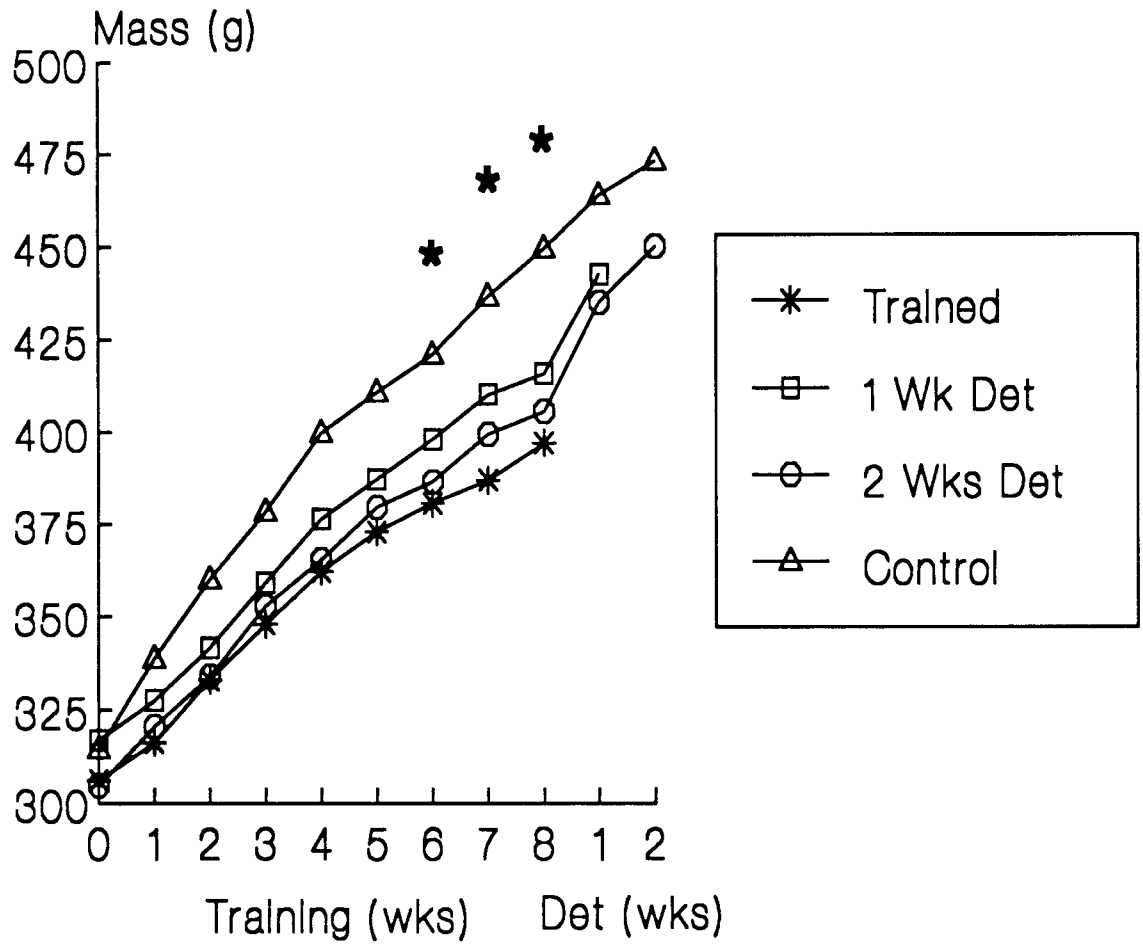

Figure 4.2. Body mass changes over the 8 week ad libitum training period and 2 week detraining period ($^{*}p < 0.05$, controls vs trained groups).

The rate of weight gain expressed as change in mass per day ($g \cdot d^{-1}$) was significantly higher in controls than in trained rats over the 8 weeks of *ad libitum* wheel running ($p < 0.001$, Table 4.2). After cessation of training, the rate of weight gain was significantly higher in the detrained rats when compared to controls ($p < 0.05$, Table 4.2). Using simple linear regression, there was no significant "dose-response" relationship between spontaneous training distance and rate of weight gain during training or detraining.

Table 4.2. Change in mass ($g \cdot d^{-1}$) for trained, 1- and 2-week detrained and control rats over 8 weeks of wheel running and 1 and 2 weeks after the cessation of training (means ± SEM).

	Weeks training									Detraining	
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**0-8**	**1**	**2**
Trained (n=23)	1.6^a ±.4	2.4 ±.3	2.2 ±.3	2.0 ±.2	1.6 ±.2	1.3^a ±.3	0.9^a ±.3	1.4 ±.2	1.7^a +.1	-	-
Detrain 1 wk (n=25)	1.5^a ±.4	2.0 ±.3	2.5 ±.3	2.5 ±.3	1.5 ±.3	1.5 ±.3	1.8 ±.4	0.8^a ±.3	1.8^a ±.1	3.8^a ±.5	-
Detrain 2 wks (n=23)	2.5 ±.6	1.8^a ±.4	3.0 ±.4	1.8 ±.2	1.8 ±.2	1.0^a ±.2	1.8 ±.2	0.9^a ±.2	1.8^a ±.1	4.2^a ±.5	2.2^a ±.3
Control (n=20)	3.5^b ±.6	3.0^b ±.5	2.6 ±.3	3.0 ±.4	1.6 ±.3	2.2^b ±.3	2.2^b ±.5	1.8^b ±.3	2.5^c ±.2	2.0^b ±.6	1.3^b ±.3

(Group means which do not share a common superscript are significantly different; ^{a} vs ^{b}, $p < 0.05$, ^{a} vs ^{c}, $p < 0.001$).

There were no differences in food energy intake between groups during training compared to food energy intake during 1 and 2 weeks of detraining (Table 4.3). Nor were there any differences in food intake between experimental rats and sedentary controls (Table 4.3).

Table 4.3. Energy intake ($kJ \cdot d^{-1}$) during the final week of wheel training and for 1 and 2 weeks following the cessation of training (means ± SEM).

		Detrained	
	Trained	**1 Week**	**2 Week**
Trained (n=11)	390.2 ±11.8	-	-
1 Week Detrain (n=13)	377.2 ±11.7	396.1 ±16.4	-
2 Week Detrain (n=11)	412.9 ±20.2	345.7 ±16.8	378.0 ±28.6
Controls (n=5)	360.8 ±20.2	333.5 ±16.8	364.6 ±28.6

Feeding efficiency, expressed as the change in mass relative to the energy ingested ($g \cdot kJ^{-1} \cdot d^{-1}$), increased significantly in rats after stopping training for 1 week ($p < 0.005$, Table 4.4). Feeding efficiency in rats which had detrained for 2 weeks was still higher than that of controls, but this was no longer statistically significant ($p=0.07$)..

Table 4.4. Feeding efficiency expressed as the change in body mass over the energy ingested ($mg \cdot kJ^{-1} \cdot d^{-1}$) in trained vs control rats and after 1 or 2 weeks of detraining (means ± SEM).

		Detrained	
	Trained	**1 Week**	**2 Week**
Trained (n=11)	3.7 ±0.8	-	-
1 Week Detrained (n=13)	2.4^{c} ±0.9	7.5^{d} ±1.4	-
2 Week Detrained (n=11)	2.9^{a} ±0.9	8.4^{b} ±1.6	$4.1^{a,b}$ ±1.4
Controls (n=5)	5.5 ±1.0	8.7 ±2.1	3.1 ±1.3

(^{a,b} < 0.05, ^{c,d} $p < 0.005$, for within group differences in feeding efficiency in the 1 week detrained group from the final week of training to 1 week of detraining)

Heparin-releasable ATLPL activity:

Heparin-releasable ATLPL activity was significantly higher in exhausted vs rested rats ($p < 0.005$, Figure 4.3). ATLPL activity was also significantly higher in 1 and 2 week detrained rats, when compared to trained rats ($p < 0.005$, Figure 4.3). ATLPL activity in trained rats, was not different than that of controls either at rest or in the exhausted state. However, there was a significant interaction effect ($p < 0.005$) between treatment (rested vs exhausted) and group (trained and detrained vs controls), which showed that control rats did not have the same magnitude of change in

ATLPL activity in response to exhausting exercise as did trained and detrained rats.

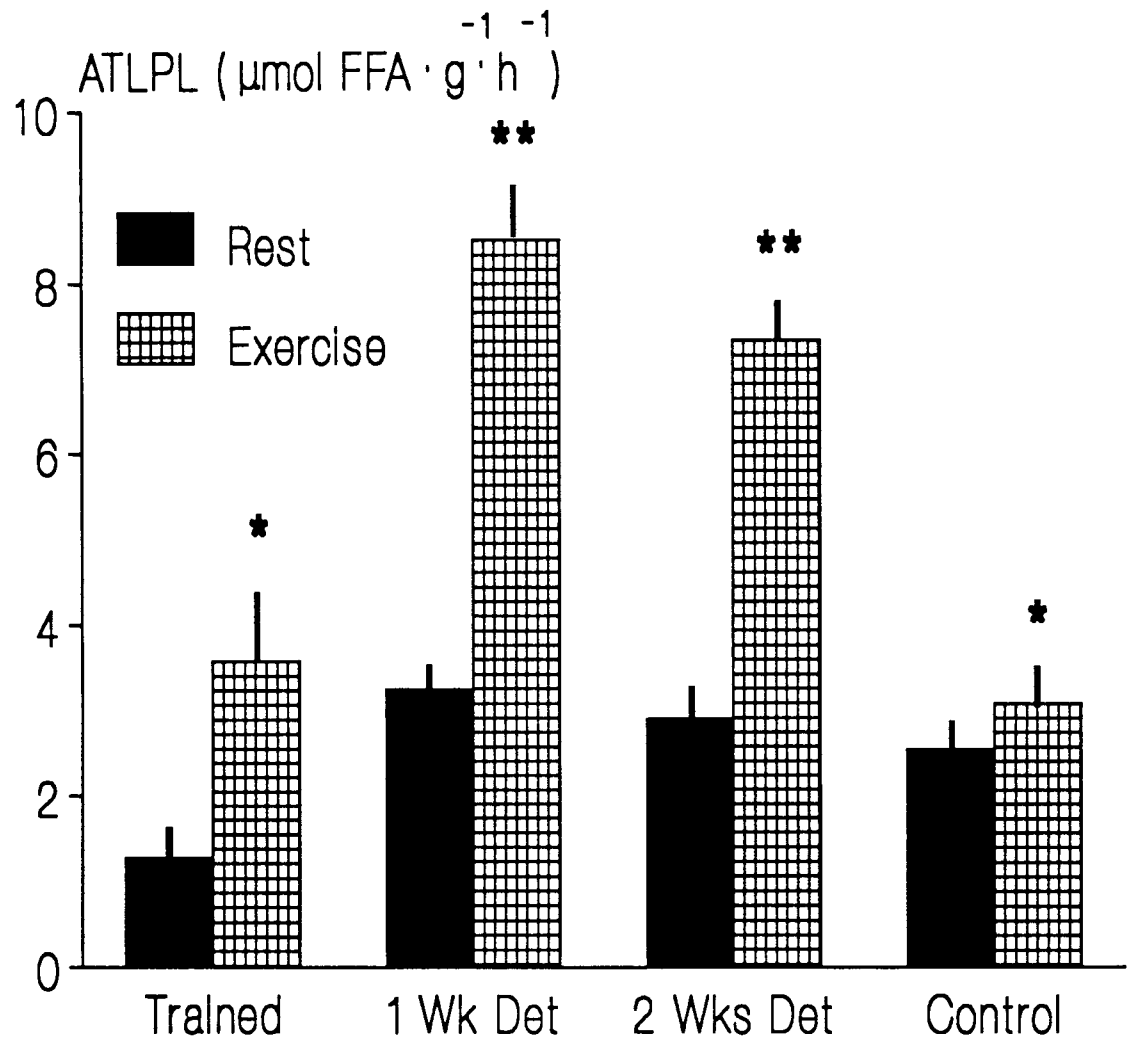

Figure 4.3. Heparin-releasable, ATLPL activity at rest and after acute, exhausting exercise in trained, 1- and 2-week detrained, and control rats (means ± SEM, * $p < 0.005$ for the main effect of state at sacrifice, rested vs exhausted, ** $p < 0.005$ for the main effect of group, detrained vs trained, $p < 0.005$ significant interaction effect between group and state at sacrifice).

ATLPL activity following an acute bout of exhausting exercise was 1.7 times higher 24 hours after the last exercise bout when compared to activity measured 3 days after cessation of training (2.9 $\pm$ 0.4 umol FFA $\cdot g^{-1} \cdot h^{-1}$ vs 5.0 $\pm$ 0.8 $umol \cdot g^{-1} \cdot h^{-1}$, $p < 0.05$, for day 3 vs day 1 respectively).

ATLPL activity was significantly and positively correlated to training distance in rested rats, 1 week after stopping training, i.e. there was a "dose-response" ($r = 0.57$, $p < 0.05$, Figure 4.4). There was also a positive correlation between training distance and ATLPL activity measured at rest in rats after 2 weeks of detraining, although this was not statistcally significant. However, there was a negative correlation between training distance and ATLPL activity in 8 week trained rats ($r = -0.52$) which was not statistically significant.

Body mass and fat pad mass and adipocyte diameter:

Body mass, epididymal fat pad mass and interscapular fat mass were compared in a randomly selected sample of trained, 1 and 2 week detrained and control rats (Table 4.5). Total body mass was significantly lower in trained rats when compared to controls and detrained rats ($p < 0.002$, and $p < 0.05$, respectively). Mean epididymal fat pad mass was significantly higher in controls than in trained rats ($p < 0.05$). However,

mean epididymal fat pad mass did not differ between 1 and 2 week detrained rats and controls or trained animals. Nor were there any group differences in interscapular fat mass.

Mean epididymal adipocyte diameter was significantly greater in controls compared to both trained and detrained groups ($p < 0.0001$, Table 4.6). One- and 2-week detrained rats had a significantly greater fat cell size compared to trained rats ($p < 0.05$, Table 4.6).

Table 4.5. Body mass and fat pad mass in trained, 1 and 2 week detrained and control rats (means ± SEM).

	Total Mass (g)	**Epididymal fat pad mass (g)**	**Interscap fat pad mass (g)**
Trained (n=4)	402.9^{a} ±12.4	1.63^{a} ±0.2	0.49 ±0.1
1 Week Detrained (n=8)	$473.8^{b,c}$ ±15.6	2.73 ±0.3	0.78 ±0.1
2 Weeks Detrained (n=7)	$465.8^{b,c}$ ±18.0	2.38 ±0.3	0.78 ±0.1
Controls (n=6)	509.6^{c} ±18.9	3.61^{b} ±0.8	0.71 ±0.3

(Group means which do not share a common superscript are significantly different; ^{a} vs ^{c}, $p < 0.002$, ^{a} vs ^{b}, $p < 0.05$).

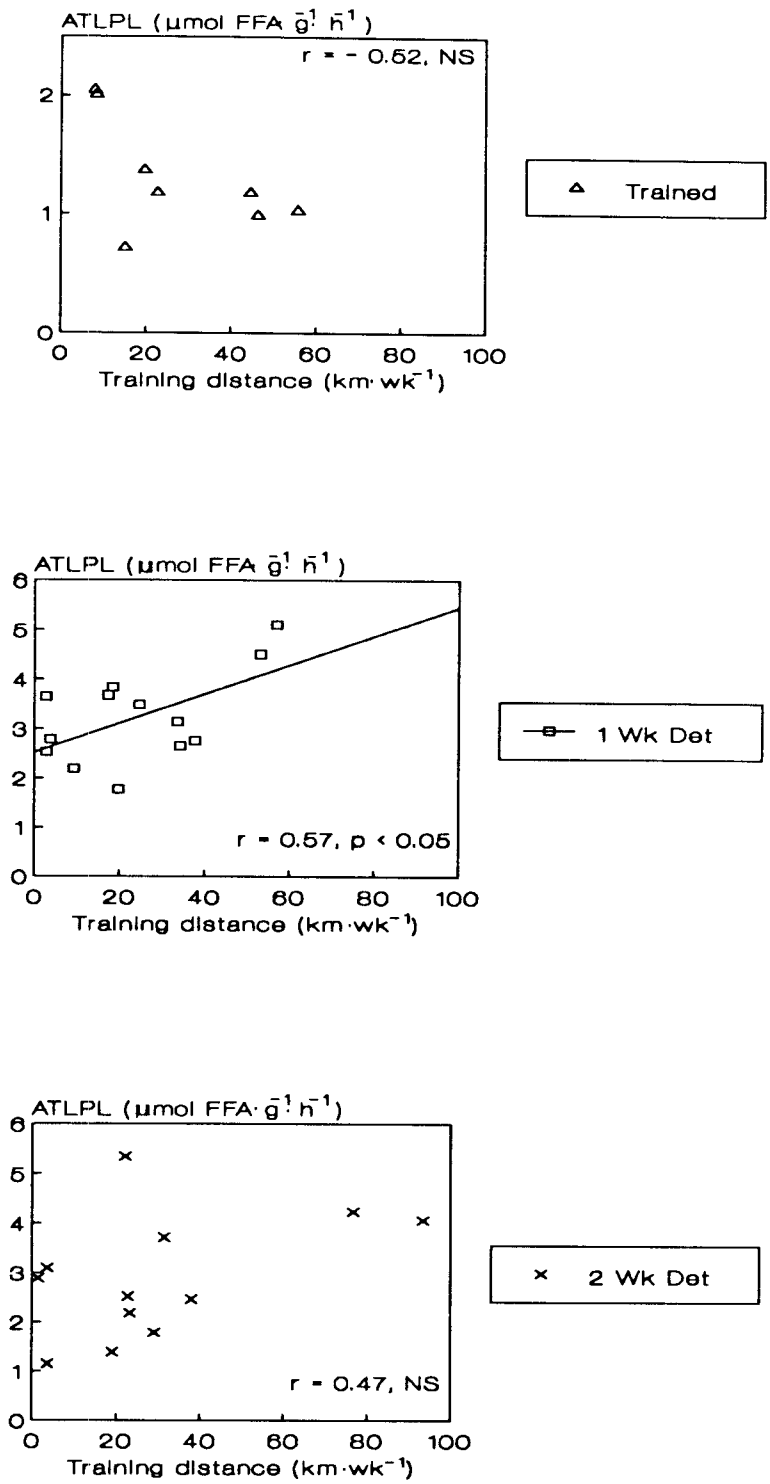

Figure 4.4. Relationship between ATLPL measured after acute, exhausting exercise and training distance in trained, 1- and 2-week detrained rats.

Table 4.6. Adipocyte diameter (μm) from epididymal fat pads of trained, 1- and 2-week detrained, and control rats (means $\pm$ standard errors of the mean).

Controls (n = 6)	**Trained** (n = 5)	**1 week Detrained** (n = 5)	**2 weeks Detrained** (n = 8)
90.9^{a} ± 6.2	61.0^{b} ± 1.4	77.7^{c} ± 2.1	$74.3^{c,d}$ ± 3.3

(Group means which do not share a common superscript are significantly different; ^{a} vs ^{b}, $p \leq 0.0001$, ^{a} vs ^{c}, $p < 0.05$, ^{a} vs ^{c,d}, $p < 0.005$, ^{b} vs ^{c}, and ^{c,d}, $p < 0.03$)

Discussion

In the present study, growing Long-Evans rats were exposed to a voluntary exercise stimulus, which was subsequently removed. The effects of this short-term intervention or perturbation in energy balance on changes in body energy stores were quantified.

Evidence for training adaptations to spontaneous wheel running:

The first important finding of this study was that spontaneous wheel running resulted in an attenuation in the rate of growth, body fat accretion and fat cell size in male Long-Evans rats, when compared to age-matched, sedentary control rats (Tables 4.5, 4.6). This study, therefore, confirmed results of previous studies which have shown that exercise training slows the rate of increase in fat cell size and body

fat accretion in both male and female growing rats, as well as lowering the rate of weight gain in male rats (Applegate et al., 1984, Arnold and Richard, 1987, Booth et al., 1974, Craig et al., 1983, Oscai et al., 1972, Oscai et al., 1973).

An important contribution of the present study is that it is the first study of detraining in rats which has quantified that rats had actually undergone adaptations to exercise training. Treadmill running time was significantly longer in trained rats when compared to both sedentary control rats, and to rats which had stopped training for 2 weeks. Therefore, rats have been accurately described as trained, detrained and sedentary, and metabolic differences may be attributed to differences in training status.

Increased feeding efficiency and lipogenic capacity: dissociation of detraining from refeeding:

Spontaneously exercising male rats in this study did not compensate for increased energy expenditure by increasing food energy intake and were leaner and weighed less than the sedentary control rats. Thus, when exercise training was terminated, it was not surprising that the rate of weight gain in these rats increased. Increased feeding efficiency, rapid fat cell accretion and increased lipogenic activity occurred in these rats which had stopped training, despite an unchanged food intake. It is not clear why, during the detraininig

period, these previously trained rats gained weight more rapidly than their sedentary counterparts, despite an equivalent food energy intake. It is possible that differences in feeding efficiency were a result of a greater positive energy balance in rats after stopping training when compared to sedentary controls.

Previous studies have described a similar increased feeding efficiency in rats and hamsters following the cessation of exercise training when "overfeeding" or eating more than sedentary, *ad libitum*-fed controls (Applegate et al., 1984, Applegate and Stern, 1987, Dohm et al., 1977, Sandretto and Tsai, 1988, Walberg et al., 1983). An enhanced feeding efficiency and rapid weight gain have also been demonstrated during *ad libitum* refeeding following a period of food energy restriction (Quig et al., 1983, Spencer et al., 1978, Taskinen and Nikkila, 1987). The mechanisms responsible for these adaptations in detrained rats have not yet been identified. Nor is it clear whether the nature of the signal for altering the metabolic sequelae following refeeding and the cessation of exercise training is the same.

Studies have shown that basal adipose tissue lipogenic activity is attenuated in the smaller fat cells of food restricted or exercise-trained individuals. However, post-prandial adipose tissue lipogenic activity and the insulin-stimulated glucose uptake by adipose tissue is actually higher

in smaller fat cells (Yost and Eckel, 1988, Savard et al., 1985, Savard and Greenwood, 1988, Taskinen and Nikkila, 1987). It has been suggested that the reduction in adipocyte size associated with chronic food restriction and/or exercise training sensitizes ATLPL response to feeding by some feedback regulation, though the details of the feedback loop are unknown (Taskinen and Nikkila, 1987). Miller et al. (1982) have suggested that the reduced fat cells have a high priority for lipid filling. This is supported by the observation of Tremblay et al. (1984) that deviations in adipocyte size from that of normal sedentary individuals set in motion regulatory mechanisms that tend to return the fat cell size to normal.

In the present study, there were significant differences in adipocyte diameter in trained, vs 1- and 2-week detrained rats and controls. The fact that ATLPL activity was also elevated in the detrained rats when compared to the trained rats is consistent with the "lipostat theory", that fat cell size regulates fat cell lipogenic activity when the training stimulus is removed.

These data are further supported by the finding of Fried et al. (1983), who studied changes in ATLPL activity in rats after a 3-day fast, and during 21 days of refeeding. Fasting resulted in an 80% reduction in adipocyte size and a decrease in total ATLPL activity. However, within 3-5 days of *ad libitum* refeeding, ATLPL activity was not different from

controls. After 10 days of refeeding, ATLPL activity was 60-100% higher than that of controls. After 20 days of refeeding, both ATLPL activity and adipocyte size were no longer different from values in *ad libitum*-fed controls.

Applegate et al. (1984) studied exercise training and short-term detraining in Osborne-Mendel rats. They found that *in vivo* lipogenesis, measured by incorporation of $^{3}H_2O$ into fat pads and total ATLPL activity, were attenuated with exercise training. After two weeks of detraining, both *in vivo* lipogenesis and ATLPL activity had returned to pre-training levels but were not higher than those of sedentary controls. They attributed the rapid rate of fat accretion in the detrained rats in their study to the higher food intake during the detraining period and the increased *in vivo* adipose tissue lipogenic rate associated with the detraining period.

Data from these two studies provide indirect evidence that the attenuation in the rate of change in adipocyte size associated with chronic food restriction and/or exercise training may be regulating the subsequent augmented lipogenic response during refeeding or following the cessation of training (Applegate et al., 1984, Fried et al., 1983).

There is also some evidence to suggest that refeeding following food restriction is not a sufficient stimulus for an overcompensation in adipose tissue lipogenic activity, and

that it is related specifically to "overfeeding" or the "overshoot" phenomenon which seems to occur when previously-food restricted rats eat more than control animals upon refeeding (Applegate et al., 1984, Applegate and Stern, 1987, Quig et al., 1983, Sandretto and Tsai, 1988). Taskinen and Nikkila (1987) found that basal and post-prandial ATLPL activity were lower following food restriction and weight reduction in obese women and only recovered partially during 180 days of refeeding a weight-maintaining, energy-controlled diet (i.e. a diet containing more food energy than the weight loss diet, but considerably less than an *ad lib* diet). Thus, increased lipogenic capacity only occurs when refeeding following food energy restriction is totally unrestricted and when the animal or organism displays a transient hyperphagia relative to non-food restricted controls.

This is perhaps the single most convincing argument for why the mechanism for enhanced feeding efficiency and *in vivo* lipogenic capacity must be different for refed-previously food-restricted rats compared to detrained-previously-trained rats whose food energy intake has remained constant.

Possible mechanisms for enhanced feeding efficiency, fat accretion and adipose tissue lipogenic activity in detrained rats:

In an attempt to identify a possible mechanism for the accelerated body weight gain and fat accretion which has been extensively described in detrained rats (Applegate et al., 1984, Craig et al., 1983, Dohm et al., 1977, Sandretto and Tsai, 1988), Arnold and Richard (1987) compared whole body energy balance in trained rats and rats which had detrained for 27 days with sedentary controls. They did this by measuring food energy intake, excreta and mass changes, and by determining the energy content of the carcasses of rats sacrificed after training and after 27 days of detraining. In addition, they measured brown adipose tissue content and GDP-binding of brown adipose tissue *in vitro*, as an indicator of changes in "regulatory thermogenesis" (Rothwell and Stock, 1979). These investigators were unable to explain the increased "metabolic efficiency" demonstrated with detraining by changes in the energy content of excreta, the energy cost of body fat and protein accretion, brown adipose tissue content or activity. They concluded that the "catch-up growth" seen in these detrained rats was due to increased energy intake per unit body mass compared to the sedentary controls.

However in the present study, if energy intake is corrected either for body mass or for "metabolic body size", there is still no difference in food energy intake between trained, detrained or control rats in Long-Evans rats fed a standard diet. Thus, the response to the cessation of training which

leads to enhanced body mass gain and fat accretion appears to be 'regulated' and related to exercise-induced adaptations.

There is little doubt that increased tissue sensitivity to insulin, which is a consequence of a reduction in adipocyte size, plays at least a permissive role in enhancing lipogenic activity during refeeding or following the cessation of training (Applegate et al., 1984, Craig et al., 1983, Schwartz and Brunzell, 1978, Taskinen and Nikkila, 1987).

Fasting plasma insulin concentrations and insulin secretion in response to a glucose load increase rapidly following the cessation of training, as tissue insulin sensitivity and glucose tolerance deteriorate (Heath et al., 1983, King et al., 1988). Indeed, Swinburn et al. (1991) have suggested that insulin resistance is the mechanism by which adipose tissue prevents any further lipid filling. They have speculated that decreased insulin sensitivity prevents further adiposity by decreasing glucose oxidation and storage, and thus, indirectly enhancing lipolysis and fat oxidation. Thus, the time course of changes in fat cell size following the cessation of training should mirror the changes in insulin sensitivity (Craig et al., 1983, Heath et al., 1983).

In the present study, we did not measure tissue insulin sensitivity. However, Craig et al. (1983) studied the time course of the effects of stopping training on insulin

sensitivity by measuring insulin-stimulated rates of glucose uptake and oxidation in isolated adipocytes of Wistar rats. They found that tissue insulin sensitivity was significantly attenuated with the cessation of training, but that even 9 days after stopping training, detrained rats were more insulin-sensitive than their sedentary counterparts. Based on these findings, and the previous discussion, it is not surprising that the adipocyte size of the detrained rats in the present study had not been entirely restored to that of sedentary controls.

However, it is unlikely that insulin is the only factor involved the regulation of adipose tissue lipogenesis. Changes in plasma insulin, in some cases, have been shown to be temporally dissociated from changes in ATLPL activity. For example, Walberg et al., (1983) found that increased ATLPL activity was present in young, obese Zucker rats prior to the onset of hyperinsulinaemia.

Adipose tissue lipoprotein lipase activity after an acute bout of exercise: time course and effects of training

Heparin-releasable ATLPL activity in spontaneously-trained rats in this study was higher 24 hours following an exhausting treadmill exercise bout than it was at 48 hours after stopping training. ATLPL activity was still attenuated 72 hrs after

maximal exercise, when compared to that sampled 24 hours after exercise.

The *in vitro* response of ATLPL following an acute bout of exercise seems to be highly variable. For example, acute, exhausting exercise in untrained rats has been associated with a reduction in total ATLPL activity, for up to 24 hours post-exercise (Barakat et al., 1981). On the other hand, Applegate and Stern (1987) found that total ATLPL activity in trained, Osborne-Mendel rats was lower 24 hours after moderate exercise than sedentary controls, and increased significantly only 72 hours post-exercise.

In the present study, there was no effect of acute exercise on ATLPL activity in sedentary control rats. This suggests that adipose tissue metabolism following acute, exhausting exercise is influenced by some physiological adaptation to exercise training which persists for up to two weeks following the cessation of training.

One possible explanation for the apparent differences in the acute effects of exhausting exercise on ATLPL activity between trained rats and control rats may be differences in circulating plasma catecholamine concentrations. Studies have demonstrated that epinephrine-infusion *in vivo* increases the heparin-releasable ATLPL activity measured in adipose tissue samples *in vitro* (Eckel, 1988). Kjaer et al. (1989) found

that plasma catecholamine concentrations in trained persons were higher during maximal exercise than in untrained persons, which may explain the attenuation of the post-exercise ATLPL response.

Moreover, the data from this study, and from that of Savard et al. (1987) suggest that the acute effect of exhausting exercise on ATLPL activity may exhibit a dose-response pattern. For example, Savard et al. (1987) found that the post-exercise increase in ATLPL activity was significantly correlated to total work output during a 90-minute cycle ergometer ride. In the present study, there was a trend for post-exercise ATLPL activity to be correlated to total treadmill run time in sedentary control rats ($r = 0.76$, $n = 6$, $p < 0.08$). There was, however, no relationship between total treadmill run time and ATLPL in trained and detrained rats, despite the fact that 2 week detrained rats had a mean treadmill running time which was not different from that of sedentary controls.

It is also not clear why the time course of changes in heparin-releasable ATLPL activity after acute exhausting exercise in this study differs from the post-exercise time course of changes in total ATLPL activity following moderate activity reported by Applegate and Stern (1987). The differences may be related to the intensity of exercise, or to

the fact that post-exercise changes in ATLPL activity in the Osborne-Mendel rats resulted from an increased food intake. However, these differences may also provide indirect evidence for the regulation of heparin-releasable ATLPL, *in vivo*.

As was mentioned previously, epinephrine-infusion *in vivo* increases the heparin-releasable ATLPL activity measured *in vitro*. However, if epinephrine is added to the incubation medium of adipose tissue which has not been pre-treated with epinephrine *in vivo*, then the measured heparin-releasable ATLPL activity actually decreases (Eckel, 1988). This suggests that epinephrine is involved in the mobilization (translocation) of the ATLPL from the intracellular pool to the capillary endothelium, *in vivo*, and this is reflected in the *in vitro* measurement of heparin-releasable (or membrane-bound) ATLPL.

It is possible that Applegate and Stern (1987) found no effect of acute exercise on ATLPL because they measured total enzyme activity. If epinephrine is involved in the translocation of the enzyme, then total ATLPL activity would not reflect this regulation, and would be unlikely to be affected by acute exercise.

Summary:

In summary, it was possible to compare *in vitro* adipose tissue lipogenic activity in trained rats vs untrained control rats or rats in a deconditioned state, and relate it to factors such as weekly training distance and feeding efficiency. The Long-Evans rats in this study did not become hyperphagic with detraining, which thus, dissociated the refeeding response from the effects of removing the exercise stimulus.

These results are in contrast to all existing studies, in which increased lipogenic activity and fat accretion in detrained rats has been linked to an increased food intake, especially in rat strains predisposed to obesity or in animals fed a high-fat diet (Applegate et al., 1984, Applegate and Stern, 1987, Arnold and Richard, 1987, Dohm et al., 1977, Sandretto and Tsai, 1988).

Therefore, the increased ATLPL activity and accelerated body mass gain which was observed in this study was attributed to the cessation of training *per se* and not due to an increase in food intake which has previously been reported to occur when training is terminated. Furthermore, the data from this study suggest that the sympathoadrenal system *in vivo* may be the effector which increases the activity of ATLPL. This theory requires further study.

In addition, this design enabled comparisons between trained and sedentary rats with regard to the control of ATLPL activity after an acute bout of exercise, and provided insight into the mechanisms which may stimulate membrane-bound ATLPL *in vivo*.

In this chapter, it has been effectively demonstrated that the cessation of exercise training results in at least a short-term augmentation of body mass gain and fat accretion which cannot be explained on the basis of differences in food energy intake. This effect seems to diminish as fat cell size "normalizes". The "internal regulator" for this response has not been identified, however, there is an apparent dose-response effect, related to the amount of training which is performed.

CHAPTER 5

EFFECT OF WEIGHT LOSS AND BODY COMPOSITION CHANGES WITH EXERCISE TRAINING AND/OR FOOD ENERGY RESTRICTION ON ENERGY EXPENDITURE IN OBESE MEN AND WOMEN

Introduction

In Chapter 3 of this dissertation, the responses to short-term perturbations in energy balance were measured directly in growing rats, during *ad libitum* eating following pre-weaning under- and over-nutrition. Although there were profound differences in body mass and rate of body fat accretion between rats raised in small and large litters immediately prior to weaning, there were no peristent effects of either intervention on post-weaning feeding efficiency, growth and body fat accretion.

Conversely, undernutrition in adult humans, previously in energy balance, has been shown to result in a decrease in resting energy expenditure. In 1969, Bray (1969) studied the metabolic response to food energy restriction and weight loss in previously obese women. He found that the mean resting energy expenditure in these women decreased by a total of 15% over a period of 21 days while they ingested 1.9 MJ of food energy per day. Despite this, mean body mass decreased by only 4-7%, and Bray pointed out that "the decline in oxygen consumption closely paralleled the slowing of weight loss". He suggested that these findings challenged two of the assumptions which form the basis of the expectation that weight loss will occur in proportion to the degree of caloric restriction, during a period of food energy restriction. These assumptions are that 1) basal energy requirements and 2) the efficiency of food energy utilization, do not change with dieting.

It is unclear whether the decline in resting energy expenditure, as a consequence of food energy restriction and weight loss, is proportional to the loss of body cell mass (de Boer et al., 1986, Donelley et al., 1991, Elliot et al., 1989, Foster et al., 1990, Fricker et al., 1991, Geissler et al. 1987, Heshka et al., 1990, Heymsfield et al., 1989, Hill et al., 1989, Luke and Schoeller, 1992, Nelson et al., 1992, Rumpler et al., 1991, Wadden et al., 1990, Webster and Garrow, 1989, Welle et al., 1984). There is some evidence to suggest that "reduced-obese" individuals have a lower energy expenditure per unit mass and fat-free mass when compared to weight-matched controls who have not undergone food energy restriction (Astrup et al., 1990, Fricker et al., 1991, Geissler et al., 1987, Heshka et al., 1990, Leibel and Hirsch, 1984, Luke and Schoeller, 1992).

Ravussin et al. (1985) used indirect calorimetry to study 24-hour energy expenditure in obese subjects before and after weight loss. These investigators were able to account for the entire attenuation of energy expenditure in their subjects on the basis of 1) the change in mass, and fat-free mass, 2) a lower thermic effect of feeding, in direct proportion to the decrease in food intake and 3) a lower thermic effect of activity, as a result of a smaller body mass.

Finally, several studies have examined the metabolic consequences of exercise training in combination with food energy restriction, or have compared exercise training with food energy restriction, in an attempt to manipulate the ratio of fat-free mass to total

body mass, while undergoing an energy deficit. The results of these interventions on resting energy expenditure and body composition are conflicting (Donnelly et al., 1991, Henson et al., 1987, Heymsfield et al., 1989, Hill et al., 1987, Hill et al., 1989, Mole et al., 1989, Tremblay et al., 1990).

Thus, the aim of the study reported in this chapter was to compare the effects of various modes of weight loss, rates of weight loss, and refeeding on the relationship between resting energy expenditure and fat-free mass (FFM). Moreover, this study examined the effects of variable food energy restriction, exercise training and refeeding on the thermogenic effect of feeding and epinephrine infusion in reportedly weight-stable persons with a high mass.

Methods

Subjects and research protocols:

Twenty-seven men and women between the ages of 27 and 58 years volunteered to undergo one of three self-selected protocols of food energy restriction for the purpose of weight loss. Subjects included 10 men and 17 women, with a mean starting mass of 100.2 ± SEM 21.6 kg, and percentage body fat of 36.5 ± SEM 6.0 %. With the exception of three of the men, all subjects were free from metabolic disease. One of the men was a non-insulin-dependent diabetic on oral hypoglycaemic agents, and the other two men were

being treated with ß-adrenergic receptor antagonists for hypertension. All subjects were cleared for participation by a medical doctor. Written, informed consent was obtained from each subject after all procedures were explained and the nature of the study was made clear. All procedures had been approved by the Ethics and Research Committee of the Faculty of Medicine of the University of Cape Town Medical School.

The three weight loss regimens included a diet-only group (D, n = 4), a diet and exercise group (DE, n = 13), and a very-low-energy diet group (VLED, n = 9). The intervention programme for each regimen is described in detail below.

Diet-only group: The diet-only group (D) was given initial dietary instruction by a registered dietitian. Each then received a booklet containing a list of food choices from the 6 dietary exchange groups, and the recommended number of servings from each exchange group for men and women, respectively (Table 5.1). The recommended daily energy intake was 4.5 $MJ \cdot d^{-1}$ for the women, and 5.2 $MJ \cdot d^{-1}$ for the men. The food energy restriction phase of this intervention programme was 12 weeks in duration. Subjects met weekly for the purposes of weighing, dietary education and to monitor adherence. All subjects underwent a voluntary period of refeeding after food energy restriction which will be described in detail.

Table 5.1 Recommended servings from dietary exchange groups for men and women in the diet-only group.

	MEN				WOMEN			
Exchange Group	**No**	**CHO (g)**	**FAT (g)**	**PROT (g)**	**No**	**CHO (g)**	**FAT (g)**	**PROT (g)**
Breads/Cereals	7	105	-	14	6	90	-	12
Dairy products	2	24	-	16	2	24	-	16
Fruits	3	30	-	-	3	30	-	-
Vegetables	1	5	-	2	1	5	-	2
Meats	4	-	12	28	3	-	9	21
Fats	5	-	25	-	4	-	20	-
Energy (kJ)		2800	1400	1000		2500	1100	900
% total energy		53%	27%	20%		56%	25%	19%

Very-low-energy diet group: The very-low-energy diet constituents are presented in Table 5.2. This dietary regimen (VLED) provided a daily energy intake of 2.0-2.5 MJ, for a period of only 4 weeks. The 4 week period of intervention was chosen for two reasons. Firstly, it was anticipated that weight losses over 4 weeks on a very-low-energy diet would be comparable to those over 12 weeks on a low-energy diet (Donnelly et al., 1991). Secondly, 4 weeks was the longest period of intervention which was not likely to result in unacceptable side effects. In fact, 1 of the 9 subjects in the VLED group did develop cardiac arrhythmias during the course of treatment.

Table 5.2. Composition of the very-low-energy formula diet (450 Diet, 450 Health Foods (Pty) LTD, 155 Jan Smuts Ave., Parkwood 2193, South Africa).

Nutrient	Men	Women
Carbohydrate ($g \cdot d^{-1}$)	75	60
Fat ($g \cdot d^{-1}$)	5	4
Protein ($g \cdot d^{-1}$)	60	48
Energy ($kJ \cdot d^{-1}$)	2470	1970
Vitamin A (% RDA)	125	100
Vitamin C (% RDA)	125	100
Vitamin B_1 (% RDA)	125	100
Vitamin B_2 (% RDA)	125	100
Niacin (% RDA)	125	100
Ca^{2+} (% RDA)	250	200
Iron (% RDA)	125	100
Folic Acid (% RDA)	125	100

Diet and exercise group: The diet and exercise group (DE) followed an identical dietary regimen to the diet-only group. Participants in the exercise programme attended 3 supervised exercise classes per week for 16 weeks. Classes consisted of a 15 minute pre-exercise warm-up period which included range-of-motion exercises. This was followed by 15 minutes of muscle strengthening activities and a 20-25 minute period of walking and jogging.

Respiratory gas measures were collected during exercise classes using Douglas bags in 6 of the 12 subjects, to estimate the

energy cost of a typical exercise session. Using indirect calorimetry, as described below, the average total energy expenditure for the entire duration of the exercise class was approximately 1500 kJ. Warm-up and stretching activities required 191 ± 42 W (11.5 ± SEM 2.5 $kJ \cdot min^{-1}$); muscle strengthening activities required 492 ± 103 W (29.5 ± SEM 6.2 $kJ \cdot min^{-1}$); and walking and jogging accounted for 660 ± 88 W (39.6 ± SEM 5.3 $kJ \cdot min^{-1}$).

The efficacy of the exercise training programme was measured in the first 8 participants using an incremental treadmill test to volitional exhaustion. The protocol has been described in detail previously (Noakes et al., 1990). Briefly, subjects were required to walk or jog on a motor-driven treadmill, starting at a speed of 2.22 $m \cdot s^{-1}$ (8 kph) and 0% gradient, and increasing in speed 0.14 $m \cdot s^{-1}$ (0.5 kph) every 30 seconds. Oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were determined each minute, throughout the test, using the respiratory gas analysis system described below. Peak VO_2 and peak treadmill speed were the parameters used to assess the efficacy of the training programme.

Voluntary "Refeeding": After each period of dietary intervention, subjects underwent a period of voluntary "refeeding". The subjects were instructed to increase their voluntary food energy intake, so that it was not different from their food energy intake per unit fat-free mass during the pre-trial period. The refeeding period was 3-4 weeks in duration. The mean reported food energy intake per unit fat-free mass prior

to the trial for all groups was 140 ± SEM 10 kJ·kg $FFM^{-1}·d^{-1}$. This was not statistically different from the mean food energy intake reported during the refeeding period, 135 ± 14 kJ·kg $FFM^{-1}·d^{-1}$. The reported food intake during the period of food energy restriction ranged from a minimum of 31 kJ·kg $FFM^{-1}·d^{-1}$ for one of the subjects in the VLED group to a maximum of 149 kJ·kg $FFM^{-1}·d^{-1}$ in one of the subjects from the DE group.

Body composition:

Four skinfold sites were used to estimate body composition using the regression equations of Durnin and Womersley (1974). These included: triceps, biceps, subscapula and suprailiac. The biceps and triceps skinfold thicknesses were measured as vertical folds in the midline of the upper arm, halfway between the acromion process and the olecranon process. The subscapular skinfold thickness was measured as an oblique fold just below the inferior angle of the scapula. The suprailiac skinfold was measured as a slightly oblique fold, taken 3 cm above the anterior, superior iliac crest. Fat-free mass was calculated as the difference between estimated fat-mass and total body mass.

Resting energy expenditure:

All subjects were familiarized with the measurement techniques on a pre-trial visit. Resting oxygen consumption (VO_2) was measured in the morning, in the post-absorptive state, after a minimum of 30 minutes of supine rest. Subjects in the DE group were asked

to refrain from physical activity on the day prior to each measurement of resting energy expenditure. Respiratory exchange measures were collected for a 30 minute period and the mean VO_2, VCO_2 and the respiratory exchange ratio (VCO_2:VO_2, RER) were determined for the resting state.

VO_2 was measured using a ventilated-hood, open-circuit system for indirect calorimetry. The subject's head was placed in a clear plastic hood (internal volume: 23 litres), while lying in the supine position. Unidirectional air flow was ensured via flaps located around the neck/head region and room air was drawn through the hood by a suction diaphragm pump at a flow rate of approximately 40 litres·min $^{-1}$, or 3-4 times the resting minute ventilation. This flow rate ensured that the oxygen content of the air leaving the hood was not lower than 95% of ambient air. The flow rate was calibrated using a Tissot spirometer (Warren E. Collins, Inc., Braintree, Mass) before and after each test.

The vaccuum pump directed expired air to a small mixing chamber. Mixed, expired air was sampled continuously for oxygen and carbon dioxide content using an Ametek S-3A/1 Oxygen Analyzer and Ametek CD-3 Carbon Dioxide Analyzer (Pittsburgh, Pennsylvania), respectively. Analyzers were calibrated before and after each test using analytical grade gases of known concentration. VO_2, VCO_2, and RER were then calculated each minute for the duration of the trial using a microcomputer and software (Craig Mason-Jones, Lateral Alternative, Cape Town, South Africa). Respiratory exchange data were used to calculate energy

expenditure and substrate utilization using conventional conversion equations (Weir, 1949).

For the purposes of the remainder of this dissertation, absolute resting energy expenditure measured by indirect calorimetry will be reported as W, or relative to mass or fat-free mass, as $W \cdot kg^{-1}$. While it is recognized that W (Watts or $J \cdot sec^{-1}$) is the SI unit for the rate of energy production or power, the use of W is more commonly associated with mechanical energy production. Therefore, absolute energy expenditure will also be reported as $kJ \cdot min^{-1}$ or extrapolated to $MJ \cdot d^{-1}$ in the text and figures which will allow more ease of comparison with reported food energy intake in the present study, as well as with previous studies in the literature.

In addition, measured resting energy expenditure was compared to the average resting energy expenditure predicted from a regression equation determined on a sample population of 160 women and men from this laboratory (REE, W = 17.66 + 1.065 FFM or $MJ \cdot d^{-1}$ = 1.562 + 0.092FFM, Chapter 10). The predicted value was subtracted from the measured value and expressed as W. Ravussin et al. (1989) have previously reported a lower than average measured minus predicted resting energy expenditure as a "risk factor" for subsequent weight gain during long-term follow-up.

Glucose-induced increment in resting energy expenditure:

The glucose-induced increment in energy expenditure was measured in the D and DE groups, only. After a period of 50 minutes of supine rest in the post-absorptive state, subjects were then fed a chilled glucose solution, consisting of 100g glucose diluted in 400 ml of water. The solution was fed through a drinking tube while subjects remained in the supine position.

The post-glucose increment in energy expenditure was monitored from the 30th to the 120th minute post-ingestion. Respiratory exchange samples were taken every other 10 minute interval for 10 minutes. In this way, two subjects could undergo testing simultaneously. The gas collection periods were from minutes 30-40, minutes 50-60, minutes 70-80, minutes 90-100, and minutes 110-120. The area under the curve was calculated for the period from 30 to 120 minutes following glucose feeding. The area under the curve for resting energy expenditure was estimated for the same time span, based on the assumption that resting metabolic rate remained constant and was not subject to significant variation in a test of such short duration. The glucose-induced increment in energy expenditure was considered to be the total W increment in energy expenditure over resting energy expenditure for the period from 30 to 120 minutes post-ingestion.

Serial blood samples were obtained using an indwelling cannula in the antecubital vein which was kept patent with a slow saline

infusion. Plasma was stored at -20° C, for later analysis of glucose and insulin concentrations in response to glucose ingestion. Glucose was determined enzymatically, using the Beckman Autoanalyser (Beckman Instruments Inc., Diagnostic Systems Group, Brea, California), and insulin was measured using a radioimmunoassay (Phadeseph Insulin RIA, Pharmacia Diagnostics, Upsala, Sweden).

Epinephrine-induced increment in energy expenditure:

The epinephrine-induced increment in resting energy expenditure was measured for all three trials. In the case of the D and DE trials, these tests took place on a separate day to the initial determination of resting energy expenditure, and the glucose-induced increment in energy expenditure. Prior to determination of resting energy expenditure, a plastic cannula to be used for epinephrine infusion was introduced into the antecubital vein. On the opposite side, another cannula was introduced into the antecubital vein for the purpose of serial venous blood sampling for the determination of free fatty acid concentrations.

After a minimum of 30 minutes of supine rest, respiratory exchange measures were collected for a 30 minute period, using a ventilated-hood, open-circuit system for indirect calorimetry, and the mean VO_2, VCO_2 and RER were determined for the resting state.

For the first hour of each trial, during the determination of resting energy expenditure, 0.9% NaCl (saline) was infused at a rate of 0.5 $ml \cdot min^{-1}$. Thereafter, epinephrine (adrenalin tartrate) in 0.9% saline was infused at a rate of $0.03 \mu g \cdot kgFFM^{-1} \cdot min^{-1}$ for 60 minutes. This infusion dose has been found previously to result in an increase in plasma concentrations of epinephrine and norepinephrine, reaching steady-state over a relatively short time. This dose also results in an increase in resting oxygen consumption, thus, providing an indirect measure of *in vivo* catecholamine sensitivity.

Indirect calorimetry, as described previously, was used to determine the increment in resting energy expenditure as a result of epinephrine infusion, for the duration of the 60 minute infusion. Blood samples were obtained via the indwelling cannula every 15 minutes throughout the infusion for the determination of serum free fatty acid concentrations. Heart rate was monitored by ECG throughout the infusion.

Serum free fatty acid concentrations were determined by enzymatic colourimetric assay (Boehringer Mannheim 1082-914 test combination).

Statistical analyses:

All data are expressed as means and standard errors of the mean ($\pm$ SEM). Cross-sectional group comparisons, for variables such

as "rate of weight loss" were made using a one-way analysis of variance. Tukey's post-hoc analyses was performed where significant F ratios were found to determine which groups were significantly different from one another. For comparisons between groups and across time, a two-way analyses of variance for repeated measures was performed. In this way, the main effects of group and time (test period) could be examined after correcting for the effects of subjects nested within the groups. Post-hoc tests were performed as described above. In addition, the two way analyses quantified the interaction effect of groups over time.

Simple linear regression was performed to compare the slope and intercept for the regression of resting energy expenditure against fat-free mass, across treatment periods.

Results

Peak VO_2 and peak treadmill speed:

Peak oxygen uptake and treadmill speed, measured only in the DE group, were significantly higher after training than before training ($p < 0.03$). Peak VO_2 was 20.5 ± 1.8 ml $O_2 \cdot$STPD $kg^{-1} \cdot min^{-1}$ prior to training and 24.7 ± 2.7 ml O_2 STPD$\cdot kg^{-1} \cdot min^{-1}$ after training. Similarly, peak treadmill speed increased from 2.83 ± 0.22 $m \cdot s^{-1}$ (10.2 ± 0.8 kph) before training to 3.25 ± 0.22 $m \cdot s^{-1}$ (11.7 ± 0.8 kph) after training. There was no difference

in the mean peak RER in response to exercise before or after the training programme (1.03 ± 0.03 vs 1.07 ± 0.01 for pre- and post-training, respectively).

Changes in mass and body composition:

Changes in body mass and body composition for the three treatment groups are presented in Table 5.3. Starting mass and fat-free mass were significantly higher in the DE group, compared to the D and VLED groups ($p < 0.05$). The DE group had a significantly greater decrease in body mass after treatment than either the D or VLED groups (-11.7 ± 1.5 kg vs -5.0 ± 1.0 and -5.6 ± 0.7 kg, respectively, $p < 0.004$). However, when initial body mass was used as a covariate, these differences were no longer significant. Thus, the heaviest people lost the most weight and the overall weight loss was not different between groups.

Fat-free mass decreased significantly after each treatment ($p < 0.0001$), but there were no differences in the loss of fat-free mass between groups. There were also no differences in the ratio of fat-free mass to total mass lost between treatment groups (Table 5.3). Starting mass had no effect on the changes in body composition with each treatment and was not significantly correlated to the ratio of fat-free mass to total mass lost after treatment.

Resting energy expenditure and the regression of resting energy expenditure against FFM:

Day-to-day variability in 3 pre-trial determinations of resting energy expenditure was calculated in 7 subjects. The coefficient of variation was 3.39%.

Absolute resting energy expenditure (W) decreased significantly in all groups after weight reduction ($p < 0.001$, Table 5.4), and did not increase with refeeding. However, when resting energy expenditure was expressed relative to total mass or fat-free mass ($W \cdot kg^{-1}$), there was no significant change in resting metabolic rate as a result of food restriction and there was no interaction between treatment groups over time (Table 5.4). The change in resting energy expenditure as a result of all treatments was significantly positively correlated to the change in mass ($r = 0.74$, $p < 0.0001$) and the change in fat-free mass ($r = 0.42$, $p < 0.04$).

Absolute resting energy expenditure was regressed against mass and fat-free mass prior to weight reduction, after weight reduction and after refeeding (Figure 5.1). When the individual slopes and y-intercepts of these relationships were compared prior to weight reduction, after weight reduction and refeeding, there were no significant differences which could be attributed to weight loss and body composition changes through dieting and/or exercise.

Table 5.3. Changes in mass and body composition in response to dietary intervention (D), exercise and dietary intervention (DE), and very-low-energy dietary intervention (VLED, means ± SEM).

Group	Time	Mass (kg)	FFM (kg)	Fat Mass (kg)	Rate of Weight Loss ($kg \cdot wk^{-1}$)	Ratio FFM: Mass Loss
D (n=4)	Pre	[a] 91.0 ±8.0	[a] 55.7 ±5.5	35.3 ±3.3		
	Post	86.1 ±7.7	54.0 ±4.9	32.1 ±3.6	0.42^{c} ±.09	0.52 ±.27
	Refeed	86.6 ±8.5	55.3 ±5.7	31.3 ±3.2		
DE (n=12)	Pre	[b] 113.1 ±5.5	[b] 71.2 ±4.0	42.2 ±3.9		
	Post	101.4 ±5.1	67.7 ±3.5	34.3 ±4.3	0.80^{c} ±.11	0.28 ±.09
	Refeed	100.9 ±5.2	67.5 ±3.8	33.1 ±4.4		
VLED (n=8)	Pre	[a] 83.3 ±3.4	[a] 54.2 ±2.5	28.9 ±1.8		
	Post	77.7 ±2.8	52.5 ±2.4	25.1 ±1.6	1.36^{de} ±.19	0.23 ±.10
	Refeed	77.0 ±2.6	52.7 ±2.4	24.3 ±1.5		

(Means which do not share a common superscript are significantly different; [a] vs [b] $p < 0.05$ for FFM, DE > D and VLED, Pre > Post and Refeed, [c] vs [d] $p < 0.002$ for rate of weight loss for VLED and D, [c] vs [e] $p < 0.01$ for rate of weight loss for VLED and DE)

This was true, whether or not the data from the D and VLED groups were combined or considered separately. Thus, the changes in resting energy expenditure were proportional to the change in mass and fat-free mass (Figure 5.1).

Table 5.4. Resting energy expenditure (W, $kJ \cdot min^{-1}$, $W \cdot kg^{-1}$, $W \cdot kg\ FFM^{-1}$, means ± SEM).

Group	Trial	W	$kJ \cdot min^{-1}$	$W \cdot kg^{-1}$	$W \cdot kgFFM^{-1}$
D (n=4)	Pre	^{a}78.8 ±8.0	4.73 ±.48	0.87 ±.05	1.42 ±.05
	Post	^{b}75.3 ±8.5	4.52 ±.51	0.88 ±.08	1.40 ±.08
	Refeed	^{b}77.8 ±7.8	4.67 ±.47	0.92 ±.08	1.42 ±.10
DE (n=12)	Pre	^{a}99.0 ±7.2	5.94 ±.43	0.90 ±.03	1.38 ±.05
	Post	^{b}88.5 ±4.5	5.31 ±.27	0.90 ±.03	1.32 ±.03
	Refeed	^{b}90.0 ±4.5	5.40 ±.27	0.92 ±.03	1.35 ±.05
VLED (n=9)	Pre	^{a}66.5 ±2.0	3.99 ±.12	0.80 ±.03	1.23 ±.03
	Post	^{b}62.8 ±3.0	3.77 ±.18	0.82 ±.03	1.20 ±.05
	Refeed	^{b}59.7 ±2.2	3.58 ±.13	0.78 ±.03	1.15 ±.07

(^{a} vs ^{b}, $p_1 < 0.001$, REE, W_1 Pre > Post, Refeed, NS difference in REE, $W \cdot kg^{-1}$, and $W \cdot kg\ FFM^{-1}$)

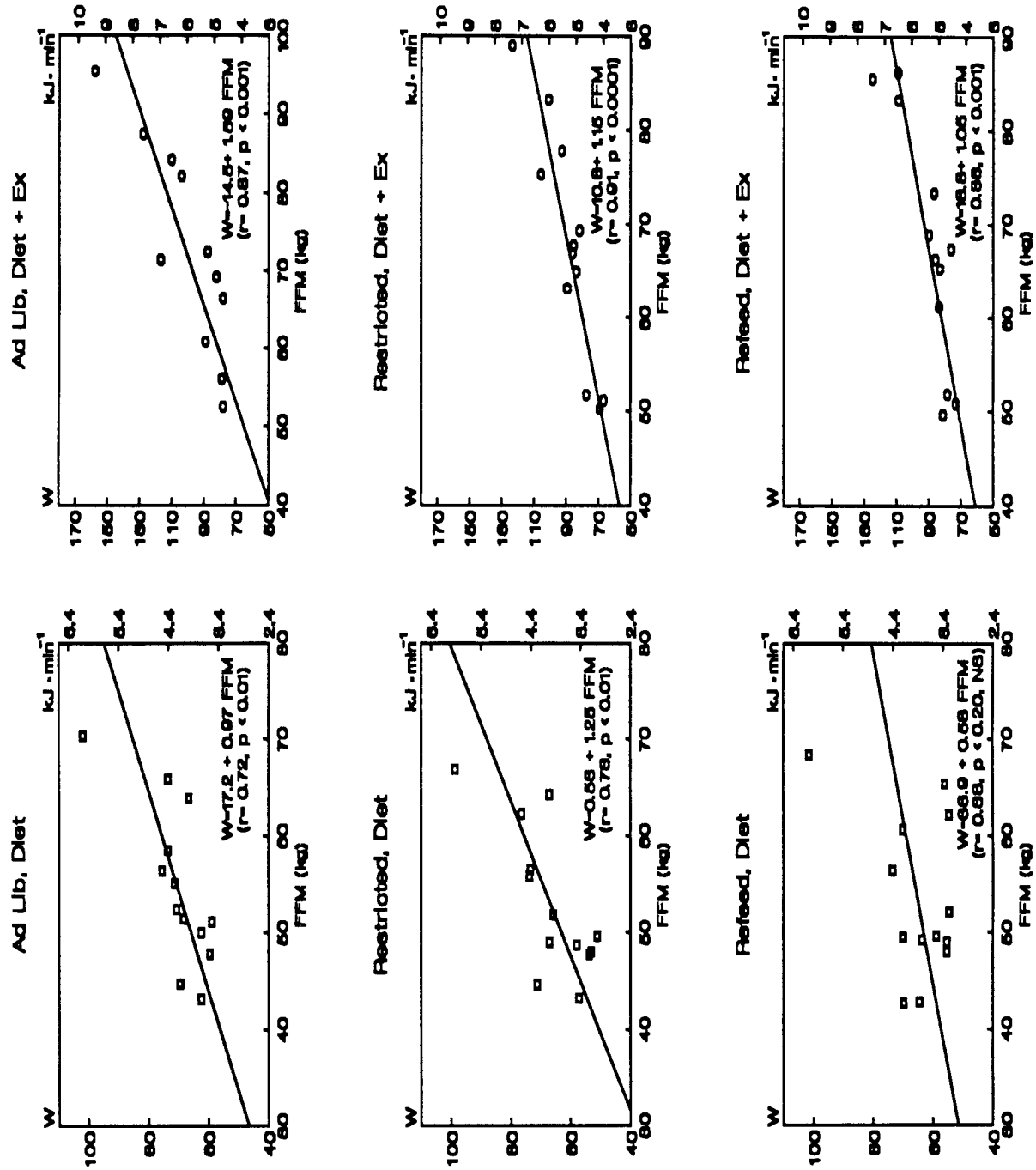

Figure 5.1. Resting energy expenditure regressed against FFM for the DE group and the D and VLED groups combined, prior to dieting, after dieting, and after "refeeding". The slope and y-intercept of this relationship were not different after dieting or refeeding.

Measured resting expenditure compared to predicted resting energy expenditure:

The measured-minus-the-average predicted resting energy expenditure was not significantly different prior to intervention when compared to the periods of food energy restriction and refeeding in all groups (-0.24 ± 2.49 vs -4.31 ± 1.66 and -4.72 ± 2.32 W, for pre- and post-intervention, and refeeding, respectively).

However, there was a significant and negative correlation between the measured-minus-the-average predicted resting energy expenditure and the amount of weight lost during intervention (Figure 5.1a., $r = -.062$, $p < 0.01$).

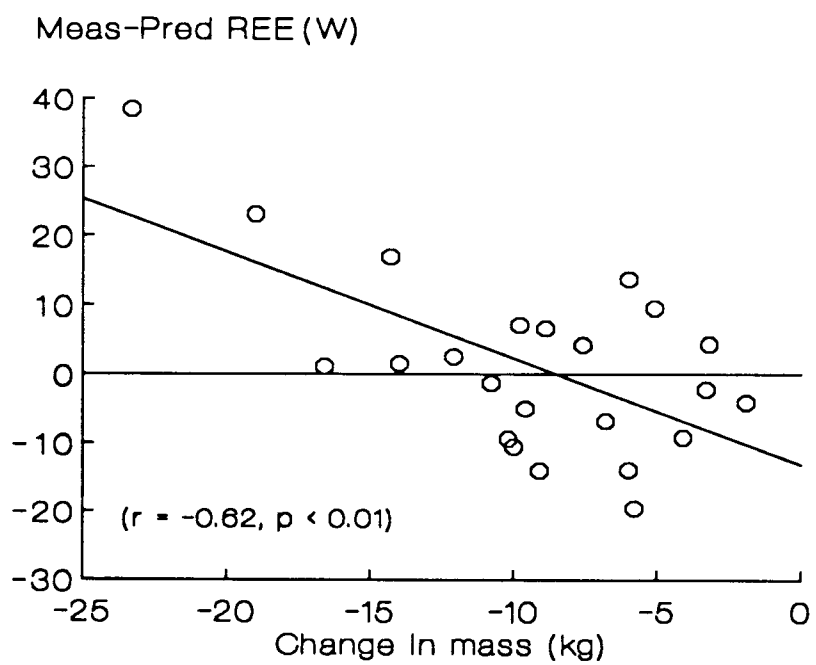

Figure 5.1a. The relationship between the measured-minus-the average-predicted resting energy expenditure and weight lost following intervention.

Glucose-induced increment in resting energy expenditure:

There was a tendency for the glucose-induced increment in energy expenditure in both the D and DE groups to be attenuated by food energy restriction and to return to baseline levels after refeeding, but this was not statistically significant (Figure 5.2, Figure 5.3, $p < 0.06$). Even after covarying for changes in resting energy expenditure and fat-free mass, glucose-induced thermogenesis was not significantly altered as a result of food energy restriction and exercise training. Nor was there any significant relationship between the change in mass or fat-free mass and the change in the glucose-induced increment in energy expenditure.

The respiratory exchange ratio was lower at rest in the fasting state, and at 30, 90, and 120 minutes following glucose ingestion in both groups, after weight loss ($p < 0.05$), prior to refeeding. Total carbohydrate (CHO) and fat oxidation were calculated, and total CHO oxidation in response to glucose ingestion was attenuated during the period of food energy restriction in both groups ($p < 0.05$, Figure 5.4). Total fat oxidation in response to an oral glucose load did not change with weight loss and food energy restriction. Three-four weeks of partial refeeding did not entirely restore glucose oxidation rates at rest or in response to glucose feeding.

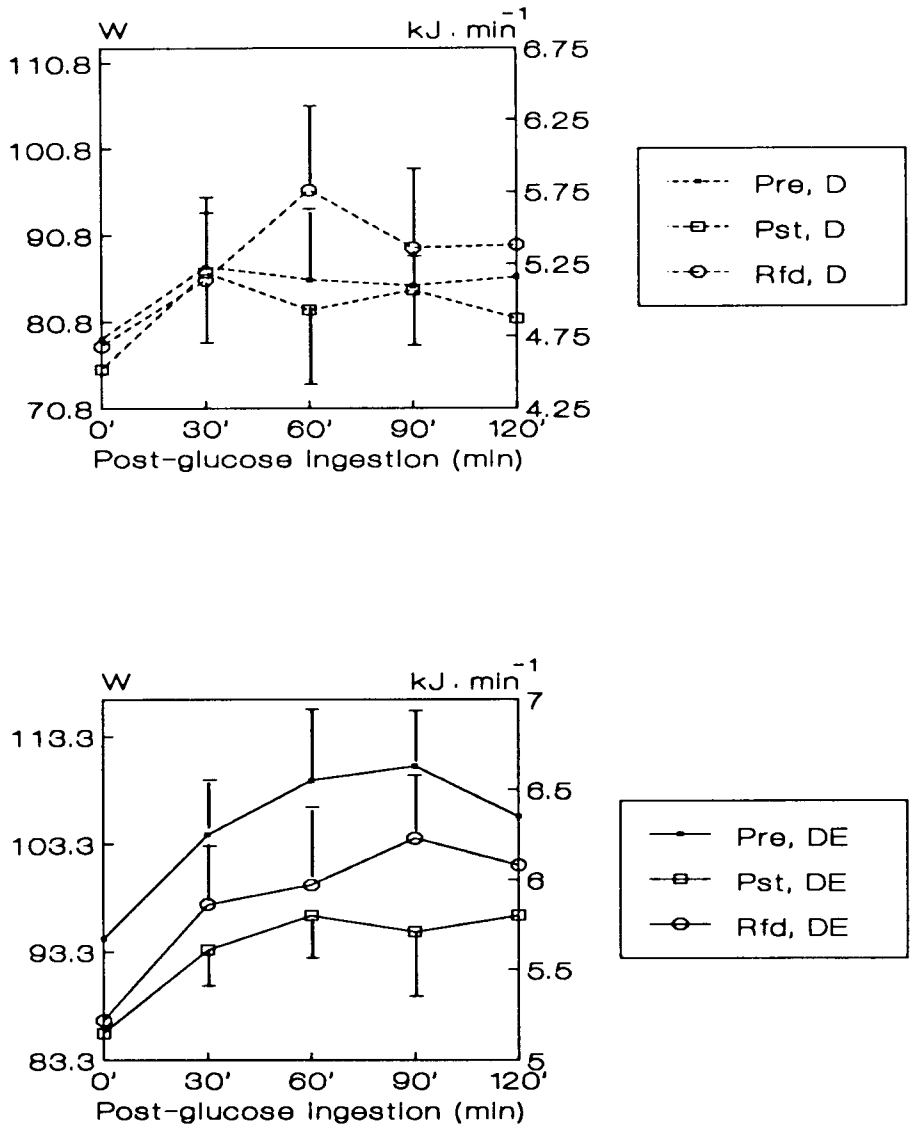

Figure 5.2. Glucose-induced increment in resting energy expenditure (W, or $kJ \cdot min^{-1}$) prior to weight loss, after weight loss and refeeding for diet-only (D) and exercise and diet (DE) groups.

Fasting plasma glucose concentration and the change in plasma glucose concentration following glucose ingestion were not different between groups as a result of weight loss through exercise and/or dietary intervention (Table 5.5).

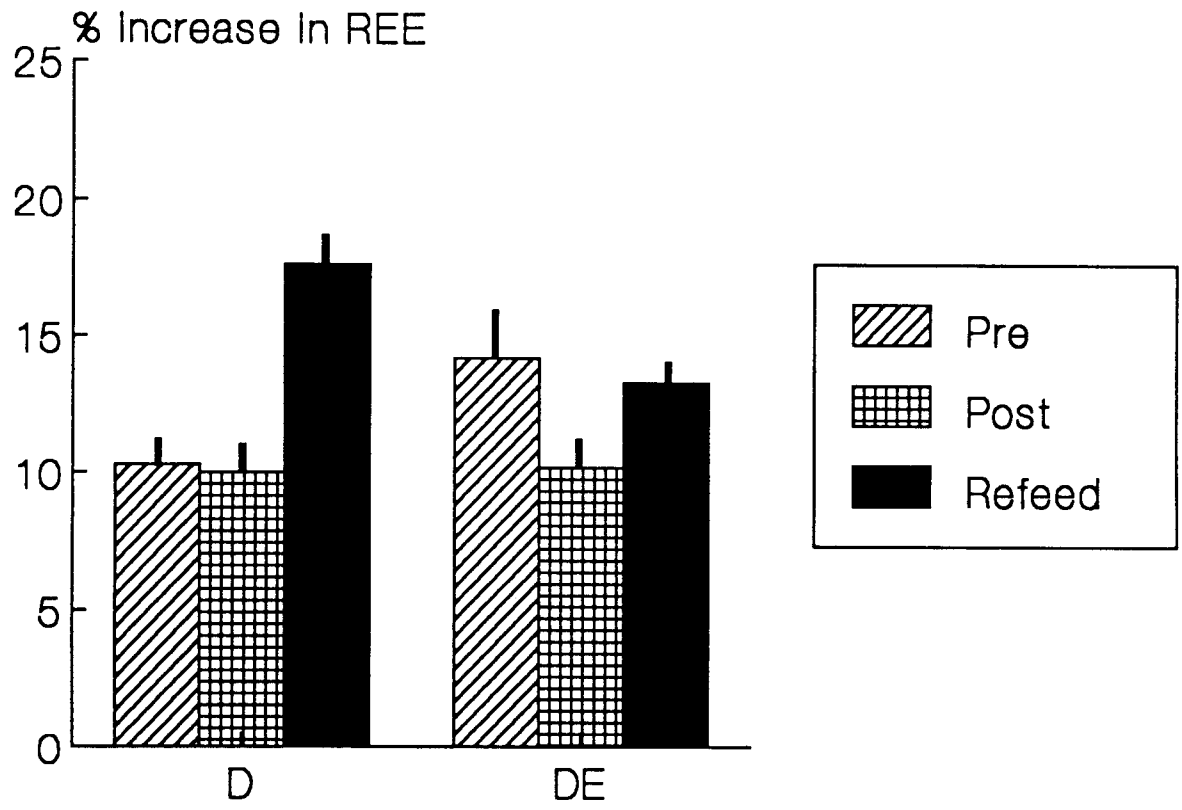

Figure 5.3. Glucose-induced increment in resting energy expenditure (% increase from 30-120 minutes post-glucose ingestion) for D and DE groups, prior to weight loss, after weight loss and during refeeding.

Conversely, fasting plasma insulin concentration was significantly lower in both treatment groups following weight loss ($p < 0.05$, Table 5.5). However, the insulin response to glucose ingestion was not attenuated with weight loss, as a result of exercise and/or dietary intervention (Table 5.5).

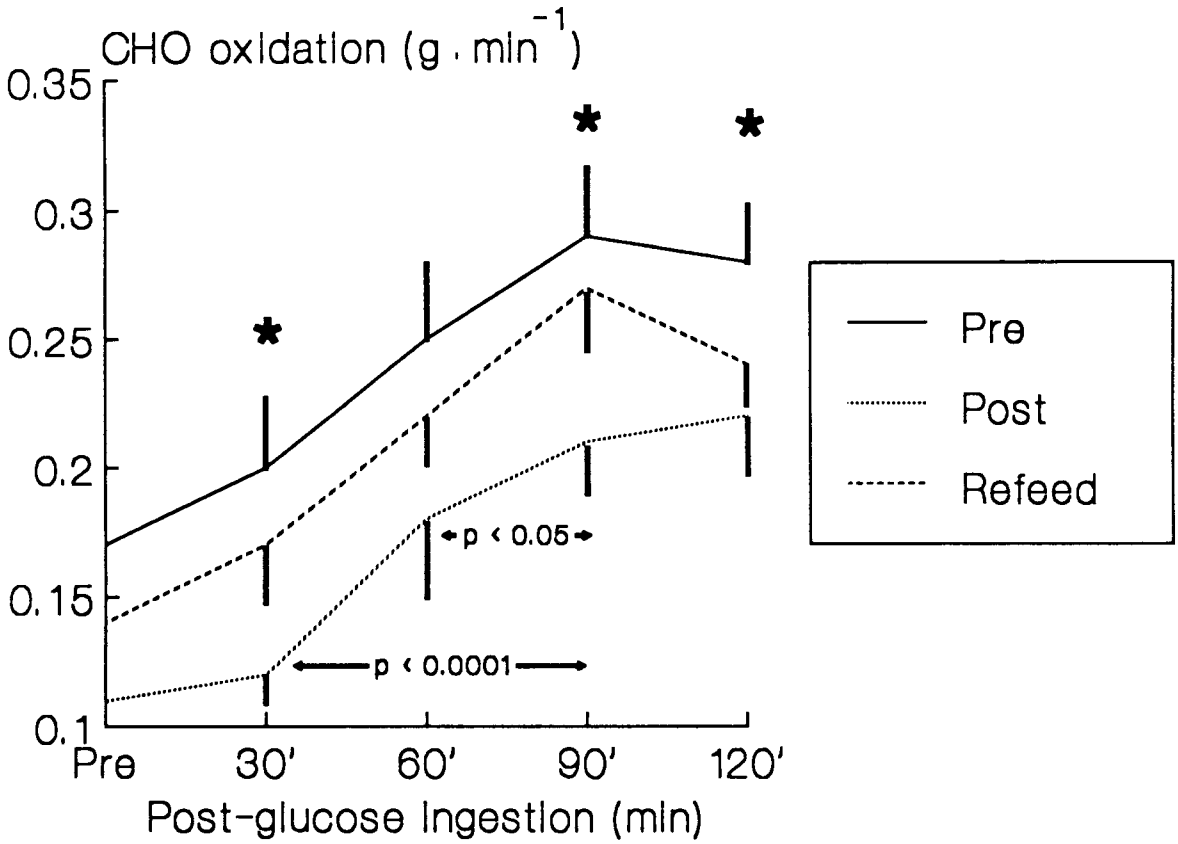

Figure 5.4. Total carbohydrate oxidation at rest and in response to glucose feeding in D and DE groups combined, before and after weight loss and during refeeding (* $p < 0.05$ for pre vs post at 30, 90 and 120 minutes post-ingestion).

Epinephrine-stimulated increment in energy expenditure:

The mean epinephrine-induced increment in energy expenditure was higher across all treatments in the D (combined with VLED) group when compared to the DE group ($p < 0.001$). There was no significant effect of dietary intervention, weight loss or refeeding on the epinephrine-stimulated increment in energy

expenditure, when expressed as a percentage increase in area under the curve over resting metabolic rate (Figure 5.5a). This was true, even after covarying for resting energy expenditure.

Table 5.5 Plasma glucose and insulin concentrations at rest and in response to glucose ingestion in D group compared to the DE group, prior to intervention, after weight loss, and refeeding (means ± SEM).

		Glucose (mM)				
Group	**Trial**	**Pre**	**30′**	**60′**	**90′**	**120′**
D	**Pre**	5.3 ±.4	8.6 ±.4	8.2 ±.8	8.1 ±1.7	7.5 ±1.5
	Post	5.1 ±.3	7.5 ±.2	7.2 ±.7	7.2 ±.8	8.0 ±.7
	Refeed	5.0 ±.2	7.7 ±.4	8.1 ±.6	8.0 ±.6	7.2 ±.5
DE	**Pre**	5.8 ±.4	7.9 ±.6	7.8 ±1.1	7.5 ±2.5	7.3 ±.7
	Post	5.1 ±.4	7.5 ±.9	8.4 ±.9	7.4 ±.7	7.3 ±.6
	Refeed	5.3 ±.4	7.1 ±.6	7.6 ±.9	6.5 ±1.3	7.1 ±1.0

		Insulin ($\mu U \cdot ml^{-1}$)				
Group	**Trial**	**Pre**	**30′**	**60′**	**90′**	**120′**
D	**Pre**	11.0[a] ±2.5	64.9 ±16.5	81.6 ±31.8	68.2 ±22.2	50.3 ±11.9
	Post	7.8[b] ±3.6	50.5 ±19.5	61.9 ±15.7	65.7 ±16.6	68.7 ±13.7
	Refeed	7.9[b] ±5.2	52.7 ±16.5	64.0 ±16.9	67.4 ±20.6	79.2 ±25.4
DE	**Pre**	17.4[a] ±5.1	60.5 ±10.1	72.3 ±14.2	78.1 ±19.2	69.2 ±13.7
	Post	7.4[b] ±1.7	51.0 ±10.5	58.3 ± 7.3	64.2 ± 8.9	48.8 ± 9.5
	Refeed	7.9[b] ±1.1	60.2 ± 9.2	61.0 ± 7.8	57.6 ± 5.8	57.5 ±11.0

([a] vs [b], means which do not share a common superscript are significantly different, $p < 0.05$).

When energy expenditure was compared at specific time intervals throughout the epinephrine infusion (15, 30, 45 and 60 minutes), there was a significant time by treatment interaction effect ($p < 0.005$, Figure 5.5b). There was little change in energy expenditure (W or $kJ \cdot min^{-1}$) in the diet-only intervention groups (D and VLED) during food energy restriction. In the DE group, there was a marked reduction in energy expenditure during epinephrine infusion with food energy restriction and weight loss.

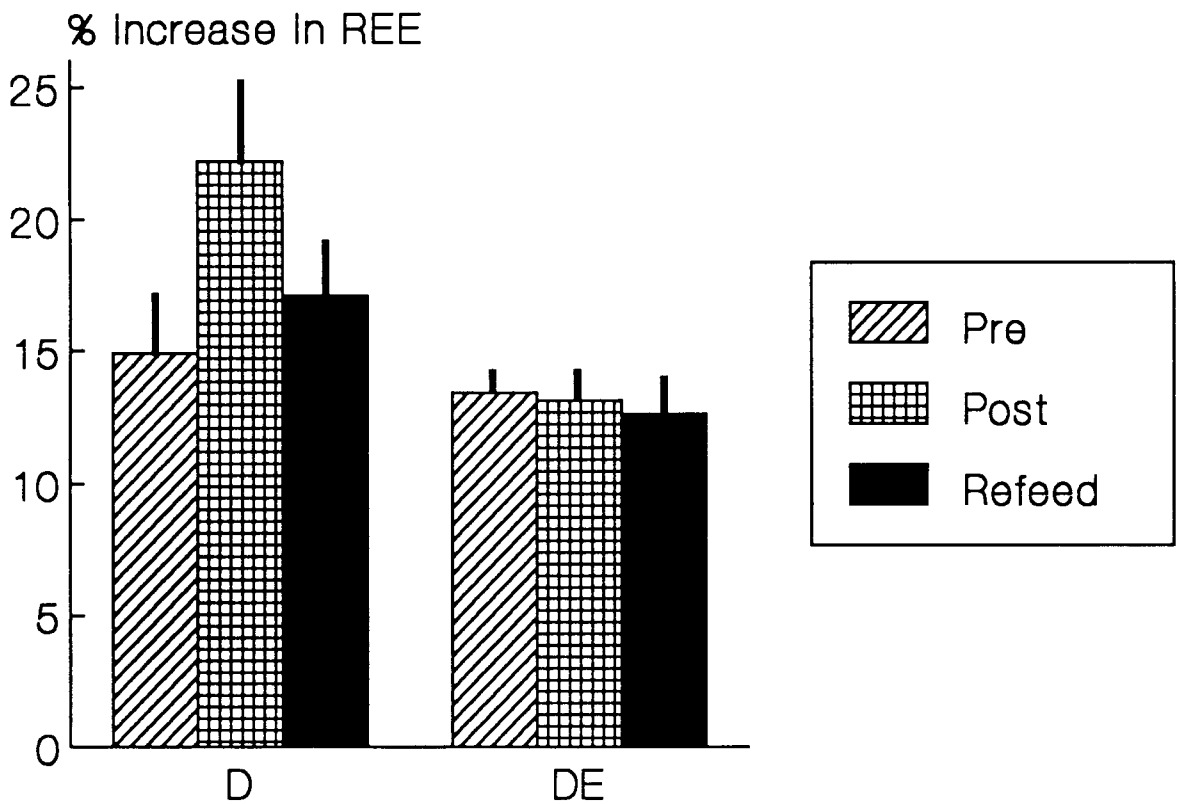

Figure 5.5a. Percent increase in energy expenditure in response to epinephrine infusion, expressed as total area under the curve (W), for the D and VLED groups combined (D) compared to the DE group.

However, after refeeding, epinephrine-induced energy expenditure in the DE group returned to near-baseline levels, despite the fact that there was no increase in body mass which could have accounted for this increase in energy expenditure.

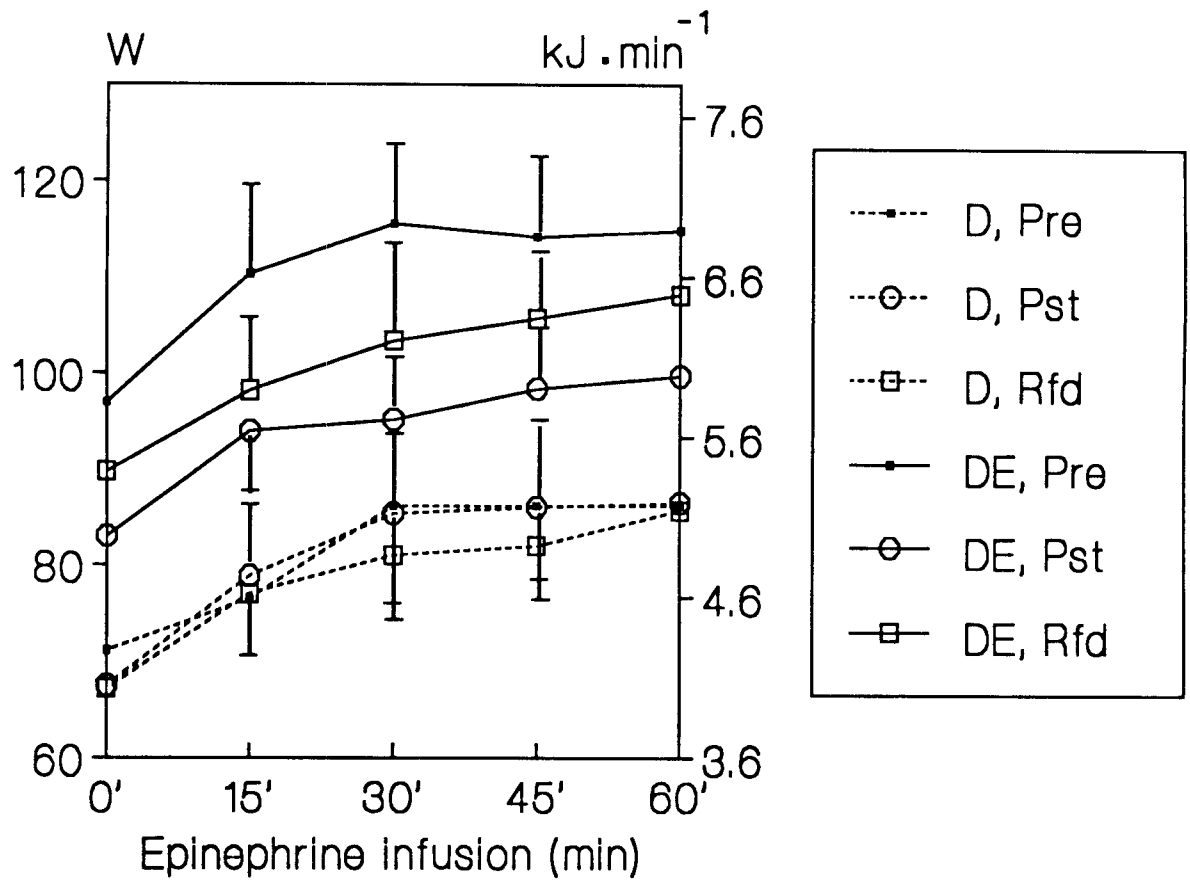

Figure 5.5b. Epinephrine-induced increment in resting energy expenditure during 0-60 minutes infusion for D and VLED groups combined, vs DE group, prior to intervention, after weight loss, and refeeding (Significant interaction effect for time by treatment, $p < 0.005$).

There was no effect of dietary intervention or exercise training on the relative contribution of carbohydrate and fat to total oxidative metabolism during epinephrine infusion. Nor were there any differences between groups, before and after weight loss, in fasting plasma free fatty acid concentrations (FFA) and the change in plasma FFA concentrations in response to epinephrine infusion (Table 5.6).

Table 5.6. Fasting plasma free fatty acid concentration ($mmol \cdot l^{-1}$) prior to and during epinephrine infusion in the D (combined with VLED) group compared to the DE group (means ± SEM).

Group	Trial	Pre-infusion	Epinephrine-Stimulated
D	Pre	0.61^{a} ±.07	1.04^{b} ±.10
	Post	0.69^{a} ±.08	1.19^{b} ±.12
	Refeed	0.64^{a} ±.09	1.06^{b} ±.11
DE	Pre	0.59 ±.09	0.84 ±.13
	Post	0.51^{a} ±.05	1.15^{b} ±.16
	Refeed	0.49^{a} ±.06	0.99^{b} ±.17

(^{a} vs ^{b}, $p < 0.05$, means which do not share a common superscript are significantly different).

Discussion

The effect of weight loss, rate of weight loss, method of weight loss and changes in body composition on resting energy expenditure:

The most important finding of the present study was that the change in resting energy expenditure subsequent to voluntary exercise and/or voluntary food energy restriction and weight loss was not different between groups undergoing moderate food restriction for 12 weeks, exercise training or very-low-energy dieting for 3-4 weeks. When resting energy expenditure was expressed per unit body mass, or per unit fat-free mass, there was no significant decrease in resting metabolic rate, as a result of food energy restriction and weight loss.

It is, however, important to examine the significance of the lack of change in REE, expressed per unit FFM, after changes in mass and fat-free mass with food energy restriction in the present study. If the ratio of REE to FFM does not change under conditions where FFM is significantly reduced, this suggests that the individual has a lower-than-average predicted resting energy expenditure per unit FFM. This is largely a consequence of the positive y-intercept of the regression of REE against FFM, which makes it impossible to "standardize" the expression of REE to FFM under conditions where FFM or the constituents of FFM are changing. This problem is dealt with in detail in Chapter 10,

and further explains the need to examine the change in the slope of the relationship between REE and FFM.

The slope and intercept of the linear regression of resting energy expenditure and fat-free mass did not change significantly as a result of weight loss and changes in body composition. Thus, the reduction in absolute resting metabolic rate with food energy restriction in this study could be attributed largely to the loss of total body mass and lean body mass, and was not unexpected.

The original observation of Bray in 1969, that the "decline in oxygen consumption closely paralleled the slowing of weight loss" and the attenuation of absolute resting energy expenditure are now widely accepted as a part of the metabolic sequelae resulting from food energy restriction and weight loss (Belko et al., 1987, de Boer et al., 1986, Donahoe et al., 1984, Donelley et al., 1991, Elliot et al., 1989, Foster et al., 1990, Franssila-Kallunki et al., 1992, Fricker et al., 1991, Geissler et al. 1987, Heshka et al., 1990, Heymsfield et al., 1989, Hill et al., 1989, Luke and Schoeller, 1992, Nelson et al., 1992, Rumpler et al., 1991, van Dale and Saris, 1989, Wadden et al., 1990, Webster and Garrow, 1989, Welle et al., 1984). The data from the present study suggest that the attenuation of resting energy expenditure with food energy restriction was proportional to "metabolically active tissue" or body cell mass and was not a result of a hypometabolic state. These data also suggest that neither the degree of energy deficit (very-low energy diet vs low-energy

diet) nor the mode of energy deficit (diet or exercise) influenced the relationship between the change in resting energy expenditure and body cell mass.

Results from previous studies which have compared the effects of food energy restriction to those of exercise combined with food energy restriction on resting energy expenditure, are not consistent. This lack of agreement may be attributed to differences in experimental design, subject selection, differences in intervention protocols, the degree of energy deficit, and the degree of adherence. It is not within the scope of this dissertation to evaluate the psychosocial aspects of adherence to dietary and exercise intervention. Therefore, for the purposes of this discussion, changes in resting energy expenditure will be considered in relation to changes which have occurred in body mass and total body energy stores for the various modes of intervention.

Several previous studies have investigated the effects of exercise training when combined with food-energy restriction on the relationship between loss of body mass, fat-free mass and the change in resting energy expenditure. Mole' and colleagues (1989) examined the effects of food energy restriction and refeeding on resting energy expenditure in a small sample of high-mass men and women. Subjects underwent two weeks of very-low food energy intake (2.1 $MJ \cdot day^{-1}$), followed immediately by 2 weeks of exercise combined with food energy restriction, and then another 2 weeks of partial refeeding (4.8 $MJ \cdot day^{-1}$). Mean

resting energy expenditure, expressed absolutely, or relative to $mass^{0.67}$, was significantly depressed during the diet-only period, and not different from baseline during the diet-and-exercise period.

These investigators concluded that exercise added to food energy restriction attenuated the decline in metabolic rate which occurred as a result of food energy restriction alone. However, resting energy expenditure was measured daily throughout the exercise training period, and it is possible that the effects of exercise training on resting energy expenditure demonstrated in that study were a result of the residual effects of the last bout of exercise (see Discussion, Chapter 6).

In the study by Hill and colleagues (1987), the effects of exercise training and/or very-low-energy intake on metabolic rate, thyroid function and body composition were compared under metabolic ward conditions. In that study, absolute resting energy expenditure declined in both groups over the 6 week period of food energy restriction, despite the fact that the exercise group lost less fat-free mass than the diet-only group.

In a similar study (Heymsfield et al. 1989), two groups of women were food restricted for 5 weeks. One group expended an estimated additional 1.45 $\pm$ 0.25 MJ per day in supervised exercise. The additional energy deficit in the form of exercise did not have any effect on the rate of weight loss. However, the decline in resting metabolic rate over the 5 weeks was more rapid

in the exercising group, and there was a significant attenuation of resting metabolic rate relative to fat-free mass. Heymsfield et al. (1989) attributed this decline in resting metabolic rate, relative to fat-free mass, to an increased body water retention, and an overestimation of the metabolic activity of the fat-free mass.

One possible explanation for the differences between the studies of Hill et al. (1987) and Heymsfield et al. (1989) and the present study, may be that exercise training results in a greater glycogen-water storage. Consequently, there may be a smaller-than expected decrease in fat-free mass with exercise training without any change in the metabolic activity of the fat-free mass. This is further supported by data from these studies which showed that exercise training had no effect on nitrogen balance.

Henson et al. (1987) found that both absolute resting metabolic rate and metabolic rate expressed per unit fat-free mass, were depressed with low energy dieting in overweight women. In that study, exercise training of sufficient duration and intensity to increase exercise capacity did not attenuate the decline in resting metabolic rate as a result of weight loss. Henson and colleagues (1987) found that the decrease in resting energy expenditure was greater than that which would have been predicted on the basis of change in fat-free mass alone.

However, the raw data may be extracted from Figure 3 in the paper by Henson et al (1987), and a regression equation for resting

energy expenditure against fat-free mass may be plotted for each of the treatment periods; prior to dieting, after 3 weeks of dieting, after exercise training, and after the resumption of dieting. The Y-intercept and slope of these regressions did not change with changes in treatment and energy balance (Figure 5.6).

Thus, these findings suggest, as in the present study, that the decline in resting energy expenditure with food energy restriction in moderately overweight individuals is proportional to the change in fat-free mass, and that exercise training in combination with food energy restriction does not alter this relationship.

Changes in resting energy expenditure: degree of food energy restriction and relative weight loss

Previous studies have also attempted to compare the effects of various levels of food energy restriction on resting energy expenditure and weight loss. Wadden et al. (1990) and Foster et al. (1992) compared very low energy diets, similar to the one in the present study (2 $MJ \cdot day^{-1}$) with diets consisting of 2.8 $MJ \cdot day^{-1}$, 3.4 $MJ \cdot day^{-1}$ (Foster et al., 1990) and 5 $MJ \cdot day^{-1}$ (Wadden et al., 1990) for a period of 12-16 weeks. After each period of food energy restriction, food intake was gradually increased to 4.2 $MJ \cdot day^{-1}$ (Foster et al., 1992) or between 5.2 and 6.2 $MJ \cdot day^{-1}$ (Wadden et al., 1990).

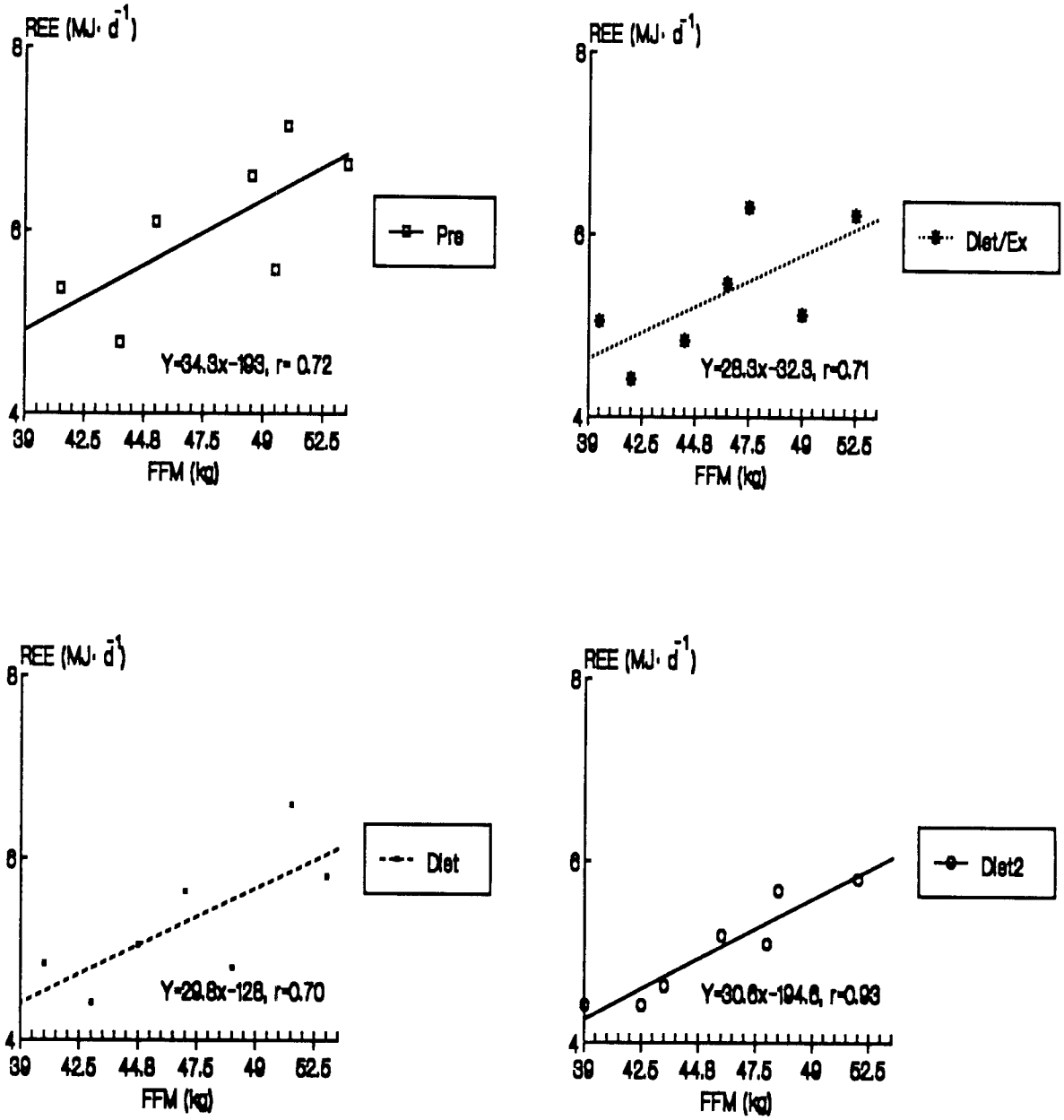

Figure 5.6. The relationship between resting energy expenditure ($MJ \cdot d^{-1}$) and fat-free mass (kg) in women prior to weight reduction (Pre), after 3 wks ingesting a diet (3.3 $MJ \cdot d^{-1}$, Diet), after 3 wks of dieting and exercise (5 $d \cdot wk^{-1}$, Diet/Ex), and 3 additional weeks of dieting with no exercise (Diet2). Data was extracted from Figure 3, Henson et al., 1987.

There was a significantly greater weight loss and loss of fat-free mass and attenuation of resting energy expenditure (absolute and per kg fat-free mass) in the subjects ingesting the very-low-energy diet compared to those persons ingesting the low energy diet. However, after a period of 6 weeks "refeeding", there were no differences between dietary treatments. Differences between these studies and the study reported in this chapter may be explained, in part, by the shorter duration of food energy restriction in those subjects ingesting the very-low-energy diet (Foster et al., 1992, Wadden et al., 1990).

In a recent study by Heshka et al.(1990), it was suggested that the standardization of expression of the change in resting metabolic rate with weight loss and loss of fat-free mass should be examined further. They concluded that 40% of the variance in REE with weight loss was not accounted for by changes in FFM, and found that the change in REE, after adjusting for a change in FFM, was related to the change in body fat with weight loss.

Thus, they suggested that subjects should be compared on the basis of percentage weight loss relative to starting mass. They found that in individuals losing more than 25% of their starting mass there was a greater decline in REE, after adjusting for differences in FFM, than in those subjects losing less than 25% of their starting mass. However, in the present study, there was no difference in the relationship between REE and FFM, even when the sample was subdivided into a group of subjects losing less

than 10% of their starting mass, compared to a group of subjects losing at least 10% of their starting mass.

There are circumstances, however, under which the change in resting energy expenditure cannot be explained on the basis of changes in fat-free mass during food energy restriction. In the study by Keys et al. (1950), young men volunteers underwent 24 weeks of semi-starvation followed by a period of 12 weeks of refeeding. Figure 5.7 illustrates the relationship between the original data for resting oxygen uptake plotted against the fat-free mass for each subject, prior to intervention, after 24 weeks of starvation, and after 12 weeks of refeeding.

The expected linear relationship between resting energy expenditure and fat-free mass is demonstrated in these subjects prior to intervention ($r = 0.56$, $p < 0.001$). However, it is clear that following semi-starvation, and refeeding, this relationship is no longer linear. These differences may be related in part to the changes in total body water which may accompany these extremes of undernutrition and subsequent refeeding. This may result in an overestimation of the fat-free mass. In the study by Keys et al. (1950), subjects were gaining weight during refeeding. Thus, this also illustrates that the interpretation of the relationship between resting energy expenditure and fat-free mass may be inaccurate if subjects are not in energy balance at the time of measurement.

Resting energy expenditure: effects of refeeding following food energy restriction

In the present study, absolute resting energy expenditure during refeeding following food energy restriction was lower than that measured prior to dietary intervention. However, when energy expenditure was expressed relative to changes in body size and fat-free body mass, there were no differences. This occurred despite (i) subjects failing to gain weight during refeeding, (ii) the DE group continuing to exercise, and (iii) reported food energy intake per kg FFM not being different prior to intervention and during refeeding. Thus, these data do not support the popular notion that food energy restriction results in a persistent attenuation of energy expenditure and an increased metabolic efficiency.

Boyle et al. (1978) studied the effects of food energy restriction and subsequent refeeding in Sprague Dawley rats. They found that in the first week of refeeding, the rats demonstrated a 10-20 fold higher feeding efficiency and weight gain compared to controls. Leibel and Hirsch (1984) compared the food energy required for "reduced-obese" subjects to remain weight-stable for a period of 7 days with that required by non-obese, non-reduced persons. Reduced-obese subjects selected were those who had previously undergone food energy restriction (2.7 $MJ \cdot day^{-1}$) for an average of 202 days. The food energy required for the reduced-obese subjects to maintain weight was significantly less than the non-obese, non-reduced group, when

expressed per m^2 body surface area. Leibel and Hirsch (1984) suggested that increased efficiency resulted in the poor long-term efficacy of dietary intervention for weight loss.

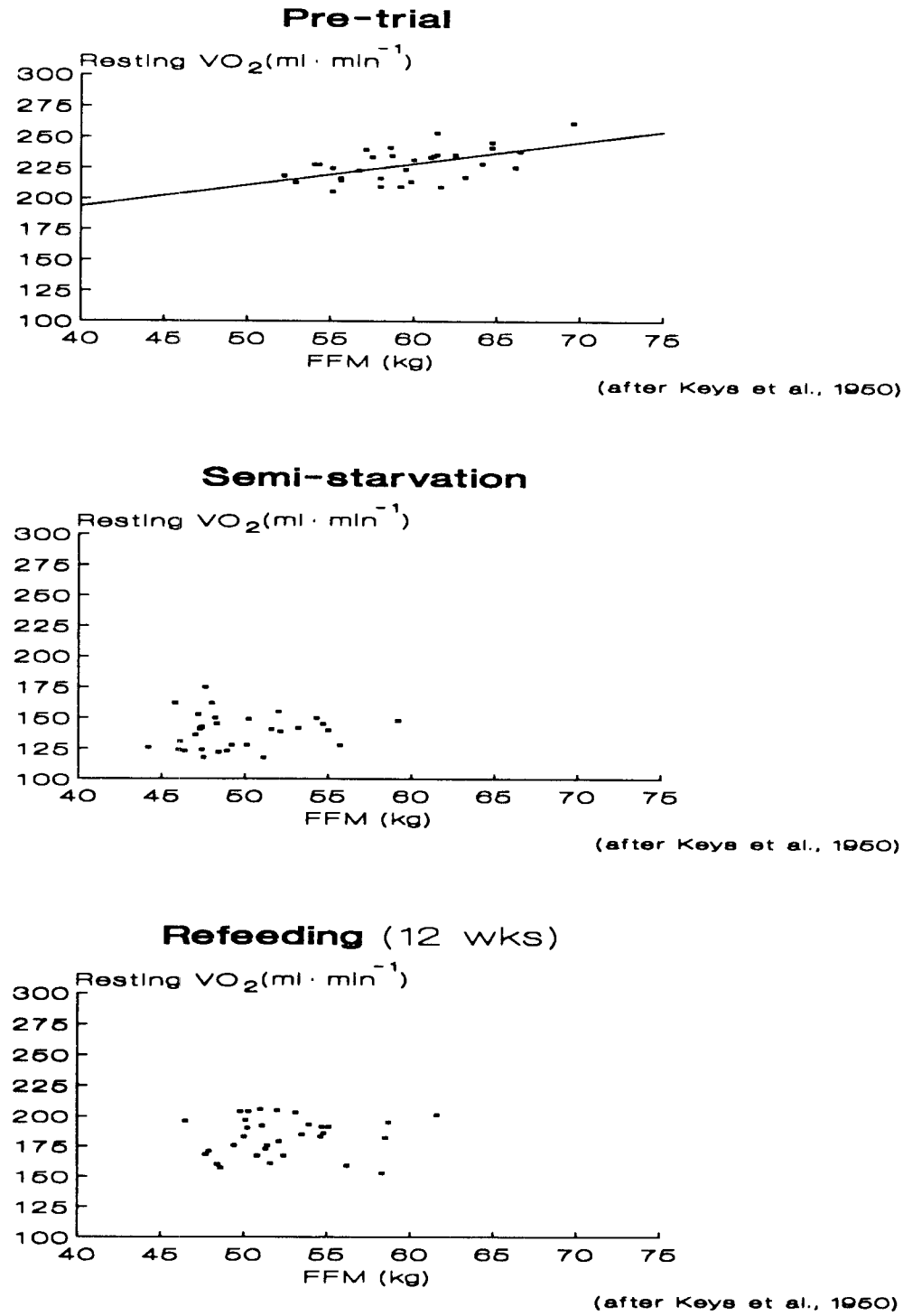

Figure 5.7. The relationship between resting oxygen uptake and fat-free mass in young men prior to undergoing semi-starvation, after 24 weeks of semi-starvation and after 12 weeks refeeding (Data extracted from Keys et al., 1950).

In studies in which subjects undergo "partial refeeding", or in which food energy intake is still below that which was reportedly ingested during the *ad libitum* period of weight maintenance prior to intervention, absolute resting energy expenditure is attenuated (Elliot et al.,1989, Foster et al.,1990, Froidevaux et al., 1992, Keys et al., 1950, Mathieson et al.,1986, Mole et al.,1991, Wadden et al., 1990).

However, if as in the present study, food energy intake during refeeding is adjusted for changes in body mass or fat-free mass, or if resting energy expenditure is expressed relative to fat-free mass, there is little difference in pre- and post-treatment resting energy expenditure (Foster et al., 1990, Mathieson et al., 1986, Wadden et al., 1990).

It is evident that the "degree" of refeeding influences the change in body weight and resting energy expenditure following food energy restriction. Keys et al. (1950) studied healthy young men after period of 24 weeks of semi-starvation. During the refeeding period, subjects gained weight and resting energy expenditure increased. However, in subjects who were only partially-refed, the change in resting metabolic rate with refeeding was delayed, and the magnitude of this change was attenuated.

Measured-minus-average-predicted resting energy expenditure: effects of food energy restriction and refeeding:

Another important finding in the present study, was that measured resting energy expenditure was not different than the average resting energy expenditure predicted from a heterogeneous sample of 160 men and women from this laboratory (Chapter 10) even after weight loss and food energy restriction.

In a recent study by Ravussin and coworkers (1988), a lower-than-average predicted resting metabolic rate in a sample of Southwest American Indians, after adjusting for age, mass and body composition, was identified as a "risk factor" for subsequent weight gain during a 21 month follow-up period.

These findings may be interpreted to suggest that individuals at risk for gaining weight have a genetic predisposition for a lower energy expenditure and subsequent body weight gain. It is also possible that the lower-than-average predicted energy expenditure suggests that the subjects were food energy restricted or not in energy balance during their initial assessment. This possibility is further suggested by the observation in the study by Ravussin et al. (1988) that the resting energy expenditure was "normalized" following a period of weight gain.

In the present study, the lack of evidence for a significantly lower-than-average predicted resting energy expenditure provides further support for the argument that changes in resting energy

expenditure following weight loss were proportional to changes in body cell mass, and not the result of a "hypometabolic state" or enhanced metabolic efficiency.

However, persons with a higher measured-minus-average-predicted energy expenditure in the present study lost more weight than those whose measured-minus-average-predicted weight loss was closer to zero or negative. This finding may indirectly support the study by Ravussin and coworkers (1988), suggesting that a higher-than-average metabolic rate predicts long-term success in weight loss. Conversely, these results may suggest that persons who lost less weight may have already been "restrained eaters" or partially food restricted at the start of the study.

Thermic effect of glucose feeding: effects of food restriction, weight loss and refeeding

Another important finding from this study was that the glucose-induced increment in resting energy expenditure was attenuated with food energy restriction and returned to baseline levels during partial refeeding. Although this effect did not reach statistical significance ($p < 0.06$, repeated measures ANOVA), data from a subsequent chapter of this dissertation (Chapter 6) suggests that, in fact, this response was physiologically meaningful. In Chapter 6, it was found that repeated exposures to the procedure for glucose-induced thermogenesis resulted in a significant reduction in the metabolic response to glucose feeding on the third exposure. Thus, the enhanced metabolic

response to refeeding in the present study may have been partially masked by this effect of repeated exposures.

These results are consistent with the findings of Astrup et al. (1990), Belko et al. (1989), den Besten et al. (1988), and Nelson et al. (1992). Nelson et al. (1992) studied the thermic effect of mixed-meal feeding in 24 moderately-overweight post-menopausal women, prior to weight reduction, following weight reduction and after 10 days of refeeding. Energy expenditure was measured for 6 hours after the ingestion of a liquid meal in which the energy content was adjusted for differences in fat-free mass between subjects and treatment periods. The thermic effect of feeding was significantly attenuated immediately following weight reduction, but had returned to baseline levels after 10 days of refeeding.

Conversely, studies by Bessard et al. (1983) and Schutz et al. (1984b) suggest that the attenuated thermic effect of glucose and mixed-meal feeding is present in both "so-called" obese and reduced-obese individuals, even after partial refeeding for reduced-weight maintenance.

Thus, Schutz et al. (1984b) found that after weight reduction and 2-3 weeks of weight stabilization, persons who were considered by the investigators still to be obese, demonstrated a lower thermic effect of feeding than normal weight controls. These investigators suggested that this increased efficiency of fuel utilization may have been a contributing factor in the aetiology

of obesity in these subjects. It is important to note that the subjects selected for dietary intervention in this study all had a family history of overweight. Thus, it is likely that the lowered thermic effect of feeding in this study was partially genotype-dependent. This genotype-dependence of the thermic effect of feeding has been elegantly demonstrated in the studies of short-term overfeeding involving pairs of monozygotic twins by Poehlman et al. (1986).

Bessard et al. (1983) studied the thermic effect of feeding in women selected on the basis of a high mass and a childhood history of overweight. There was no significant change in the thermic effect of feeding following dietary restriction, and subsequent partial refeeding for reduced weight maintenance. The investigators then "corrected" the thermic effect of feeding for spontaneous physical activity measured by radar in a respiration chamber. The "corrected" thermic effect of feeding was significantly lower after weight loss in the overweight subjects. However, there was no change in the "corrected" thermic effect of feeding in women of average weight serving as controls.

In these two studies, statistical comparisons between groups and over time were made using paired t-tests, instead of analyses of variance for repeated measures. This would enhance the likelihood of Type I error, or a rejection of the null hypothesis when it should be accepted (Huck, Cormier and Bounds, 1974). In addition, the thermic effect of feeding was calculated as the mean increment in energy expenditure over the post-ingestion

period, instead of calculating a total incremental area under the curve. These differences in statistical analyses and the method of calculating the thermogenic response to feeding, as well as differences in the energetic content of the meal and the duration of the post-feeding feeding response may account for differences in interpretation between the present study, and those of Bessard et al (1983) and Schutz et al. (1984b).

Metabolic response to epinephrine infusion:

There was no significant difference in the overall epinephrine-induced increment in resting energy expenditure before or after weight loss and refeeding in the present study. However, there was a significant interaction effect between the diet groups (D and VLED) and the DE group, when expressing the thermic response to epinephrine as W at 15 minute intervals throughout the infusion. In the diet-only group, despite a significant attenuation of resting metabolic rate, there were no changes in the thermic effect of epinephrine before and after weight loss. This is comparable to a study by Bazelmans et al. (1985) in which obese subjects demonstrated no difference in norepinephrine flux, in response to 10 days of undernutrition or overnutrition, and despite significant gains and losses in body mass.

Conversely, the absolute energy expenditure in the DE group in response to epinephrine infusion was attenuated with weight loss and partially restored to baseline with refeeding. This

difference between the exercise-and-diet group (DE) and the diet-only groups may be a result of training-induced adaptations in the sensitivity of the sympathetic nervous system (Kjaer et al., 1989).

In a study by Finer et al. (1985), obese subjects demonstrated a significantly greater attenuation (nearly 50%) of the norepinephrine-induced increment in energy expenditure during a 45 minute norepinephrine infusion, than that which could be attributed to a change in resting energy expenditure following weight loss. Differences between this study and the present study may be attributed to the fact that in the Finer study, the norepinephrine dose remained constant, whereas in the present study, the epinephrine dose was adjusted for changes in fat-free mass.

Conclusions:

In the present study of free-living individuals undergoing one of three interventions for food energy restriction and exercise training, the single best predictor of weight loss, was starting mass. More importantly, there was no treatment-specific effect on the sparing of lean body mass, and the changes in resting energy requirement were proportional to the overall changes in mass and fat-free mass, across all treatment groups.

Nor did this study corroborate the findings of previous studies which suggest that the "reduced-obese" have a reduced daily

energy expenditure, and a persistent thermogenic defect. In addition, there was no apparent increase in efficiency of weight gain during a period of refeeding following food energy restriction, which has been described directly in rat studies (Bjorntorp and Yang, 1982, Boyle et al., 1978, Fried et al., 1983) and indirectly in humans (de Boer et al., 1986, Eckel and Yost, 1987, Mathieson et al., 1986, Yost and Eckel, 1988).

CHAPTER 6

SHORT-TERM DETRAINING: EFFECTS ON BODY COMPOSITION, RESTING METABOLIC RATE AND THE GLUCOSE-INDUCED INCREMENT IN ENERGY EXPENDITURE IN ENDURANCE-TRAINED ATHLETES

Introduction

In a previous chapter of this dissertation (Chapter 4), growing rats in positive energy balance were exposed to a voluntary exercise stimulus, which was subsequently removed. These rats demonstrated an accelerated rate of gain in body mass and body fat, not only when compared to trained rats, but also when compared to sedentary controls. This enhanced metabolic efficiency occurred despite the fact that there was no difference in food energy intake between groups.

In the present chapter, "highly-trained" and "moderately-trained", weight-stable, runners and triathletes were asked to stop training for a period of two weeks, and to maintain a constant food energy intake. Changes in body mass, body composition, resting- and glucose-stimulated energy expenditure were used to document changes in energy balance.

This protocol had the potential to perturb energy balance 1) by changing the energy "requirements" for maintaining energy balance, or 2) by reversing metabolic adaptations to exercise training. This response is complicated by the well-documented effects of an acute bout of exercise on post-exercise energy expenditure (Bielinski et al., 1985) and intermediary metabolism (Heath et al., 1983).

A single bout of exercise is associated with a measurable post-exercise increment in resting energy expenditure. This effect may last for a period of up to 24 hours post-exercise depending on the intensity and duration of the exercise bout (Bielinski et al., 1985, Maehlum et al., 1986, Sedlock et al., 1990, Segal et al., 1985).

Regular exercise training in freely-eating persons has been shown to result in an increase in resting metabolic rate when compared to 1) pre-training levels or 2) to resting energy expenditure in untrained persons (Tremblay et al., 1985, Tremblay et al., 1986, Poehlman et al., 1988). In addition, endurance exercise training is a powerful stimulus for the reduction of fat cell size and an overall reduction in body fat under conditions of *ad libitum* food intake in both animals and humans (Booth et al., 1974, Pollock et al., 1975).

However, there is also evidence which suggests that there is no effect of exercise training on resting energy expenditure (Broeder et al., 1992, Davis et al., 1983, Gilbert et al., 1991, Hill et al., 1984). Moreover, there is some evidence that highly-trained persons may have a blunted metabolic response to various thermogenic stimuli. For example, exercise training is associated with a reduction in catecholamine secretion (Koivisto et al., 1982) and attenuated oxygen consumption during standard submaximal exercise (Lambert and Noakes, 1989). Tremblay and coworkers, and

others have repeatedly demonstrated that highly-trained "runners" have a lower increment in energy expenditure in response to glucose and mixed-meal feeding when compared to untrained controls (Tremblay et al., 1985, Tremblay et al., 1986, Poehlman et al., 1988). However, other investigators have not been able consistently to reproduce this finding (Davis et al., 1983, Hill et al., 1984, Poehlman et al., 1989).

Tremblay and coworkers (1988) found that a 3-day interruption of training resulted in an attenuation in resting energy expenditure and an increase in the thermic effect of glucose feeding in endurance-trained athletes. However, there are no previous studies which have characterized the effects of short-term detraining over a period of two weeks on resting metabolic rate and the glucose-induced increment in energy expenditure in highly-trained persons.

Thus, the aim of this study was to characterize adaptations in resting energy expenditure and the glucose-induced increment in energy expenditure in response to short-term detraining in endurance-trained athletes. The importance of factors such as the training 'load', food intake, age and body composition on these adaptations were also quantified.

Methods

Subjects and experimental protocol:

Twenty-six men participated in this study. Subjects were divided into four groups based on their weekly training 'load' (measured as the number of hours per week in which they engaged in vigorous physical activity) and their willingness to stop training for 2 weeks. Group 1 (Run-TR) consisted of six subjects who were moderately-trained, long-distance runners. None of these runners exceeded a training load of 11 hours of running per week, and all had been training consistently for a minimum of three years. These runners continued to train for the duration of the study. Group 2 (Run-DET) consisted of 8 similarly-trained runners drawn from the same population as Group 1, who elected to stop training for two weeks. Group 3 (Tri-DET) was comprised of 4 highly-trained triathletes. These subjects all trained for a minimum of 14 hours per week, and were asked to detrain for the two week experimental period. Group 4 (Control) consisted of eight university students who volunteered to act as control subjects for the various measurements over time. These subjects were not formally engaged in any weekly training regimen, but were healthy and active.

Subjects were informed of the nature of the study and written, informed consent was obtained. All procedures had been

previously approved by the Ethics and Research Committee of the Medical Faculty of the University of Cape Town.

All experimental subjects were tested on 3 separate occasions. The pre-test took place not more than 10 days, and not less than 7 days prior to participation in either a marathon or an ultra-triathalon. In this way, athletes were unlikely to be affected by a reduction in training volume or altered food intake. The post-testing took place one week and two weeks following participation in these events. Control subjects were tested over a 3 week period, with one additional trial included to test the thermogenic response to the ingestion of a sweetened placebo.

Subjects were instructed to keep their daily food intake constant throughout the experimental period. Each subject was given a weekly menu which was based on their previously reported daily energy intake. The foods on the menu either duplicated the meals, snacks and beverages of the pre-test period or provided equivalent exhanges where possible to increase the number of food choices. Subjects were asked to place a mark in a space provided next to each menu item or to note any substitutions which were made. The purpose of this was to control for any variation in food and nutrient content of the diet between experimental trials.

At each testing period, the following measurements were taken: body mass, body composition, resting energy expenditure, and the glucose-induced increment in energy expenditure.

Procedures:

Body composition: Four skinfold sites were used to estimate body composition using the regression equations of Durnin and Womersley (1974). These included: triceps, biceps, subscapular and suprailiac skinfolds.

Dietary record: Each volunteer was given a standard 500g food scale and asked to record the weights and volumes of all food and beverages ingested during a 7-day period. Household measures were used when metric measurement was not possible. Food records were coded and analyzed using reference food composition and quantity tables (Research Institute for Nutritional Diseases, Parow, South Africa) and a computerized dietary analysis program (Floro Diet Data Program, Durban, South Africa).

From these food records, weekly menus were derived. Subjects were asked to place a mark after each food item, meal, snack

or beverages, or to note any major deviations from this menu for the duration of the trial.

Training 'load': Training 'load' was estimated for each runner or triathlete based on the number of minutes per week which they trained, and the average training intensity and mode. For example, runners training at a pace of 3.3 $m \cdot s^{-1}$ (12 kph) were presumed to expend 14.5 $W \cdot kg^{-1}$. A triathlete was presumed to expend 11.8 $W \cdot kg^{-1}$ during cycling training (Bannister and Brown, 1968). Relative training volume, expressed in "Training Load Units (TLU) was calculated from these assumed rates of energy expenditure during training. (One TLU is approximately equal to 1 MJ energy expenditure on exercise above the REE per week).

Resting energy expenditure: Resting oxygen consumption (VO_2) was measured in the post-absorptive state, after familiarization with the procedure. Subjects were instructed not to train for the 24-hour period preceding each test, nor to stop training for more than 48 hours prior to each test. After a minimum of 30 minutes of supine rest, respiratory exchange measures were collected for a 30 minute period using a ventilated-hood, open-circuit system for indirect calorimetry. The mean VO_2, carbon dioxide production (VCO_2) and RER were determined for the resting state. The measurement technique was described in full in Chapter 5 (**Methods**). Respiratory exchange data were used to calculate

energy expenditure using the conversion equations for energy equivalents (Weir, 1949).

Glucose-induced thermogenesis: The glucose-induced increment in energy expenditure was measured in 20 of the subjects. Glucose-feeding was not carried out in the following subjects: 2 Control, 3 Run-DET and 1 Run-TR, due to time constraints. After the period of 50 minutes of supine rest in the post-absorptive state, subjects were fed a chilled glucose solution, consisting of 100g glucose diluted in 400 ml of water. The solution was ingested through a drinking tube while subjects remained in the supine position.

The post-glucose increment in energy expenditure was monitored from the 30th to the 120th minute post-ingestion, and expressed as the total area under the curve for energy expenditure. The area under the curve for resting energy expenditure was extrapolated for the same period of time, based on the assumption that resting metabolic rate remained constant and was not subject to significant variation in a test of such short duration. The glucose-induced increment in energy expenditure was expressed either as the total W increment in energy expenditure over resting energy expenditure for the period from 30 to 120 minutes post-ingestion or as the percentage increment (area:area) over resting metabolic rate.

Control subjects underwent an additional test for comparison of the effects of a sweetened placebo (saccharin) vs glucose feeding. The test protocol was identical, with the exception that subjects ingested 400 ml of water sweetened with artificial sweetener (Sweetex^{R}, sodium saccharin).

Statistical analyses:

All data are expressed as means and standard errors of the mean (± SEM). Initial cross-sectional group comparisons were made using a one-way analysis of variance. Tukey's post-hoc analysis was performed where significant F ratios were found to determine which groups were significantly different from one another. For comparisons between groups and across time, a two-way analyses of variance for repeated measures was performed. In this way, the main effects of group and time (test period) could be examined after correcting for the effects of subjects nested within the groups. Post-hoc tests were performed as described above. In addition, the two way analyses quantified the interaction effect of groups over time.

Results

Group characteristics:

Descriptive characteristics of subjects in each group are presented in Table 6.1. There were no differences in body mass and fat-free mass between trained subjects and untrained controls. There was, however, a significant difference in percentage body fat when the runners were compared to the triathletes and controls ($p < 0.01$). This was probably a result of the significant age differences between these same groups ($p < 0.0001$). There were also no differences in reported daily energy intake ($MJ \cdot d^{-1}$) and the gram ratio of ingested available carbohydrate to fat. The triathletes had a significantly higher estimated weekly training 'load' than either group of runners (45.3 ± 1.2 vs 26.9 ± 3.8 and 24.8 ± 4.2 $TLU's \cdot wk^{-1}$, respectively, $p < 0.05$).

The control group provided a means of determining the test-to-test variability for the different measures. For example, the coefficients of variation for mass, fat-free mass and percentage body fat were 0.57%, 0.65% and 2.01%, respectively. The coefficient of variation for resting energy expenditure (W or $kJ \cdot min^{-1}$) for 4 measurements over a period of 4 weeks was 7.09%.

Table 6.1 Descriptive characteristics of subjects within groups (Moderately trained runners= Run-TR, Moderately-trained runners-detrained= Run-DET, Highly-trained triathletes-detrained = Tri-DET, and sedentary controls = Control, Ratio = grams carbohydrate: grams fat ingested, means ± SEM).

Group	Age (yrs)	Mass (kg)	FFM (kg)	%Fat	Intake ($MJ \cdot d^{-1}$)	Ratio (CHO:FAT)	Training ($TLU \cdot wk^{-1}$)
Run-TR (n=6)	41.3^{a} ±2.5	74.0 ±1.7	62.2 ±1.2	15.8^{a} ±0.7	15.4 ±1.5	3.6 ±0.7	26.9^{a} ±3.8
Run-DET (n=8)	40.8^{a} ±2.6	74.4 ±2.1	61.5 ±1.8	17.9^{b} ±0.7	12.3 ±1.2	3.2 ±0.1	24.8^{a} ±4.2
Tri-DET (n=4)	26.5^{b} ±1.5	71.9 ±1.1	63.2 ±0.7	12.1^{c} ±0.5	13.3 ±0.5	4.3 ±0.6	45.3^{b} ±1.2
Control (n=8)	19.9^{b} ±0.8	68.1 ±1.3	61.9 ±1.1	11.7^{c} ±0.7	11.3 ±0.6	2.8 ±0.2	-

(^{a,b,c} Means which do not share a common superscript are significantly different; age, $p < 0.0001$; % fat, $p < 0.01$; Training, $p < 0.05$)

The thermic response to glucose-feeding was compared with the 2-hour increment in energy expenditure resulting from the ingestion of an artificially sweetened placebo. The mean percentage increment in 2-hour energy expenditure for the placebo trial was 2.64 ± 0.91%, which was significantly less ($p < 0.003$) than the 3 subsequent glucose-feeding trials (10.52 ± 1.94%, 14.12 ± 2.29%, and 12.64 ± 1.23% increment over resting energy expenditure). The thermic response to glucose-feeding was, however, highly variable (coefficient of variation, 35.7%).

Effects of detraining on body composition and resting energy expenditure:

There were no statistically significant effects of stopping training for 2 weeks on body mass, fat-free mass and percentage body fat in the runners and triathletes (Table 6.2).

The triathletes had a significantly higher mean initial resting energy expenditure than the runners or sedentary controls, when expressed absolutely or relative to total body mass (Table 6.3, $p < 0.03$ and $p < 0.04$, respectively). However, when resting energy expenditure was expressed relative to fat-free mass, these differences between groups were no longer statistically significant (1.26 ± 0.08, 1.37 ± 0.07, 1.49 ± 0.07, and 1.31 ± 0.02 $W \cdot kg\ FFM^{-1}$ for Run-TR, Run-DET, Tri-DET, and Controls, respectively, $p < 0.07$).

Resting energy expenditure was significantly lower in all groups at 1 week, when compared to baseline ($p < 0.03$, Table 6.3). However, the triathletes were the only group which demonstrated a further reduction in resting metabolic rate 2 weeks after stopping training ($p < 0.03$, Table 6.3).

Respiratory exchange ratio (RER) was significantly higher in the Tri-DET group across all trials ($p < 0.01$, 0.89 ± 0.02 vs 0.80 ± 0.01, 0.83 ± 0.02, and 0.82 ± 0.01 for the Run-TR, Run-

DET and Control group, respectively). Resting RER was not different over the different testing periods and there were no interaction effects of group over time. RER increased during the 120 minutes following glucose feeding in all groups. However, there were no significant effects of group, time or any significant interactions between group and time on substrate oxidation in response to glucose feeding.

Table 6.2 Body mass and fat-free mass from baseline,and subsequent testing at 1 and 2 weeks for each group (means ± SEM).

Group	**PRE**		**1 wk**		**2 wks**	
	Mass (kg)	**FFM** (kg)	**Mass** (kg)	**FFM** (kg)	**Mass** (kg)	**FFM** (kg)
Run-TR (n=6)	74.4 ±3.0	62.5 ±2.2	74.0 ±3.3	62.3 ±2.3	73.5 ±3.2	61.8 ±2.3
Run-DET (n=8)	74.0 ±3.9	61.3 ±3.0	74.9 ±3.9	61.9 ±2.9	74.5 ±3.7	60.9 ±2.7
Tri-DET (n=4)	71.8 ±1.9	63.6 ±1.2	71.6 ±2.0	62.9 ±1.5	72.3 ±2.1	63.1 ±1.4
Control (n=8)	68.0 ±2.2	61.8 ±2.0	67.9 ±2.3	61.7 ±2.1	68.3 ±2.4	62.2 ±2.1

The effects of detraining on the glucose-induced increment in energy expenditure:

There was no significant effect of stopping training on the glucose-induced increment in energy expenditure. There was, however, a significant time effect across all groups (Table 6.4, $p < 0.0003$). The glucose-induced increment in energy expenditure was consistently lower in all groups at the third

testing period, suggesting adaptation or a learning effect took place.

Table 6.3 Resting energy expenditure (W) for each group at pre-testing, 1 and 2 wks post-testing (means ± SEM).

Group	REE (W) PRE	1 wk	2 wks	GROUP (main effects)
Run-TR (n=6)	78.7[a] ± 5.0	77.3 ± 3.7	82.7 ± 4.3	79.5 ± 2.5
Run-DET (n=8)	83.0[a] ± 2.3	81.2 ± 2.8	85.8 ± 3.7	83.3 ± 1.7
Tri-DET (n=4)	94.8[b] ± 4.0	91.2 ± 3.2	84.7* ± 6.7	90.2 ± 2.8
Control (n=8)	80.0[a] ± 1.8	76.5 ± 1.8	83.5 ± 3.3	80.0 ± 1.5
TIME (main effects)	82.8 ± 1.8	80.3[a] ± 1.7	84.2[b] ± 2.0	-

Group	REE ($kJ \cdot min^{-1}$) PRE	1 wk	2 wks	GROUP (main effects)
Run-TR (n=6)	4.72[a] ±0.30	4.64 ±0.22	4.96 ±0.26	4.77 ±0.15
Run-DET (n=8)	4.98[a] ±0.14	4.87 ±0.17	5.15 ±0.22	5.00 ±0.10
Tri-DET (n=4)	5.69[b] ±0.24	5.47 ±0.19	5.08* ±0.40	5.41 ±0.17
Control (n=8)	4.80[a] ±0.11	4.59 ±0.11	5.01 ±0.20	4.80 ±0.09
TIME (main effects)	4.97 ±0.11	4.82[a] ±0.10	5.05[b] ±0.12	-

([a,b] Means which do not share a common superscript are significantly different; Pre, $p < 0.03$; TIME, $p < 0.03$, * significant interaction effect of GROUP x TIME, $p < 0.03$)

Table 6.4. The glucose-induced increment in energy expenditure from 30-120 minutes post-glucose ingestion, expressed as the percentage increase over the resting area under the curve, as well as the absolute kJ increment over resting energy expenditure for the same time period (means ± SEM).

Group	Pre	1 wk	2 wks	Group (main effects)
		% increment		
Run-TR (n=5)	18.0 ± 4.4	18.4 ± 0.6	7.7 ± 1.2	14.7 ±1.9
Run-DET (n=5)	14.6 ± 1.4	16.1 ± 3.0	8.4 ± 2.2	13.0 ±1.5
Tri-DET (n=4)	15.0 ± 3.3	13.8 ± 1.4	8.4 ± 0.4	12.4 ±1.4
Control (n=6)	11.4 ± 2.5	15.3 ± 2.7	12.6 ± 1.2	13.1 ±1.3
TIME (main effects)	14.5^{a} ±1.5	16.0^{a} ± 1.1	9.5^{b} ± 0.8	-

Group	Pre	1 wk	2 wks	Group (main effects)
		kJ increment		
Run-TR (n=5)	92.6 ±23.8	92.6 ± 6.5	37.9 ± 7.4	74.4 ±10.5
Run-DET (n=5)	80.0 ±10.5	89.0 ±16.8	41.6 ±10.6	70.2 ±8.9
Tri-DET (n=4)	90.8 ±20.7	79.0 ± 8.9	42.1 ± 5.3	70.6 ±9.4
Control (n=6)	58.9 ±13.4	80.5 ±17.3	67.9 ± 7.7	69.1 ±7.6
TIME (main effects)	79.0^{a} ±8.6	85.3^{a} ± 6.7	48.6 ± 4.8	-

(^{a,b} Means which do not share a common superscript are significantly different, $p < 0.0003$)

Discussion

This study was designed to characterize the metabolic response to short-term detraining in endurance-trained athletes. Many of the physiological and metabolic adaptations associated with endurance training may directly or indirectly influence energy balance in the athlete. For example, exercise training may result in adaptations in food intake (Tichenal, 1989), adrenergic sensitivity (Winder et al., 1978), body composition (Parizkova', 1977, Pavlou et al., 1985, Pollock et al., 1975), insulin sensitivity (Heath et al., 1983, LeBlanc et al., 1981) and the post-exercise increment in energy expenditure (Maehlum et al., 1986). In addition, the time course of these adaptations may differ, and the combined effects of each may be additive or may actually work in opposition in order to regulate energy balance.

The cessation of exercise training provides a metabolic "challenge" from which it may be possible to gain information about the role of exercise training in body weight regulation and energy balance.

Resting metabolic rate: differences between trained subjects and untrained controls

The first important finding in the present study was that resting metabolic rate in highly-trained triathletes in

training was higher than that of sedentary controls and moderately-trained and, in this case, older runners.

This finding is consistent with several previous studies which have demonstrated that exercise training is associated with an increased resting energy expenditure, compared to sedentary, age-matched controls (Poehlman et al. 1986, Poehlman et al., 1988, Poehlman et al., 1990, Tremblay et al., 1983, Tremblay et al., 1985, Tremblay et al., 1986). However, there are a number of other studies which have found that there is no effect of exercise training or maximal aerobic capacity on resting energy expenditure (Broeder et al., 1992a, Broeder et al., 1992b, Davis et al., 1983, Gilbert et al., 1991, Hill et al., 1984, Le Blanc et al., 1984, Lundholm et al., 1986, Poehlman et al., 1990, Schulz et al., 1992, Sharp et al., 1992). The interpretation of these studies is complicated by the differences in experimental design which exist between them.

For example, in each of the previous cross-sectional studies which have investigated the effects of exercise training on resting metabolic rate, some or all of the following variables were controlled by subject selection and experimental design: age, mass, gender, body composition, level of fitness, training 'load', time elapsed from the last bout of exercise, food intake, the timing of meals, time of day and method of measurement of energy expenditure, and current energy balance

status. However, there has been little standardization of protocol between studies, and these variables have not been controlled similarly.

Furthermore, many cross-sectional studies have equated "fitness" or exercise training with a quantitative measure of maximal oxygen consumption during incremental exercise testing (Broeder et al., 1992a, Davis et al., 1983, Hill et al., 1984, Poehlman et al., 1989, Poehlman et al., 1990, Sharp et al., 1992). Previous studies from this laboratory suggest that maximal oxygen consumption is not the best predictor of fitness or performance (Noakes, 1988, Noakes et al., 1990, Scrimgeour et al., 1986), nor does it accurately reflect training status (Lambert et al., 1989). While it is preferable to compare the metabolic responses to training along a continuum of "fitness" as opposed to some arbitrary cutoff points determined by the investigators, it is important that the variables selected to represent training accurately reflect training.

In the present study, subjects were grouped according to "training load", from competitive triathletes, training a minimum of 14 hours per week, to recreational runners training less than 11 hours per week, and individuals who did no formal training. When resting energy expenditure was compared in a combined group of all of the trained subjects to that of the untrained controls, there were no significant differences.

This suggests that grouping subjects according to "training load" provides additional information on the dose-response relationship between training and resting energy expenditure.

It is possible to draw some conclusions regarding the effects of exercise training on resting energy expenditure based on the combined findings of the present study and previous research. Firstly, the effects of exercise training on energy expenditure may be transient, and attenuated when measured more than 48 hours following the last exercise bout. In two previous studies by Broeder et al. (1992a, 1992b), in which resting energy expenditure measurements were not taken within 48 hours of the last exercise bout, there were no differences between trained and untrained subjects.

Results from studies which have measured resting energy expenditure between 16 and 48 hours following the last exercise bout are equivocal, and interpretation may be limited by poor statistical power in studies with small numbers (Hill et al., 1984) and the interaction between 'training load' and the duration of the post-exercise increment in energy expenditure (Tremblay et al., 1986). In the study by Gilbert et. al. (1991), endurance-trained athletes who averaged 4.5-7.0 hours per week in training did not demonstrate a higher resting energy expenditure than age-matched controls who only participated in leisure activities. This was despite the fact that resting metabolic rate was measured between 24 and 48

hours following the last exercise bout, and is consistent with the responses of the Run-TR, and Run-DET groups in the present study.

Thus, it appears that provided resting metabolic rate is measured over 16 hours after the last bout of exercise, then the effects of the previous exercise bout cannot explain any differences in metabolic rate between trained and untrained, age-matched controls. However, it is is also unlikely that the higher resting metabolic rate demonstrated by the highly-trained triathletes in the present study was a residual effect of the last bout of exercise. In previous studies which report a significant and prolonged post-exercise increment in energy expenditure, there was a concomitant reduction in the respiratory exchange ratio, suggesting a significant increase in relative fat oxidation (Bahr et al., 1987, Bahr et al., 1990, Bahr et al., 1991, Bielinski et al., 1985, Maehlum et al., 1986, Wolfe et al., 1990), possibly related to post-exercise ketosis or increased rates of fatty-acid/triglyceride substrate cycling (Bahr et al., 1990, Koeslag et al., 1981). The respiratory exchange ratio in the Tri-DET group from this study was higher under all conditions than the moderately-trained runners and sedentary controls.

Therefore, it is possible that in some previous studies differences in resting energy expenditure between trained vs untrained persons may have been explained by the time elapsed

from the last bout of exercise. However, it is clear that this mechanism cannot explain differences which exist between highly-trained triathletes and moderately-trained runners and controls in this study.

It is also possible that age differences between the moderately-trained runners and triathletes may explain initial differences in resting energy expenditure. However, recent studies which have reappraised the food energy requirements for men have found that age had little predictive value for resting energy expenditure in men between the ages of 18 and 80 yrs (Chapter 10, Cunningham, 1980, Owen et al., 1987, Ravussin et al., 1986).

Poehlman et al. (1991) suggested that one of the mechanisms which may be responsible for differences in resting energy expenditure in trained vs untrained persons is a higher food energy intake or a higher "energy flux". This was supported indirectly by a previous study (Poehlman et al., 1988) in which reported food energy intake was greater in highly-trained endurance athletes compared to sedentary controls. However, reported food energy intake in the present study was not higher in the Tri-DET group compared to the other groups, nor was it correlated to resting energy expenditure when all groups were combined. Thus, differences in resting metabolic rate between Tri-DET and other trained groups in the present

study cannot be explained by differences in food energy intake.

In a longitudinal study by Bingham et al. (1989), healthy, previously sedentary young adults underwent 9 weeks of exercise training, resulting in a 28% increase in total daily energy expenditure. They were unable to demonstrate any significant increase in resting energy expenditure following 9 weeks of exercise training. However, it is important to note that the subjects were in negative nitrogen balance at the end of the training period. Thus, increases in resting energy expenditure with training are unlikely to occur with exercise training if subjects are not in energy balance.

Differences in absolute resting energy expenditure between groups of trained and untrained individuals may be attributed to differences in fat-free body mass. In the present study, resting energy expenditure tended to be higher in the Tri-DET group even when expressed per unit fat-free mass ($p < 0.07$). After covarying for differences in fat-free mass between individuals in groups, the resting energy expenditure was higher in the Tri-DET group compared to the moderately trained runners ($p < 0.03$). Thus, it is unlikely that differences in fat-free mass and body composition entirely accounted for the initial differences in resting energy expenditure between groups in the present study.

Resting energy expenditure: response to stopping training

The second important finding in the present study was that highly-trained triathletes, previously training for more than 14 hours per week, demonstrated a significant reduction in resting metabolic rate after two weeks of detraining (Table 6.3). This response was contrasted against little or no change in resting energy expenditure in the moderately-trained runners who also detrained for two weeks, and occurred despite the fact that food energy intake remained relatively constant from training to detraining.

This finding provides the strongest evidence that the higher baseline rate of resting energy expenditure found in the Tri-DET group from the present study was a response to training and not the result of a prolonged post-exercise increment in energy expenditure following the last bout of exercise. Further evidence provided by the present study, that the decline in metabolic rate with detraining was not simply a residual effect of the last bout of exercise was that the attenuation in resting metabolic rate progressed over the course of the 2 weeks of detraining.

These results also suggest that the degree to which the cessation of training influences resting metabolic rate might be "dose-response"-related. In a previous study by Tremblay

et al. (1985), athletes training between 6 and 12 hours per week were contrasted with those training from 12 to 16 hours per week and then compared with healthy, untrained controls. Resting metabolic rate was significantly higher in the athletes with the highest "training load" as in this study.

It is unlikely that the attenuation of resting energy expenditure with detraining resulted from a change in body composition in response to the cessation of training. Fat-free mass remained unchanged in the triathletes over the course of detraining. However, it is possible that the constituents of fat-free mass change as a result of stopping training. For example, detraining may result in changes in body water, which is a constituent of fat-free mass, and thus, the "metabolic activity" of this tissue may be influenced.

It is possible that the decline in resting energy expenditure may have been related to some insensible reduction in food energy intake during detraining on the part of the triathletes. Parizkova (1977) studied gymnasts and found that during periods of reduced training (summer vacation) the women gymnasts spontaneously reduced food energy intake. Subjects in the present study were instructed to, and were assisted in, maintaining a constant food intake. If detraining athletes did reduce their food intake, then a similar response might have been expected to occur in the moderately-trained athletes, but this seemed not to be the case.

The mechanism which is responsible for the decline in resting metabolic rate associated with the cessation of exercise training in highly-trained athletes has not been elucidated. However, it has been argued that the effect was not simply the result of the residual effects of a single bout of exercise. It has also been demonstrated that the decline in resting metabolic rate with detraining was not related to changes in body composition and self-reported food energy intake. Thus, it is likely that the metabolic response to stopping training is linked to one or more physiological adaptations to exercise training which were not characterized in the present study. Conversely, stopping training may have resulted in changes in food energy intake, or daily energy expenditure or body energy stores which were not measurable with the present methodology.

Glucose-induced increment in energy expenditure:

In this study, there were no initial differences in the glucose-induced increment in energy expenditure between groups. Highly-trained athletes, moderately-trained runners and sedentary young control subjects had a similar increase in resting energy expenditure in response to a 100 g oral glucose load.

Some studies have found that the thermic response to mixed-meal and glucose-feeding in endurance-trained athletes is blunted when compared to sedentary, age-matched controls (LeBlanc et al., 1984, LeBlanc, 1986, Poehlman et al., 1988, Tremblay et al., 1983, Tremblay et al.,, 1985). Conversely, studies by Davis et al. (1983), Hill et al., (1984) and Lundholm et al. (1986) have found that the thermic effect of feeding is significantly enhanced as a result of endurance training. The main apparent difference between these conflicting studies appears to be the delay between the last training bout and the test period. In studies by LeBlanc et al. (1984, 1986) and by Tremblay et al. (1983, 1985), athletes were tested within 16 hours after the last training session. In the other studies, this delay was longer.

In a recent study by Poehlman et al. (1988), the thermic effect of a mixed-meal was determined in 3 groups of subjects: untrained controls, moderately-trained runners and competitive runners. The energy content of the meal was adjusted for differences in fat-free mass and the thermic effect of feeding was expressed per unit body mass. They found that the thermic effect of feeding was highest in the moderately-trained runners, and lower in the controls and the highly-trained group. The mechanism responsible for differences in the thermic effect of feeding between the moderately-trained group and the high- and low- trained groups may be related to differences in tissue insulin sensitivity, plasma

catecholamine secretion, genotype or even, the number of previous exposures to the procedure.

Several physiological adaptations associated with endurance training may directly or indirectly influence the metabolic response to glucose feeding. Increased tissue insulin sensitivity as a result of exercise training is associated with increased glucose disappearance from the blood (Heath et al., 1983) and may thus enhance the thermogenic response to glucose-feeding. Acheson et al. (1984) studied glucose-induced thermogenesis using a hyperinsulinaemic, hyperglycaemic glucose clamp in healthy men. They found that 30% of the glucose-induced increment in energy expenditure could be blocked by administering a ß-adrenergic receptor antagonist, despite the fact that glucose storage and oxidation remained unchanged. Thus, that study implicates the sympathetic nervous system as one of the effector pathways in the regulation of the facultative component of the post-feeding increment in energy expenditure. Training-induced adaptations in adrenergic sensitivity may influence the facultative component of this response.

The reason for the discrepancy between this study and other studies, where both blunted and enhanced responses to glucose-feeding have been demonstrated as a result of endurance training is unclear. Factors which are not consistent between previous studies include: the period of time since the last

exercise bout, the duration of the measurement of the post-feeding increment, the energy and nutrient load, the type of control subjects chosen, the method by which area under the curve for glucose-induced thermogenesis was calculated, and the sensitivity and accuracy of one's measurement techniques.

For example, some studies have only followed energy expenditure during the post-feeding period for 90 minutes (Tremblay et al, 1983); others have followed it for up to 8 hours (D'Alessio et al., 1988). Feeding stimuli have included glucose solutions (75 g, Tremblay et al., 1985) and mixed meal feeding (6.9 MJ, Tremblay et al., 1983) and in some studies, the energy intake was calculated per kg fat-free mass (Poehlman et al., 1988). Some studies have compared the "end-rate" oxygen consumption (Davis et al., 1983) or the "end-TEF" (Hill et al., 1984) which is the percentage increase in energy expenditure over baseline during the 30 minute interval between 150 and 180 minutes after mixed-meal ingestion.

Other studies have found that the rate of glucose oxidation and the glucose-induced increment in energy expenditure is lower in trained individuals. This has been attributed to an increase in non-oxidative glucose disposal in the glycogen-depleted state (Tremblay et al., 1985). In the present study, glucose-induced thermogenesis was measured a minimum of 24-hours following the last training bout, and thus, non-oxidative glucose disposal may have been attenuated.

The cessation of training had no effect on glucose-induced thermogenesis in the present study. Had there been a more marked change in fat-free mass or in the percentage body fat, it might have been expected that the response to glucose-feeding would have actually decreased in the detraining athletes as a result of increased fat cell size with decreased insulin sensitivity (Craig et al., 1983).

Perhaps, the most significant finding with regard to glucose-feeding in this study was that there was a great deal of test-to-test variation, and that repeated exposures to glucose-feeding resulted in a significant reduction in the magnitude of the response, despite deliberate familiarization with the protocol before the experiment. The results from this study suggest that there is an attenuation of the metabolic response to glucose-feeding with repeated exposures. This has important implications for laboratories which recycle "professional subjects" and post-graduate students for various research protocols and could, perhaps, explain variability in findings in other studies.

Summary:

There was no evidence that the short-term cessation of training in freely-living, highly-trained athletes resulted in

a rapid accretion of body weight, body fat and the increased efficiency of fuel utilization. However, short-term detraining in highly-trained endurance athletes on a constant diet does seem to influence the regulation of resting energy expenditure. This occurs independently of changes in mass, fat-free mass, food energy intake, and in response to a single bout of exercise. This effect appears to be dose-response-related to the athlete's training 'load'. The glucose-induced increment in energy expenditure does not appear to be similarly regulated during this short period of cessation of training.

CHAPTER 7

EPINEPHRINE-INDUCED INCREMENT IN RESTING ENERGY EXPENDITURE, AND CHANGES IN SUBSTRATE OXIDATION IN EXERCISE-TRAINED VS UNTRAINED PERSONS

Introduction

Previous chapters of this dissertation have demonstrated that exercise training in growing rats results in an attenuated feeding efficiency and that stopping training while maintaining a constant food energy intake results in either an increase in body energy stores in rats or a decrease in resting energy expenditure in highly-trained men (Chapters 4 and 6).

Despite this evidence for the adaptability of resting energy requirements, there was no evidence to corroborate the results of previous studies which have found that energy expenditure in response to mixed-meal or glucose-feeding in highly-trained persons is blunted when compared to untrained or sedentary controls (Tremblay et al., 1985, Tremblay et al., 1986, Poehlman et al., 1988).

Differences between the present studies and existing studies were attributed, in part, to the energy balance status of the subjects, the time elapsed following the last exercise bout, glycogen status of the skeletal muscle, genotype differences and the possibility of habituation to the technique (Chapter 6, LeBlanc, 1986, Poehlman et al., 1985, Tremblay et al., 1985).

In this study, the metabolic effects of *in vivo* epinephrine infusion was compared in trained runners and age-matched, sedentary controls. Exercise training has been shown to result in a reduction in oxygen uptake and heart rate and an attenuated sympathoadrenal response during standard submaximal exercise (Kjaer et al., 1989, Koivisto et al., 1982, Lambert and Noakes, 1989, Winder et al., 1978). At the same time, other studies have suggested that repeated exposure to an exercise stimulus (i.e. training) results in an increased sensitivity of target tissues to plasma catecholamine concentrations, but a lower state of arousal, with a consequent decrease in plasma catecholamine release (Thompson and Blanton, 1987).

Furthermore, there are examples of studies in which subjects have undergone short-term chronic exposure to sympathomimetic drugs including: ephedrine chloride (Astrup et al., 1985, Astrup et al., 1986) and terbutaline sulphate (Acheson et al., 1988). Astrup et al. (1985, 1986) found that chronic oral administration of ephedrine in healthy young subjects over 2 weeks, resulted in 1) a sustained elevation of resting metabolic rate, 2) an enhanced thermogenic response to acute ephedrine administration but 3) no difference in the glucose-induced increment in energy expenditure.

These findings suggest that the repeated exposure to sympathomimetic drugs in healthy young adults results in a

similar increase in target tissue sensitivity to circulating catecholamines as that found by some investigators after repeated exposure to exercise.

Thus, data from previous studies suggests that enhanced target tissue sensitivity to plasma catecholamines may result in an enhanced thermogenic response to epinephrine infusion in physically-trained persons. The aim of the present study was to investigate further the effect of exercise training on the regulation of energy expenditure.

Methods

Subjects:

Six trained and 8 untrained control subjects were recruited from the volunteers from the previous study (Chapter 6). Two additional sedentary controls were also recruited from the Department of Physiology faculty from the University of Cape Town Medical School.

Subjects were informed of the nature of the study, and written consent was obtained prior to participation. All procedures were approved by the Ethics and Research Committee of the Faculty of Medicine of the University of Cape Town Medical School.

Subjects were asked to report to the laboratory in the early morning in the post-absorptive state and were instructed not to train during the 24-hour period preceding the test.

Training 'load' and body composition:

Training 'load' was estimated as in the previous chapter (Chapter 6), based on the number of minutes per week which the athletes trained, and the average training intensity (Bannister and Brown, 1968). Training Load Units (TLU's) represented the estimated amount of energy expended during physical training for each trained subject per week. Untrained control subjects in this study were not regularly engaged in any form of physical activity.

Body composition was estimated using the skinfold equations of Durnin and Womersely (1974), as described previously (Chapter 5).

Resting energy expenditure and the epinephrine-induced increment in energy expenditure:

Prior to determination of resting energy expenditure, a plastic cannula was introduced into the antecubital vein for epinephrine infusion. Another cannula was introduced into the antecubital vein on the opposite side for venous blood

sampling for the subsequent determination of free fatty acids concentrations.

Resting energy expenditure and the epinephrine-induced increment in energy expenditure were measured as described previously (Chapter 5). After a minimum of 30 minutes of supine rest, expired air was collected for a 30 minute period, using a ventilated-hood, open-circuit system for indirect calorimetry, and the mean oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) were determined for the resting state.

For the first hour of each trial, during the determination of resting energy expenditure, 0.9% NaCl (saline) was infused at a rate of 0.5 $ml \cdot min^{-1}$. Thereafter, epinephrine (adrenalin tartrate) in 0.9% saline was infused at a rate of $0.03 \mu g \cdot kgFFM^{-1} \cdot min^{-1}$ for 60 minutes.

Indirect calorimetry, as described previously, was used to determine the increment in resting energy expenditure as a result of epinephrine infusion, for the duration of the 60 minute infusion. Blood samples were obtained via the indwelling cannula every 15 minutes throughout the infusion for the determination of serum free fatty acid concentrations. Heart rate was monitored by ECG throughout the infusion.

Serum free fatty acid concentrations were analyzed by an enzymatic colourimetric assay (Boehringer Mannheim 1082-914 test combination).

Statistical analyses:

All data are expressed as means and standard errors of the mean (± SEM). Resting energy expenditure, the epinephrine-induced increment in energy expenditure were compared between groups using a student's T-test for independent samples. Changes in heart rate, substrate oxidation and serum free fatty acid concentrations during epinephrine infusion were compared between groups using two-way analyses of variance for repeated measures. In addition, the two way analyses quantified the interaction effect of groups over time.

Results

Resting energy expenditure:

There were no significant differences in age, body mass, fat-free mass and percentage body fat between the trained and untrained groups (Table 7.1).

Table 7.1. Subject characteristics between trained and untrained groups (Means ± standard errors of the mean).

Group	Age (yrs)	Mass (kg)	FFM (kg)	% fat	Training TLU·wk^{-1}
Untrained (n = 8)	24.5 ±3.1	72.7 ±1.6	62.1 ±1.5	13.8 ±1.2	-
Trained (n = 6)	30.2 ±2.5	69.8 ±1.5	62.7 ±0.9	10.2 ±1.6	34.7 ±5.5

Nor were there any differences in resting energy expenditure between trained and untrained persons, whether expressed absolutely (W or kJ·min^{-1}) or relative to mass, and fat-free mass (Table 7.2).

Table 7.2. Resting energy expenditure of trained and untrained subjects (Means ± standard errors of the mean).

Group	W	kJ·min^{-1}	W·kg^{-1}·	W·kg^{-1}· (FFM)
Untrained (n = 8)	78.4 ±2.9	4.70 ±0.17	1.08 ±0.04	1.26 ±0.04
Trained (n = 6)	81.4 ±3.5	4.88 ±0.21	1.16 ±0.04	1.29 ±0.04

Response to epinephrine-infusion:

The epinephrine-induced increment in energy expenditure from 15 minutes to 60 minutes infusion was also not significantly different between the groups ($p < 0.09$, Table 7.3). There was also no significant relationship between the resting energy expenditure and the magnitude of the epinephrine-induced

increment in energy expenditure for either group studied separately or when the data from both groups were combined.

Table 7.3. Increment in energy-expenditure over resting energy expenditure during epinephrine-infusion from 15-60 minutes (Means ± SEM).

	Epinephrine-induced	
Group	**% increment**	**kJ increment**
Untrained (n = 8)	17.2 ± 1.8	43.6 ± 4.7
Trained (n = 6)	12.9 ± 1.6	32.4 ± 4.3

There was, however, a significant difference in heart rate response to epinephrine infusion between trained subjects and controls (Figure 7.1, $p < 0.001$). The heart rate at rest, and in response to epinephrine was significantly lower in the trained group, compared to sedentary controls.

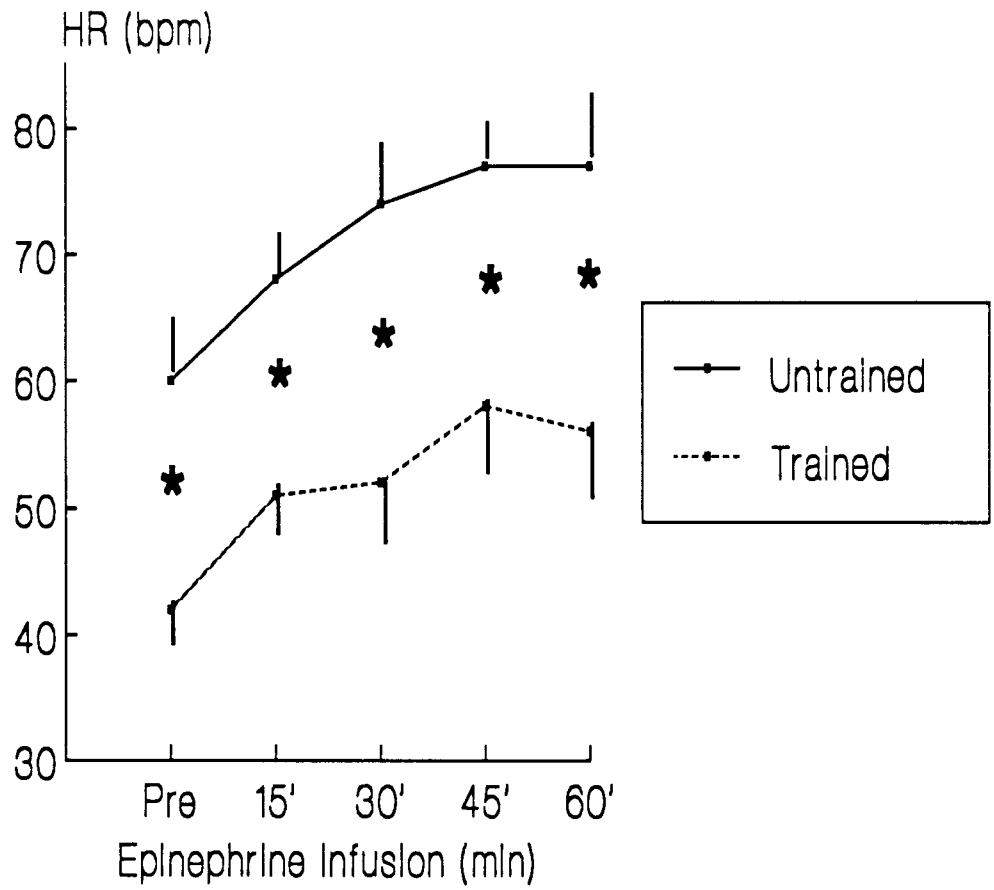

Figure 7.1. Heart rate (HR, beats per minute, bpm) response to epinephrine infusion in trained vs untrained subjects (means $\pm$ SEM, * $p < 0.001$, the overall heart rate at rest and during epinephrine infusion was attenuated in trained subjects compared to sedentary controls).

The respiratory exchange ratio (RER) rose significantly during the first 15 minutes following the start of the epinephrine infusion in both trained and untrained subjects ($p < 0.01$, Table 7.4). However, there was a significant group x time interaction effect, and the acute increase in RER after 15

minutes of epinephrine infusion was higher in the trained compared to the the untrained group ($p < 0.01$).

Table 7.4. The respiratory exchange ratio of trained and untrained subjects at baseline and during epinephrine infusion (0.03 $\mu g \cdot kg\ FFM^{-1} \cdot min^{-1}$).

Group	Pre	15′	30′	45′	60′
Untrained (n = 8)	0.83 ±.01	0.90* ±.03	0.87 ±.03	0.86 ±.02	0.85 ±.02
Trained (n = 6)	0.84 ±.02	0.98** ±.01	0.84 ±.02	0.83 ±.01	0.80 ±.02

(* $p < 0.01$ for 15′ vs all times for both groups; ** $p < 0.01$, for group x time interaction effect)

Rate of total carbohydrate oxidation increased in both groups from baseline to 15 minutes following the start of the epinephrine infusion ($p < 0.01$, Figure 7.2a). However, there was a significant group x time interaction effect ($p < 0.01$, Figure 7.2a), and carbohydrate oxidation decreased progressively in the trained group from 15 to 60 minutes after the start of the epinephrine infusion.

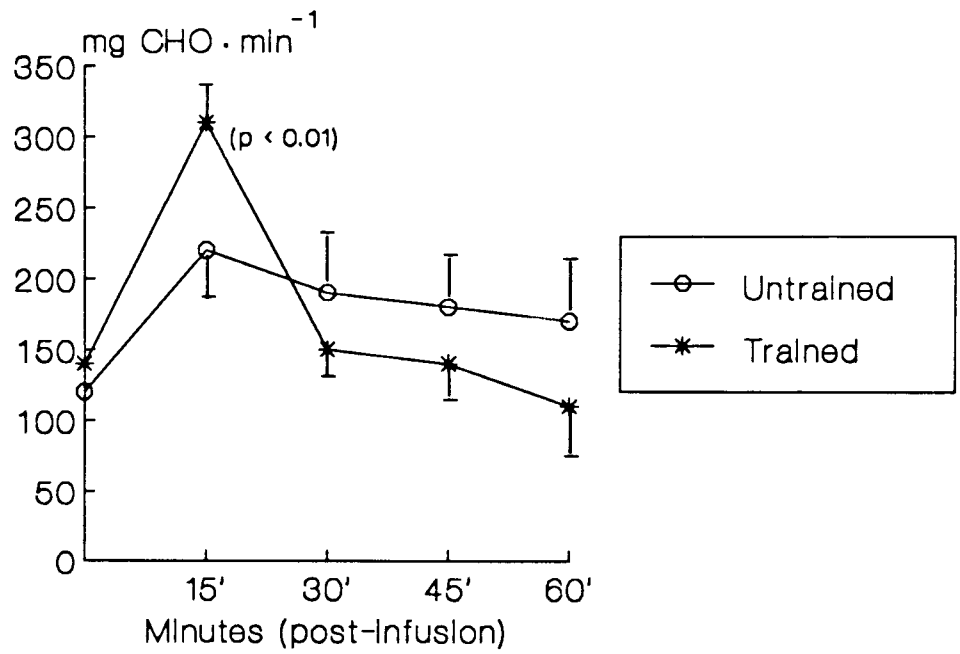

Figure 7.2a. Rate of total carbohydrate oxidation (mg CHO·min^{-1}) during a constant epinephrine infusion. CHO oxidation increased initially in both groups in response to infusion (15 minutes, $p < 0.01$). CHO oxidation decreased progressively in the trained group throughout the remainder of the infusion period ($p < 0.01$).

Fat oxidation decreased significantly in both the trained and untrained groups 15 minutes after the start of the epinephrine infusion ($p < 0.01$, Figure 7.2b). Fat oxidation was markedly attenuated in the trained group, 15 minutes after the start of the epinephrine infusion, resulting in a significant group x time interaction effect ($p < 0.01$). As the infusion progressed, fat oxidation returned to pre-infusion levels in both groups.

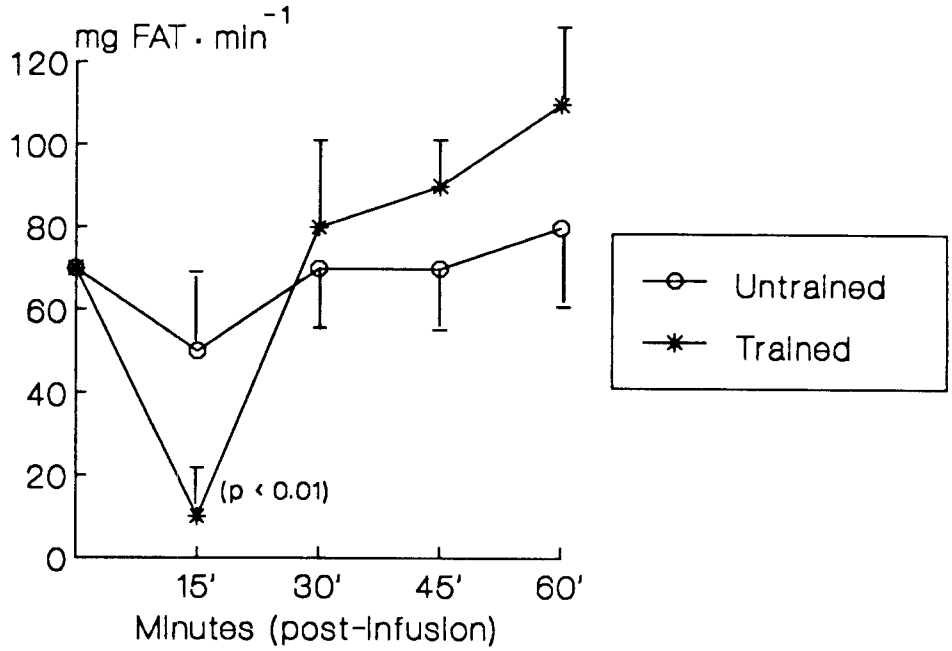

Figure 7.2b. Fat oxidation during epinephrine infusion in trained and untrained subjects. The rate of fat oxidation decreased 15 minutes after the start of the epinephrine infusion more in the trained group. Rate of fat oxidation returned to pre-infusion levels during the remainder of the infusion period in both groups ($p < 0.01$).

Fasting serum free fatty acid concentrations were significantly lower in the trained group compared to the untrained group ($p < 0.01$, Figure 7.3). In response to epinephrine infusion, serum free fatty acid concentrations increased significantly in both groups. However, serum free fatty acid concentrations remained significantly lower in the trained group vs untrained subjects throughout the infusion ($p < 0.03$, Figure 7.3).

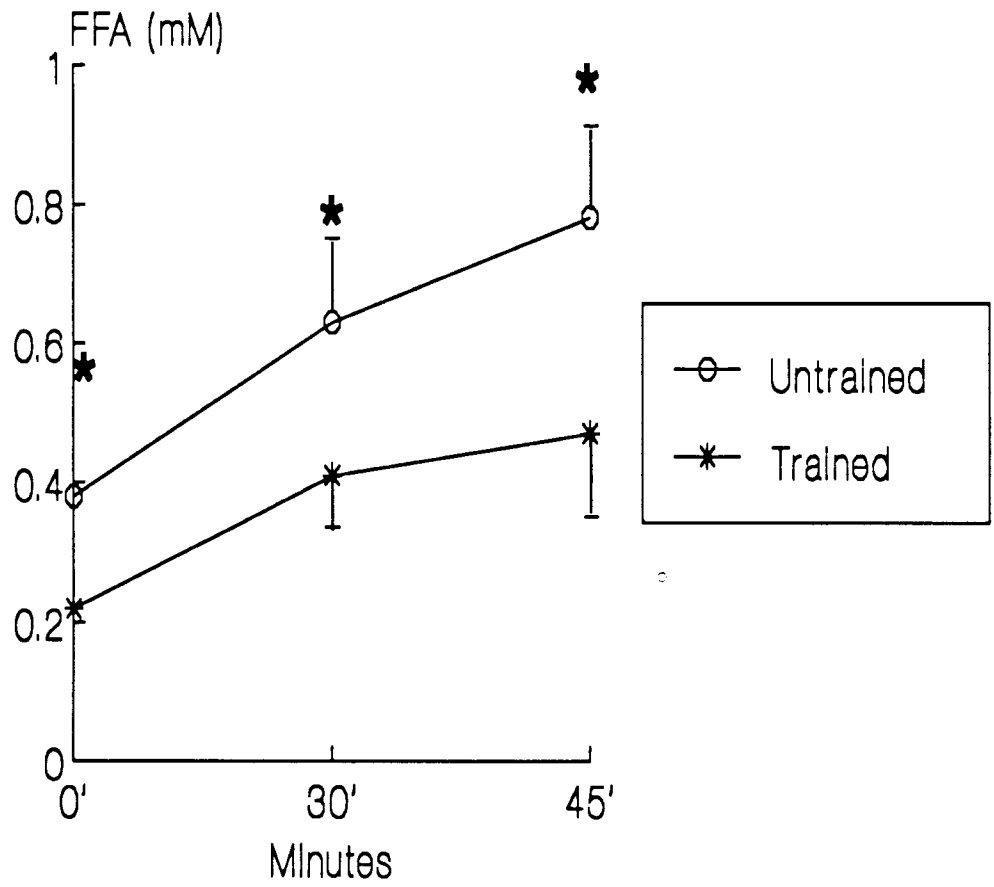

Figure 7.3. Serum free fatty acid concentrations ($mmol \cdot l^{-1}$) in trained vs untrained subjects in response to epinephrine infusion (* $p < 0.03$, [FFA] lower in trained vs control group prior to infusion and during infusion).

Discussion

The purpose of the study reported in this chapter was to examine further the effects of physical training on the

metabolic response to a known thermogenic stimulus. No previous studies have compared the thermogenic response to epinephrine infusion in physically-trained persons with that of sedentary controls. In the present study, there was no evidence to suggest that the increment in resting energy expenditure in response to epinephrine infusion (0.03 $\mu g \cdot kg\ FFM^{-1} \cdot min^{-1}$) was different between the trained subjects and sedentary controls.

In the dose-finding study by Sjostrom et al., (1983), the calculated percent change in responses from basal conditions to those during epinephrine infusion for metabolic and cardiovascular parameters varied from a 15% decrease in diastolic blood pressure to a 35% increase in metabolic rate, a 47% increase in heart rate and a 188% increase in plasma free fatty acid concentrations. These changes corresponded with responses in the present study. Heart rate increased between 24 and 27% in untrained and trained subjects; free fatty acid concentrations increased nearly 200% in both trained and untrained groups; and absolute metabolic rate increased by a mean of 12.9% for trained subjects and 17.2% for untrained subjects.

Thus, the magnitude of change for each of these parameters with epinephrine infusion was not different between trained and untrained persons. Although it was not possible to measure plasma catecholamine concentrations in the present

study, Sjostrom et al. (1983) found that infusion of epinephrine at a rate of 0.03 $\mu g \cdot kg\ FFM^{-1} \cdot min^{-1}$ resulted in an increase in circulating epinephrine concentrations, reaching a steady-state mean of 375 $pg \cdot ml^{-1}$ (346 $\pm$ 33 $pg \cdot ml^{-1}$ at 10 minutes, 401 $\pm$ 37 $pg \cdot ml^{-1}$ at 30 minutes and 377 $\pm$ 35 $pg \cdot ml^{-1}$ at 60 minutes). It has been demonstrated previously that there is no difference in catecholamine clearance rate between trained and untrained persons (Kjaer et al., 1985). An increased clearance would have shifted the dose-response curve for the metabolic effects of epinephrine infusion to the right, and thus, it is unlikely that differences in clearance rate accounted for the apparently similar thermogenic response to epinephrine in the two subject groups in the present study.

The results of previous studies which have compared the effects of various thermogenic stimuli, including exercise, meal-feeding and caffeine ingestion, in trained vs untrained persons are not consistent (Freedman-Akabas, 1985, LeBlanc et al., 1985, Poehlman et al., 1985, Poelman et al., 1988, Poehlman et al., 1989, Tremblay et al., 1983). For example, Le Blanc et al. (1985) found that the thermogenic response to caffeine in trained subjects was higher than in non-trained subjects. In addition, Le Blanc et al. (1985) showed that lipid mobilization and fat oxidation were enhanced in the trained subjects during the 2 hours following caffeine ingestion.

On the other hand, Tremblay and coworkers (Tremblay et al., 1985, 1986, Poehlman et al., 1985, Poehlman et al., 1988) have described a consistent attenuation of the thermogenic response to mixed-meal and glucose-feeding as well as caffeine ingestion, in highly-trained athletes compared to sedentary controls. There are no apparent systematic differences in data collection between these studies and the present study which might explain the differences in results. Nor is there a systematic over- or under-estimation of response to thermogenic stimuli, as the finding is consistent whether expressed as a kJ increment over resting energy expenditure, as a percentage of resting energy expenditure, or after covarying for resting energy expenditure. Nor can differences be attributed to differences in fat-free mass, which were similar in all of these studies, including the present study (Segal et al., 1985).

It is also somewhat surprising that the thermogenic response to epinephrine infusion was not enhanced in the trained group in the present study. Astrup et al. (1985) and Zurlo et al. (1990) have demonstrated that skeletal muscle can be a major thermogenic organ. For example, during physical exercise skeletal muscle may account for over 90% of whole body oxygen uptake (Stainsby and Lambert, 1979). Moreover, in a study of 5 healthy young men, Astrup et al. (1985) found that skeletal muscle accounted for more than 70% of the increase in whole body oxygen uptake in response to ephedrine ingestion.

Individuals who undergo exercise training are regularly exposed to moderately elevated levels of circulating catecholamines during physical work. This exposure may be expected to result in an enhanced catecholamine sensitivity of the skeletal muscle, the primary target tissue for the acute exercise response (Kjaer et al., 1989).

In the present study, the trained subjects had a significantly higher initial rise in respiratory exchange ratio (RER, 15 minutes following the start of the infusion) compared to sedentary controls. Such a sharp initial rise in RER following the initiation of epinephrine infusion was also demonstrated in healthy young sedentary women (Sjostrom et al., 1983). Sjostrom et al. (1983) concluded that the increase in RER was independent of changes in arterial PCO_2, and was only partially influenced by an increased respiratory rate. He suggested that the initial rise in RER reflected true oxidation of endogenous carbohydrate stores, although under non-steady-state conditions.

For the remainder of the infusion period, RER was lower in trained subjects than in controls. These findings correspond to those of Le Blanc et al. (1985) in which trained subjects ingested caffeine. These subjects also had higher initial increases in RER than controls, and these were also followed by a similar attenuation of RER during the post-ingestion period. This suggests that the metabolic responsiveness to

epinephrine may be more influenced by physical training than is the thermogenic response.

The changes in catecholamine sensitivity which have been demonstrated in previous studies in response to chronic administration of ephedrine in untrained subjects (Astrup et al., 1986), are different to those demonstrated by exercise-trained persons, who are exposed to elevated levels of circulating catecholamines each time exercise training exceeds 50% of maximal aerobic capacity (Kjaer et al., 1989).

Summary:

It is apparent from this study that the expected target tissue sensitivity to circulating catecholamines in trained subjects may result in a differentiated response in different systems to acute stimulation by epinephrine infusion. For example, the adipose tissue lipolytic response to the B_2-specific adrenergic activation by epinephrine may be different to the thermogenic effect resulting in an increase in whole body oxygen consumption.

The question may be more effectively answered in a longitudinal study, in subjects at various stages of adaptation to exercise training, in order to examine the "dose-response relationship" between training load and the epinephrine-induced increment in energy expenditure. In the

present study, however, there was no evidence for either energy "thriftiness" or enhanced thermogenesis with meal-feeding (Chapter 6) or epinephrine-infusion (Chapter 7) which may influence long-term energy balance in freely-eating, moderately-trained persons.

CHAPTER 8

METABOLIC RESPONSE TO LOCALIZED SURGICAL FAT REMOVAL IN NON-OBESE WOMEN

Introduction

Previous chapters have addressed the effects of imposing an energy deficit, in the form of food energy restriction or exercise training, or removing such a stimulus by detraining or refeeding, on resting energy expenditure and the metabolic response to glucose feeding. In this model, a form of energy "deficit" was imposed in patients electing to undergo localized fat removal by suction lipoplasty or resection. There are few data regarding the long-term efficacy of these techniques in terms of the maintenance of body fat stores, and the effect of surgical fat removal on energy balance in humans.

In animal models, oblation of adipose tissue depot stores results in a temporary increased metabolic efficiency and a consequent increase in adipose tissue lipogenic activity, hypertrophy and hyperplasia of inguinal-subcutaneous fat stores (Dark et al., 1984, Dark et al., 1985, Forger et al., 1986, Forger et al., 1988). In certain previous studies, a similar efficiency of weight gain has been demonstrated when animals or humans refeed following food restriction (de Boer et al., 1986, Hill et al., 1985). However, there is only a temporary compensation of adipose tissue mass in partially lipectomized rats and mice (Faust et al., 1976). It has not yet been established whether there is a similar adaptation in humans in response to surgical fat removal.

There is increasing evidence that the major determinant of body energy requirements in free-living sedentary persons who are not undergoing food restriction is fat-free mass (Ravussin et al., 1986). Thus, it was hypothesized that removal of fat tissue would not perturb energy balance in the same way as an energy deficit incurred through food energy restriction, where both fat and lean tissue would be lost (Pavlou et al., 1985).

Accordingly, the aim of this study was to characterize the metabolic response to surgical fat removal in weight-stable, non-obese women. The body may act to "defend" its weight by reducing energy expenditure or by going into positive energy balance and/or diverting part of food energy intake in to regenerative tissue growth or hypertrophy of remaining fat tissue. This would lend support to the idea of a physiological "set-point" (Keesey, 1989) for body mass and would suggest that total body fat stores play an important role in regulating body energy balance. If there was no evidence for compensation, this may suggest that (i) the effector which regulates adipose mass was removed with the tissue, (ii) there is no fat homeostat, or alternatively, (iii) the "sensor" is regulated by changes in cell size and not total adipocyte number.

Methods

Subjects and experimental protocol:

Seven, non-obese women scheduled for elective surgery including suction lipoplasty and abdominoplasty volunteered to participate in this study. All women were free from metabolic disease and had previously reduced body weight through food restriction, but were weight-stable for several months prior to surgery. All subjects were informed as to the nature of the study and written consent was obtained. All procedures had been approved by the Ethics and Research Committee, Faculty of Medicine, University of Cape Town Medical School.

The amount of tissue surgically removed in the lipoplasty or lipectomy procedure is presented for each subject in Table 8.2. The amount of excised tissue varied from 0.46 kg to 2.80 kg. It has been recommended that liposuction resections be limited to less than 2.5 kg, and, that in cases where over 2.0 kg of tissue is removed, that autologous blood tranfusion be performed (Hetter, 1984).

Food energy intake:

Subjects were asked to keep a 7-day record of daily food intake. The technique used has been described in detail in

Chapter 5. All food items were weighed where possible or household measures were used. Food records were coded and analyzed using the Floro Diet Data Programme (Durban, South Africa) based on the Food Composition Tables and Food Quantities Manual (Research Institute for Nutritional Diseases, Medical Research Council, Parow, South Africa). Subjects were then instructed to keep food intake constant following surgery, and a second dietary record was obtained during the post-testing period on 5 of the 7 subjects.

Resting energy expenditure and the increment in resting energy expenditure following glucose ingestion:

Resting energy expenditure and the glucose-stimulated increment in energy expenditure were measured in each subject prior to surgery and between 1 and 2 months post-operatively. These techniques have been described in detail previously (Chapter 5). Briefly, oxygen consumption (VO_2) was measured using an open-circuit, ventilated-hood system for indirect calorimetry. Mixed expired air was sampled continuously for oxygen and carbon dioxide content, and VO_2, CO_2 production (VCO_2), and respiratory exchange ratio (RER, VCO_2/VO_2) were then calculated each minute for the duration of the trial using a microcomputer and software (Craig Mason-Jones, Lateral Alternative, Cape Town, South Africa).

Resting energy expenditure was measured after a 10-12 hour fast and following a minimum of 30 minutes of supine rest. Respiratory exchange measures were collected for a further 30 minute period and the mean VO_2, VCO_2 and RER were determined for the resting state.

The glucose-induced increment in energy expenditure was determined by feeding a 100g glucose load in water with a total fluid volume of 400 ml. Respiratory exchange measures were then collected continuously for 2 hours post-prandially. The area under the curve was estimated for the increment in energy expenditure from 30 to 120 minutes following glucose ingestion. These data were compared with the area under the curve which was extrapolated for this period from the resting energy expenditure. The increment was expressed as a percentage increase in energy expenditure over resting for the 120 minute period.

Anthropometry and regional fat cell size:

Skinfold measures were obtained for the following sites both before and between 1 and 2 months post-operatively: biceps, triceps, subscapular, and supra-iliac. Percentage body fat

was estimated using the equations of Durnin and Womersley (1974).

Fat samples were obtained from the thigh and abdomen by needle aspiration after local anaesthesia. Samples weighing between 20 and 80 mg were placed in Krebs-Ringer/ HEPES buffer, pH 7.4, with 1.5 $mg \cdot ml^{-1}$ collagenase (Type II) and 3g/100 ml bovine serum albumin (Fraction V, Rodbell, 1964). Samples were incubated at 37°C for 20 minutes, and shaken gently at 5 to 10 minute intervals. Following incubation, tissue and medium were filtered through a 250 μm nylon mesh, cells were washed twice with collagenase-free buffer and resuspended in 0.5 ml buffer.

Isolated fat cell size was determined using calibrated light microscopy (Bray, 1970). A minimum of 100 cells were sized for each analysis at each site, with the exception of one post-operative femoral sample.

Statistical analysis:

A student's t-test for related samples was used to compare pre- and post-operative responses. Differences with a

probability level of less than 0.05 were considered statistically significant. Results are presented as means and standard errors of the mean.

Results

Pre- and post- surgical anthropometric data for subjects are presented in Table 8.1. There were no significant changes in body mass or in subcutaneous fat thickness in the four selected sites pre- and post-operatively. Changes in estimated percentage body fat do not reflect actual fat mass removed (Table 8.2). This would not be expected as these skinfold sites do not correspond to specific areas of localized fat removal.

Table 8.1. Pre- and 1-2 months post-operative anthropometric data (means ± SEM).

	n	PRE	POST
Mass (kg)	7	70.8 ±3.1	69.4 ±3.6
Triceps (mm)	6	20.9 ±1.4	23.0 ±2.0
Biceps (mm)	6	9.7 ±1.2	11.0 ±1.1
Subscapular (mm)	6	25.6 ±2.2	23.4 ±2.3
Supra-iliac (mm)	6	19.3 ±3.1	20.2 ±3.2
% body fat	6	32.6 ±0.9	32.9 ±0.8

Table 5.2. Mass of tissue surgically removed.

Subject	Excised tissue (kg)
JO	2.80
CS	1.14
VB	0.90
MH	0.46
CG	0.76
MZ	-
VH	-

(Excised tissue mass was not measured in 2 of the 7 subjects).

Similarly, there were no differences in pre- and post-operative regional mean fat cell diameter. Abdominal and femoral fat cell diameters remained constant before and after surgery (Table 8.3).

Table 8.3. Adipocyte diameter (μm) in the femoral and abdominal regions in four subjects pre- and post-operatively.

Subject	Abdominal		Femoral	
	PRE	POST	PRE	POST
JO	109	109	116	131
VB	93	95	128	109
VH	96	98	107	113
MH	103	102	86	101
CG	79	84	82	86
Mean	96.0	97.6	103.8	108.0
SEM	$\pm$ 5.7	$\pm$ 4.6	$\pm$ 9.8	$\pm$ 8.2

Reported energy and nutrient intake are presented in Table 8.4. While there were no statistically significant paired

differences in energy or nutrient content of the diet before surgery and during follow-up for the group, one subject reported a 43% reduction in energy intake during the follow-up period. However, previous studies have shown that subjects who reduced reported food energy intake by approximately 30% demonstrated a significant reduction in resting metabolic rate ($p < 0.01$, Chapter 5). This subject's expenditure data do not reflect food restriction. Thus, when her data are excluded, the pre- and post- operative means for energy intake are 6.02 $\pm$ 2.07 and 6.20 $\pm$ 1.79 MJ per day.

Table 8.4. Daily energy intake, extracted from 7 day dietary recall, pre-operatively and at 1-2 months post-operatively (means $\pm$ SEM).

	MJ (energy)	**Protein (g)**	**Fat (g)**	**CHO (g)**
PRE (n=5)	6.21 $\pm$0.82	58.2 $\pm$ 8.9	56.1 $\pm$ 8.7	172.4 $\pm$ 25.5
POST (n=5)	5.75 $\pm$0.83	52.8 $\pm$ 8.5	51.2 $\pm$ 8.9	163.2 $\pm$ 22.5

There was only a -2.5% difference between pre- and post-operative means in resting energy expenditure (W or $kJ \cdot min^{-1}$, Table 8.5), estimated from respiratory exchange data using the equations of Weir (1949).

When subjects were challenged with an oral glucose load, the increment in energy expenditure for 2 hours post-feeding was not different before or after surgery (11.1 $\pm$ 2.4 % vs 11.0 $\pm$

2.6 % increase over resting, Table 8.5). Nor were there any differences in the contribution of carbohydrate and fat to total oxidative metabolism at rest and in response to oral glucose feeding, pre- or post-operatively.

Table 8.5. Resting energy expenditure (REE, W or $kJ \cdot min^{-1}$) and the glucose-induced increment in resting energy expenditure, expressed as % increase in resting energy expenditure from 30 to 120 minutes post-ingestion (means ± SEM).

	n	PRE	POST
REE (W)	7	67.3 ±1.8	64.5 ±2.8
REE ($kJ \cdot min^{-1}$)	7	4.04 ±.11	3.87 ±.17
Glucose-induced % increment REE	5	11.1 ±2.4	11.0 ±2.6

Discussion

In this study, localized surgical fat removal in weight-stable, non-obese women did not significantly alter either adipocyte size or regional fat distribution in the non-operated areas. This finding is significant, particularly when one considers that total depot fat lipectomy in the animal model results in 1) increased lipogenic activity in

subcutaneous-inguinal fat depots, and 2) a regeneration of adipose tissue in non-gender-specific stores through hyperplasia and hypertrophy of subcutaneous tissue (Dark et al., 1984, Dark et al., 1985, Forger et al., 1986, Forger et al., 1988).

Oblation of depot fad pads in some mammals does appear to result in hypertrophy of the remaining adipose tissue (Dark et al., 1984, Dark et al., 1985, Forger et al., 1986, Forger et al., 1988). Evidence for regulation of adipose tissue mass based on total lipid mass or cell number has been demonstrated by increased viability of adipose tissue grafts and hypertrophy in contralateral fat depots (Leibelt et al., 1968, Shapiro and Zinder, 1972).

Conversely, partial lipectomy in rats and mice does not result in a compensatory increase in fat pad mass or total lipid mass after long-term follow-up (Faust et al., 1976). Similarly, one would not expect to see a large increase in subcutaneous fat following a human mastectomy or limb amputation, even though a significant amount of fat and lean tissue is lost as a result of the procedure. In addition, the marked differences in metabolic characteristics of regionally-distributed fat suggest that regulation of adipose tissue mass is more local (Rebuffe'-Scrive et al., 1986).

In the present study, there was no significant change in resting or glucose-stimulated energy expenditure up to 2 months following lipectomy. These results suggest that surgical lipectomy in non-obese women does not alter energy balance at rest or in response to feeding during the short-term follow-up period. These findings were not unexpected considering firstly, that fat-free mass has been shown to be the single best predictor of resting energy requirement (Ravussin et al, 1986) and that fat-free mass was not altered significantly as a result of surgery.

Although it may be argued that a follow-up period of between 1 and 2 months post-surgery may be too short, it is unlikely that there were any persistent effects as a result of the surgery, itself. Indeed, in partially lipectomized rats, the post-surgical effect was associated with increased hypertrophy in remaining depot fat at 30 days, and differences were resolved by 80 days post-surgery (Faust et al., 1976).

Thus, there were no apparent compensatory changes in adipose tissue or energy expenditure in the current human lipectomy model. This suggests that the relationship between adipose tissue mass and energy balance is different in animal vs human models. This may also be due in part, to differences in the time course of recovery following fat removal or due to differences in regulation between partial vs total lipectomy of depot fat.

Fat cells of persons who have lost weight through physical training or dieting are smaller than those of non-reduced, non-overweight controls (Eckel and Yost, 1987, Tremblay et al., 1984). Refeeding following energy restriction in rats is associated with an increased efficiency of weight gain and rapid increases in fat and total mass (Hill et al., 1985). Similarly, in obese persons who have undergone food restriction and have refed to a stable, reduced mass, adipose tissue lipogenic activity has been demonstrated to be elevated when compared to those prior to weight loss (Leibel et al., 1985, Schwartz and Brunzell, 1978). These cells have a reduced rate of lipolysis, a reduced rate of fatty-acid triglyceride recycling and an increased metabolic efficiency. Thus, studies involving food restriction and exercise training in both humans and animals (where fat cell size is reduced) strongly suggest that organisms tend to correct continually for errors in energy balance.

It is likely that adipose tissue mass autoregulates, as it remains relatively constant despite day-to-day flucuations in energy balance and is only affected when a significantly large energy deficit is incurred (Shapiro, 1984). The nature of the signal which provides feedback, and thereby informs the body's energy balance mechanisms of the level of fat stores remains unclear. However, there are a number of factors which suggest

that adipose tissue is more sensitive to changes in fat cell size than to fat cell number or total lipid mass. In both human and animal models, the reduction in fat cell number appears to have a less significant role in regulation of energy balance than lipid-filling or adipocyte size (Kral, 1975).

It is difficult to make direct comparisons between animal models where surgical removal of adipose tissue amounted to complete oblation of depot fat and this study in which the tissue removed amounted to a mean of only approximately 1.7% of total body mass. Surgical lipectomy in amounts of greater than 2.5 kg is not recommended, requiring blood transfusion and an unacceptable risk of complications (Hetter, 1984).

Summary:

Modest surgical reduction in total adipose tissue mass in reportedly weight-stable, non-obese women does not appear to result in a compensatory increase in fat cell size, metabolic efficiency or changes in regional adipose distribution. While it is recognized that there may be individual differences in regulation of adipose tissue mass or of feedback signals in obese persons and very metabolically efficient persons, in general, modest surgical fat removal in non-obese women does not influence short-term energy balance in non-obese women. Thus, the reduction in fat cell size with

dieting or exercise appears to play a more important role in the regulation of adipose tissue mass, than a reduction in fat cell number following surgical lipectomy amounting to less than 2% of total body mass.

CHAPTER 9

RESTING ENERGY EXPENDITURE AND THE THERMIC EFFECT OF FEEDING AND EXERCISE IN WEIGHT-MATCHED LARGE AND SMALL EATERS

Introduction

The law of conservation of energy implies that energy expenditure in mass-stable, body composition-stable individuals is tightly coupled to energy intake. Despite this, an apparent paradox is frequently observed in which persons with a similar body mass, and with similar levels of physical activity, may have as much as a two-fold difference in habitual energy intake (Edholm et al., 1970). Studies spanning 60 years, from the 1930's to the present (Booyens and McCance, 1957, Durnin, 1961, Edholm and Fletcher, 1955, Edholm et al., 1970, Haggarty et al., 1986, Miller and Parsonage, 1975, Rose and Williams, 1960, Warwick et al., 1988, Webb, 1985, Widdowson, 1936, Widdowson and McCance, 1936, Widdowson et al., 1954) have been unsuccessful in characterising gross differences in net energy efficiency (Kleiber, 1975) which might explain the large variation in energy which is apparently "required" for the individual maintenance of body mass and body energy stores.

Despite this, previous chapters in this dissertation (Chapters 3, 4, 5, 6, 7), have shown that organisms respond acutely to perturbations in energy balance, producing a measurable change in components of energy balance which may explain short-term differences in energy expenditure, and responses to thermogenic stimuli such as feeding or exercise.

Therefore, the aim of this study was to compare energy expenditure at rest and in response to various thermogenic stimuli in freely-eating, matched, mass-stable women selected as "large" and "small" eaters. Pairs of subjects were matched for age, height, mass, body composition and body shape, because each of these factors has been implicated as a partial determinant of individual daily energy requirements for weight maintenance (den Besten et al., 1988, Guyton, 1981, Ravussin et al., 1986, Segal et al., 1985). It was hypothesized that a compensatory increase in feeding efficiency or work efficiency as an adaptive response to chronic low energy intake would be demonstrated in the small eaters.

Methods

Subject characteristics and experimental protocols:

Subjects were selected from a pool of 45 volunteers who responded to newspaper advertisements or were recruited from university residences. All volunteers were apparently healthy, sedentary women between the ages of 20 and 42, who had been mass-stable for at least 6 months prior to the experimental trial. All volunteers were asked to keep a 7-day food diary. Height, mass and skinfold measurements were used to estimate body composition (Durnin and Womersley, 1974). Experimental subjects were selected on the basis of the "best match" for anthropometric

variables and age, and for the greatest variation in food energy intake.

Subjects were fully informed of the nature and purpose of the investigation and written consent was obtained prior to participation. Testing procedures were approved by the Ethics and Research Committee of the University of Cape Town Medical Faculty. Two separate studies were undertaken and where possible, data were combined. The two protocols are described below and graphically illustrated in Figure 9.1.

Experiment 1: Four pairs of women were matched for age, height, mass, and body composition. Subjects recorded food energy intake for a period of 7-days, after which they were provided with food packages for a period of 3-5 days to ensure weight stability and isocaloric constancy in food intake prior to the trial. Resting energy expenditure was measured, after which subjects performed 30 minutes of steady-state submaximal exercise on a cycle ergometer. Energy expenditure was measured throughout the exercise bout and continuously during two hours of post-exercise recovery.

Experiment 2: Five pairs of women were selected and matched as described previously. All subjects selected were non-smokers and did not use any form of oral contraception. These subjects were matched for waist-hip ratio (as an indicator of regional body fat distribution), as well as for age, height, mass, and body

composition. Food energy intake was recorded for 7 days, and pre-trial meals were prepared for each subject for 3-5 days prior to each trial. Resting energy expenditure was measured, after which subjects were fed a 2.1 MJ liquid mixed-meal, and the thermic effect of feeding was measured for 3 hours following ingestion. Steady-state energy expenditure during cycle ergometry was measured on a separate day.

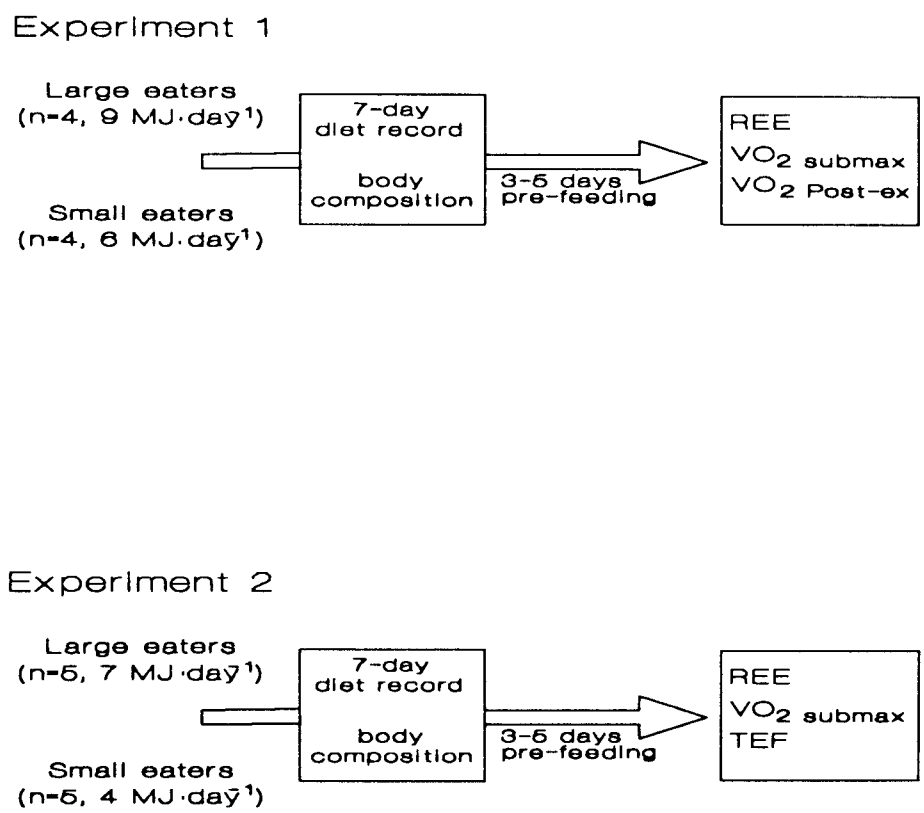

Figure 9.1. Schematic presentation of experimental protocols for Experiments 1 and 2 (REE = resting energy expenditure, VO_2 submax = submaximal oxygen consumption during steady-state cycle ergometry, VO_2 post-ex = the excess post-exercise oxygen consumption for two hours following the submaximal exercise bout, TEF = thermic effect of mixed-meal feeding for 3 hours post-ingestion).

Subject pair characteristics for both studies are presented in Table 9.1. Mean food intake was 8.95 ± 0.68 and 7.13 ± 1.28 $MJ \cdot d^{-1}$ for "large" eaters in *experiments 1* and *2*, respectively, vs 6.24 ± 1.44 and 4.09 ± 0.97 $MJ \cdot d^{-1}$ for "small" eaters in *experiments 1* and *2*.

Dietary analyses and feeding regimens:

Each volunteer was given a standard food scale and asked to record mass and volumes of all food and beverages ingested during a 7-day period. Household measures were used when metric measurement was not possible. Volunteers were encouraged to eat as normally as possible during this period. Food records were coded and analyzed using reference food composition and quantity tables (Research Institute for Nutritional Diseases, Parow, South Africa) and a computerized dietary analysis program (Floro Diet Data Program, Durban, South Africa).

In order to verify the accuracy of food intake reporting, all selected subjects were asked to ingest a laboratory-prepared diet for 3-5 days prior to the experimental trial, and throughout the experimental trial (2-5 days). This diet was comprised of prepared food and liquid meals of which the nutrient and energy content were not significantly different to that reported in the 7-day dietary record. Black, sugarless coffee and diet soft drinks were allowed in amounts which were similar to subjects'

habitual intake. Subjects weighed themselves each day, and body mass did not change significantly throughout the trial.

Table 9.1. Subject pair characteristics between matched women grouped according to food energy intake (means ± SEM).

Exp 1	Age (yrs)	Ht(cm)	Mass(kg)	% fat	Intake ($MJ \cdot d^{-1}$)
Large Eaters (n=4)	27.0 ±7.4	167.0 ± 7.8	69.0 ±13.1	31.6 ±5.0	8.95 ±.68
Small Eaters (n=4)	27.5 ±8.3	166.0 ± 6.9	71.3 ±12.5	29.8 ±5.2	6.24 ±1.44

Exp 2	Age (yrs)	Ht(cm)	Mass(kg)	% fat	Intake ($MJ \cdot d^{-1}$)	w/h ratio
Large Eaters (n=5)	19.8 ±0.4	168.6 ± 2.9	60.8 ± 2.2	26.8 ±0.5	7.13 ±1.28	.76 ±.02
Small Eaters (n=5)	22.2 ±0.7	166.8 ± 3.2	59.9 ± 2.2	27.4 ±1.3	4.09 ± .97	.73 ±.04

The energy content of the mass-maintenance diet was estimated using the standardized food composition tables as described previously. In addition, in *experiment 2*, the energy content was measured using bomb calorimetry, and corrected for measured dietary protein and estimated dietary fibre content. The prepared food was liquidized, weighed and freeze-dried. A ballistic bomb calorimeter was used to estimate the number of kJ per g of dry weight. This method has been shown to have a

measurement standard error of 1.1% in six replicate determinations (Miller and Payne, 1959).

Energy content of the bombed samples was corrected for 1) the incomplete oxidation of protein *in vivo* and 2) the estimated unavailable carbohydrate which is dietary fibre (RIND Food Composition Tables, Research Institute for Nutritional Diseases, Parow, South Africa). Freeze-dried samples of the experimental diets were then analyzed for total nitrogen content using the micro-Kjeldahl technique (Vogel, 1978).

In *experiment 2*, 24-hour urine samples were also collected. Urinary urea and creatinine were measured and urinary nitrogen excretion was estimated using standard methods of autoanalysis.

Anthropometry:

Four skinfold sites were used to estimate body composition using the regression equations of Durnin and Womersley (1974). These included: triceps, biceps, subscapula and suprailiac (described in full in Chapter 5, **Methods**)

Lean thigh volume was estimated by assuming that the thigh was a truncated cone. Limb length, thigh skinfold thickness, and circumferences immediately above the knee and just below the

gluteal fold were used to estimate lean thigh volume with the following equation (adapted from Katch and Katch, 1974).

$$\text{volume} = 1/3\ h\ (a1 + a2 + \sqrt{a1a2})$$

where: h = height between 2 girth measures, a1, a2 = surface area between 2 parallel surfaces of cone surface area = πr^2, where r is corrected for subcutaneous fat; r = (circum/2π)-(skinfold(cm)/2)

Waist-to-hip ratio (w/h) was calculated as the ratio between abdominal circumference (smallest circumference between the xiphisternum and the umbilicus) and gluteal circumference (largest horizontal circumference at the maximum protrusion of the buttocks when the feet are together, Bray 1989).

Energy expenditure at rest, during steady-state cycle ergometer exercise, the post-exercise increment in energy expenditure and the thermic response to mixed-meal feeding:

Resting oxygen consumption (VO_2) was measured in the morning, in the post-absorptive state, after subjects were familiarized with the apparatus and after a minimum of 30 minutes of supine rest, as described previously (Chapter 5). Respiratory exchange measures were collected for a 30 minute period, using a ventilated-hood, open-circuit system for indirect calorimetry.

The mean VO_2, carbon dioxide production (VCO_2) and the respiratory exchange ratio (RER) were determined at rest.

VO_2, VCO_2, and RER were then calculated each minute for the duration of the trial using a microcomputer and software (Craig Mason-Jones, Lateral Alternative, Cape Town, South Africa). Respiratory exchange data were used to calculate energy expenditure using the conversion equations for energy equivalents (Weir, 1949).

In *experiment 1*, subjects were then placed on an electronically-braked cycle ergometer (Godart NV, Bilthoven, Holland). Subjects cycled at a set workload of 50 W for 30 minutes. Expired air was sampled continuously during the final 10 minutes of the steady-state exercise bout and rates of VO_2, VCO_2 and RER were calculated for each minute.

Within two minutes of completing the cycle ride, subjects were transferred to a bed in the metabolic laboratory and energy expenditure was monitored for 2 hours after the exercise bout. Respiratory exchange data were collected for 10 minutes, every other 10 minute period. The total area under the curve for energy expenditure was estimated. The area under the curve which accounted for basal energy requirements was estimated by extending the pre-exercise metabolic rate over 2 hours. This was then subtracted from the total area under the curve to determine the post-exercise increment in energy expenditure. In addition,

the energy expenditure of large and small eaters was compared at 10, 20, 30, 40, 60...120 minutes post-exercise.

Subjects in *experiment 2* performed the steady-state cycle ergometer bout on a separate day. Workload was adjusted for lean thigh volume (10 W per litre of lean thigh volume) and no post-exercise test was performed.

In *experiment 2*, immediately following the measurement of resting metabolic rate, subjects were given a liquid meal to ingest. This meal consisted of: 200 ml full cream milk, 250 g Nestle's cream, 66 g ENSURE (Abbott's Laboratories, South Africa, powdered formula providing 0.16 g fat, 0.16 g protein and 0.62 g absorbable carbohydrate per g) and 5 g granulated white sugar. This meal therefore contained 15% protein, 40% fat and 45% carbohydrate (by energy content) for a total energy content of 2.1 MJ. The thermic effect of mixed-meal feeding was measured continuously for 180 minutes. The total area under the curve (energy expenditure x time) in the post-feeding period was calculated. The post-feeding increment in metabolic rate was calculated by subtracting the area under the curve for resting metabolic rate, extrapolated over 3 hours from the total area under the curve. In addition, the energy expenditure was compared between large and small eaters at each 15 minute interval.

Statistical analyses:

All data are expressed as means ± SEM. Paired t-tests were performed for all matching, single-measurement data such as anthropometry and energy intake. Paired t-tests were also performed for single variable comparisons. For example, the steady-state exercise oxygen consumption of large and small eaters was compared using this test. Multiple variable measurements or measurements over time were compared using a two-way analysis of variance for repeated measures. The thermic effect of feeding and exercise were compared between the groups of large and small eaters over time. When significant F-ratios were found, Tukey's post-hoc analyses were performed. A probability level of less than 5% was considered statistically significant.

Results

Food energy and nutrient intake:

Subjects were matched on the basis of age, height, mass, and body composition (Table 9.1). Mean daily reported energy intake was significantly different between large and small eaters; however, the estimated carbohydrate and protein composition (% of total energy) of *ad libitum* diets of large and small eaters based on standard food composition and quantities tables were not

different (Table 9.2). The fat composition in the diets of small eaters in *experiment 2* was significantly less than that of large eaters ($p < 0.03$).

In *experiment 2*, the energy and nutrient composition of the reported *ad libitum* diets based on standard food composition tables were compared to that of the experimental diets which were intended to have the same energy and nutrient content as the reported *ad libitum* diet. Samples of these experimental diets were analyzed by bomb calorimetry and corrected for estimated protein and fibre content. Corrected, bombed samples tended to be lower in energy content than the *ad libitum* diet estimates. However, the mean difference in energy content of food intake between groups of large and small eaters was significant for all techniques ($p < 0.001$), ranging from 35.7% and 42.6% between group difference for the various techniques.

Nitrogen intake and urea nitrogen excretion were measured in subjects from *experiment 2*. Nitrogen intake ($g \cdot 24\ h^{-1}$) was significantly higher in the large eaters than in small eaters ($p < 0.05$, Figure 9.2). The ratio between nitrogen intake and urinary urea nitrogen was not different between groups (1.10 ± 0.16 vs 1.31 ± 0.56, for large and small eaters, respectively).

Table 9.2. Nutrient composition of *ad libitum* diets of matched large and small eaters (means ± SEM).

	% of total daily MJ intake		
	Protein	**Fat**	**CHO**
Experiment 1			
Large Eaters (n=4)	14.3 ±1.4	39.2 ±5.4	44.9 ±6.4
Small Eaters (n=4)	17.1 ±3.6	36.0 ±8.3	45.9 ±8.0
Experiment 2			
Large Eaters (n=5)	15.0 ±1.7	38.9[a] ±1.9	45.6 ±2.2
Small Eaters (n=5)	17.4 ±2.7	28.6[b] ±3.0	52.7 ±2.4

([a,b] $p < 0.03$ for percentage fat intake between large and small eaters in *Experiment 2*)

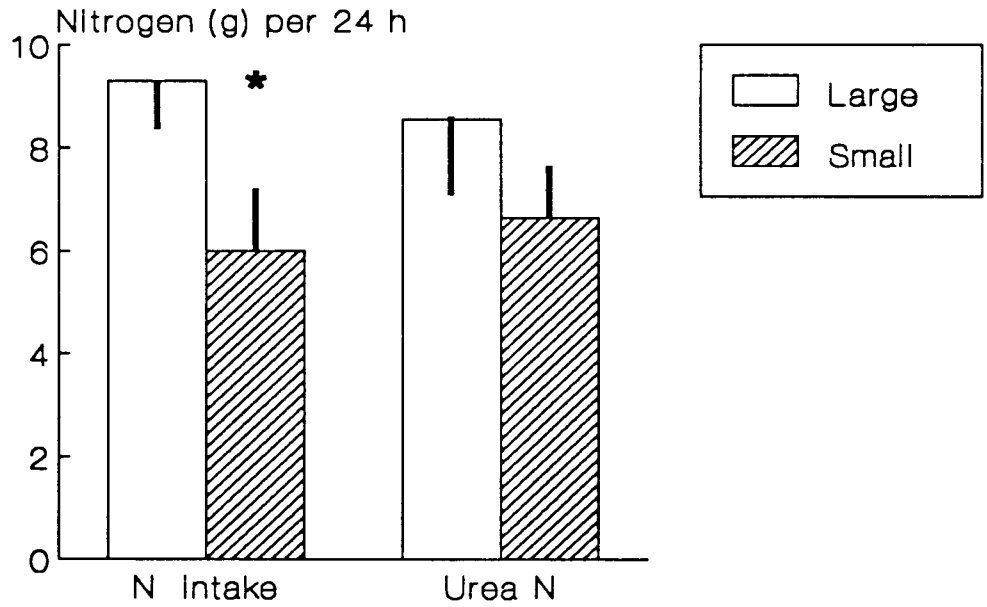

Figure 9.2. Nitrogen intake (g) and urinary urea nitrogen excretion (g) in large and small eaters from *experiment 2* (* $p < 0.05$ for nitrogen intake between large and small eaters).

Resting energy expenditure, thermic effect of feeding and exercise and the post-exercise increment in oxygen consumption:

The resting energy expenditure of large and small eaters did not differ in either experiment, whether expressed absolutely (W or $kJ \cdot min^{-1}$) or relative to body mass or fat-free mass. Individual data were combined from both studies and presented in Table 9.3.

Table 9.3. Combined data for resting energy expenditure in large vs small eaters, expressed absolutely (W or $kJ \cdot min^{-1}$) and relative to mass ($W \cdot kg^{-1}$) and fat-free mass ($W \cdot kg\ FFM^{-1}$, means ± SEM).

Large Eaters (n=9)	**W**	**$kJ.min^{-1}$**	**$W\ kg^{-1}$**	**$W \cdot kg^{-1}$ FFM**
GP	71.3	4.28	0.75	1.23
TS	53.1	3.19	0.99	1.37
KB	72.1	4.33	1.08	1.63
VP	75.7	4.54	1.16	1.57
LF	63.7	3.82	1.06	1.48
SE	61.7	3.70	1.10	1.49
TS	67.1	4.03	0.97	1.35
MF	57.3	3.44	0.99	1.33
NZ	66.5	3.99	1.09	1.47
x	65.3	3.92	1.02	1.44
SEM	±2.3	±.14	±.04	±.04
Small Eaters (n=9)	**W**	**$kJ.min^{-1}$**	**$W\ kg^{-1}$**	**$W \cdot kg^{-1}$ FFM**
HM	76.3	4.58	0.85	1.37
RF	51.1	3.07	0.93	1.24
TJ	51.8	3.11	0.75	1.01
LK	65.1	3.91	0.92	1.33
TT	57.3	3.44	0.97	1.34
SP	63.7	3.82	1.14	1.52
VW	60.8	3.65	0.89	1.33
AM	56.7	3.40	0.99	1.36
AG	71.3	4.28	1.19	1.60
x	61.6	3.70	0.96	1.34
SEM	±2.8	±.17	±.06	±.05

Small eaters expended more energy at rest, relative to their metabolisable energy intake (MEI) over 24 hours, than did large eaters ($p < 0.001$, Figure 9.3).

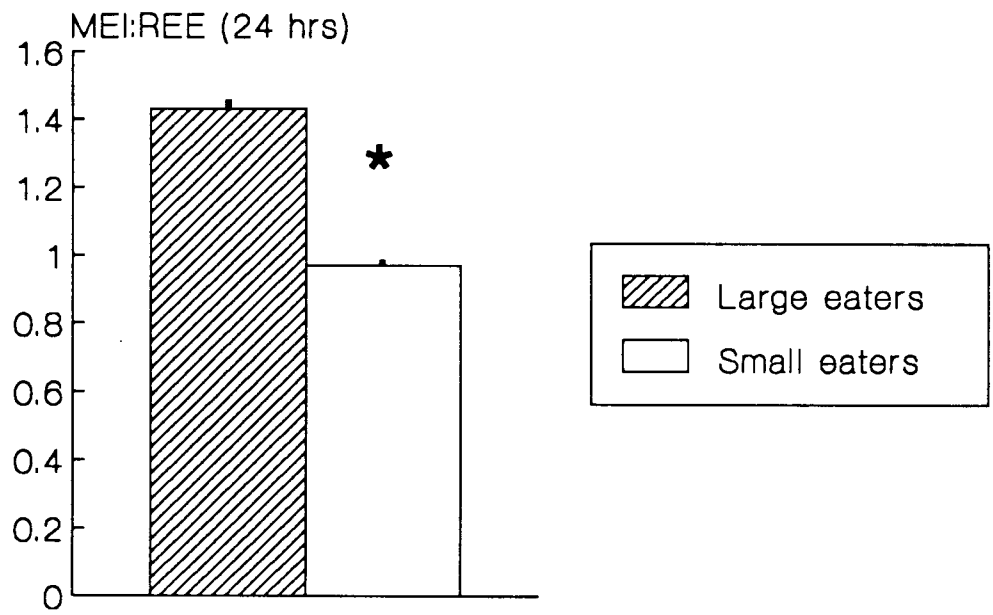

Figure 9.3. The ratio between metabolisable energy intake (MEI) and resting energy expenditure (REE) estimated for 24 hours was significantly lower in small eaters than large eaters (* $p < 0.01$, means $\pm$ SEM).

The oxygen consumption during 30 minutes of steady-state, submaximal cycle ergometer exercise was measured in both studies. There were no statistically significant differences in VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) at an absolute workload of 50 W or when the

workload was corrected for differences in lean thigh volume (Table 9.4.).

Table 9.4. Mean steady-state VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$) during submaximal cycle ergometer exercise in large and small eaters (means ± SEM).

Experiment 1	**W**	**VO_2**	**Experiment 2**	**W**	**VO_2**
Large Eaters (n=4)	50	12.3 ± 2.2	**Large Eaters** (n=5)	40 ± 3	14.6 ± 0.4
Small Eaters (n=4)	50	14.1 ± 2.0	**Small Eaters** (n=5)	43 ± 2	14.5 ± 0.7

In *experiment 1*, the post-exercise increment in energy expenditure was measured for 120 minutes. The total area under the curve post-exercise (W) was compared with the resting area (W) extrapolated for 120 minutes, and expressed as a percent increase over the resting area under the curve for 120 minutes. The exercise bout resulted in a 7.21 (± 1.66)% increment in energy expenditure over resting energy expenditure in large eaters compared with a 15.48 (± 4.80)% increment in energy expenditure demonstrated in the small eaters. However, this difference was not statistically significant.

Absolute energy expenditure (W or $kJ \cdot min^{-1}$) was also not different at any time between large and small eaters over the 120 minute period following the exercise bout (Figure 9.4). Peak

post-exercise energy expenditure occurred between 10 and 30 minutes after the cessation of exercise.

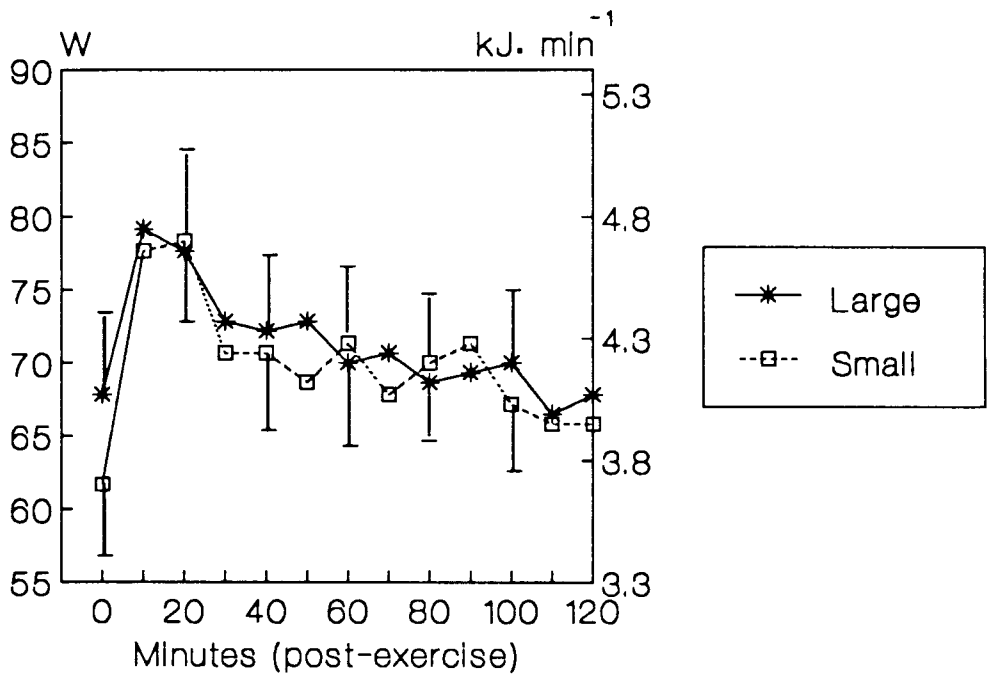

Figure 9.4. The post-exercise increment in energy expenditure for large and small eaters for 120 minutes following the standardized cycle ergometer exercise bout (means ± SEM).

The post-prandial rise in energy expenditure after a 2.1 MJ mixed meal was measured for 3 hours in *experiment 2*. The total area under the curve for energy expenditure was estimated and expressed as a percentage increment in resting energy expenditure (W) extrapolated for the same time period. The thermic effect of feeding was also expressed as a percentage of the ingested energy. There was no significant difference in the total 3-hour

area under the curve for energy expenditure following mixed meal feeding between large and small eaters (Table 9.5).

Table 9.5. Thermic response to mixed meal feeding expressed as a percentage increment over resting energy expenditure extrapolated for 3 hours, and expressed relative to the total ingested energy (2.1 MJ, means ± SEM).

	Large Eaters (n=5)	**Small Eaters (n=5)**
% increment over resting energy expenditure	13.94 ± 3.64	12.36 ± 2.42
% of total ingested energy	4.80 ± 1.40	4.20 ± 1.10

However, when the data were analyzed at 15 minute intervals over the 3 hour period, a significant interaction effect between the large and small eaters was evident after 105 minutes post-glucose ingestion (Figure 9.5, $p < 0.05$). The thermic response to feeding continued to increase in large eaters until 165 minutes. The metabolic response to feeding was lower in the small eaters from minutes 120 to 165.

In Table 9.6, various factors associated with body size, shape and composition, as well as food intake, were correlated to energy expenditure at rest and in response to thermogenic stimuli in mass-stable women. In this study, by design, there was no relationship between food intake and measures of body size or morphology. Nor was there any relationship between food intake and resting energy expenditure or the thermic effect of feeding or exercise.

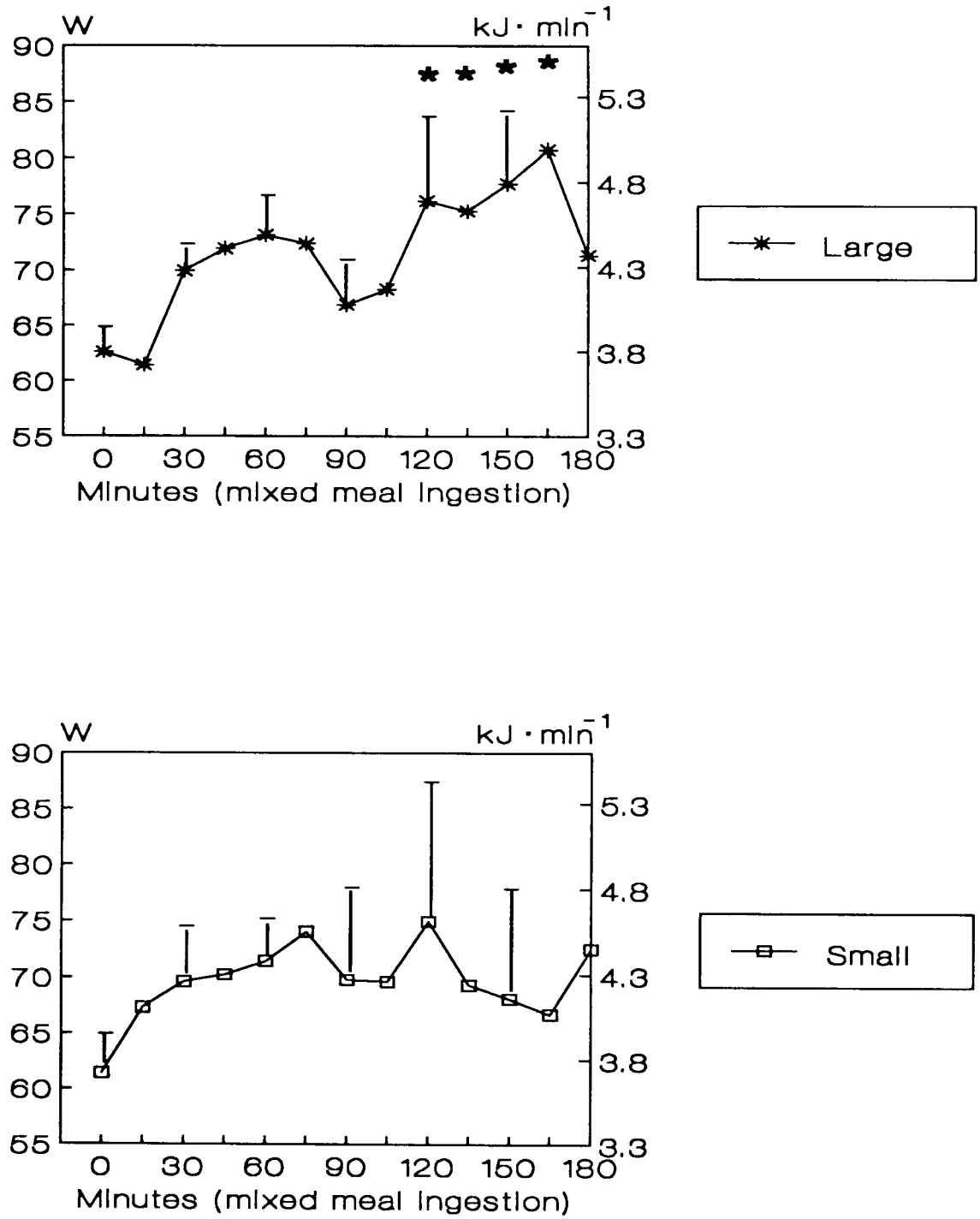

Figure 9.5. The thermic effect of mixed meal feeding in large and small eaters. There was a significant interaction effect for group x time from 120 to 165 minutes post-ingestion (* $p < 0.05$). Large eaters had a higher thermic response to feeding over this period than small eaters.

As expected, resting metabolic rate was significantly correlated to total mass, fat-free mass (FFM) and to % body fat (FAT%, $p < 0.03$). In addition, the magnitude of the post-exercise increment in energy expenditure was significantly negatively correlated to resting energy expenditure ($p < 0.05$).

The thermic response to feeding was also significantly correlated to the waist-to-hip ratio ($p < 0.03$) and negatively correlated to % body fat ($p = 0.05$).

Table 9.6. Multiple correlation matrix for factors associated with resting energy expenditure, body composition and response to thermogenic stimuli (* $p < 0.05$, REE = resting energy expenditure, FFM = fat-free mass, w/h = waist-to-hip ratio, TEF = thermic effect of feeding, TEE = thermic effect of exercise).

	REE	**MJ·D^{-1}**	**FAT%**	**FFM**	**MASS**	**AGE**	**w/h**	**TEF**	**TEE**
(n)	(18)	(18)	(18)	(18)	(18)	(18)	(10)	(10)	(8)
REE	1.0	.12	.51*	.55*	.57*	.20	-.37	.14	-.69*
MJ·D^{-1}	-	1.0	.02	.06	.04	-.08	.21	.30	-.49
FAT%	-	-	1.0	.69*	.87*	.69*	-.57*	-.62*	-.52
FFM	-	-	-	1.0	.96*	.51*	-.54	-.31	-.02
MASS	-	-	-	-	1.0	.66*	-.66*	-.49	-.21
AGE	-	-	-	-	-	1.0	-.55	-.35	.02
w/h	-	-	-	-	-	-	1.0	.71*	NA

Discussion

In this study, sedentary women were matched for age, height, mass, body composition and regional adipose distribution. Pairs of subjects were selected to include a "large eater" and a "small eater". The classification of small and large eaters was based on a relative comparison of food energy intake between members of the same pair. It was assumed that these subjects were in energy balance which was indirectly supported by the fact that body mass did not change over the course of the experimental trial. Thus, it was hypothesized that small eaters would demonstrate some degree of energy "thriftiness" as a compensatory response to a chronic, relatively low energy intake.

However, there was no difference in resting energy expenditure between groups, whether expressed absolutely (W or $kJ \cdot min^{-1}$) or relative to total mass or lean body mass. In fact, for each kJ of metabolizable energy eaten, the small eaters expended *more* energy during supine rest than did large eaters (MEI:REE, Figure 9.2).

The comparison between large and small eaters was taken one step further in order to corroborate these observations. There was some concern that differences in energy intake between matched pairs was not sufficiently large to detect differences in energy efficiency. Furthermore, there was some overlap between large and small eaters (i.e. energy intake in some small eaters was

greater than that of the large eater of another pair). Mean resting energy expenditure expressed relative to total mass and to fat-free mass was, therefore, re-calculated by grouping subjects so that there was no overlap in daily energy intake. Thus, the two non-matched groups of subjects were comprised of those with energy intakes of $\leq$ 5.2 $MJ \cdot d^{-1}$ (n=8) and those with energy intakes of $\geq$ 6.7 $MJ \cdot d^{-1}$ (n=10). There was no difference in resting energy expenditure after "re-grouping" large and small eaters so that there was no overlap in individual energy intake between groups (Table 9.7).

In fact, in this study, the only demonstratable difference in metabolic efficiency between large and small eaters occurred in response to the ingestion of a standard 2.1 MJ mixed meal. There was a significant group x time interaction and the thermic effect of feeding was higher in large eaters when compared to small eaters only after 105 minutes post-glucose ingestion.

Table 9.7. Energy intake and resting energy expenditure in large and small eaters after "re-grouping" according to levels of energy intake and without matching (means $\pm$ SEM).

	Intake ($MJ \cdot d^{-1}$)	Resting energy expenditure $W \cdot kg^{-1}$	$W \cdot kg\ FFM^{-1}$
"Small eaters" (n=8)	4.00 $\pm$0.36	1.00 $\pm$0.04	1.40 $\pm$0.04
"Large eaters" (n=10)	8.52 $\pm$0.46	0.99 $\pm$0.05	1.38 $\pm$0.06

Thus, 2 "problems" emerge: 1) that persons of similar mass and levels of physical activity may have wide variations in energy intake, and 2) that persons with very different food energy intake may have similar resting, post-prandial, and exercise-induced energy expenditure.

In fact, the very existence of "metabolic efficiency" is suggested by the large individual variation in measured basal and 24-hour energy expenditure, even when corrected for body size (Flatt, 1978). Edmundson (1977) observed that energy expenditure and work output were not apparently influenced by varying levels of energy intake in East Java workers and concluded that "the external work value of food may depend on who is eating the food".

Durnin (1961) summarized the individual data from 6 previous studies, including groups of young women, housewives, office clerks, coal miners, army cadets and students. These subjects represented groups with widely varying daily physical activity. Energy intake in each case was measured using the weighed-food method for 1 week, and energy expenditure was estimated using the activity diary method coupled with indirect calorimetry for various activities. He demonstrated that, in the vast majority (over 85%) there was no relationship between reported food intake and estimated energy expenditure on any given day. Nor did

subjects appear to compensate for a day of low energy intake or physical activity by modifying energy intake on subsequent days.

On the other hand, Edholm et al. (1955) showed that energy expenditure estimated using activity diaries and indirect calorimetry was correlated to energy intake 2 days following the measurement period in army cadets. This suggests that there is some "matching" of energy intake and expenditure.

Miller and Parsonage (1975) found that 9 of 29 women confined under metabolic ward conditions remained weight-stable over a 3 week period, ingesting a relatively constant food energy intake between 5.8 and 6.3 MJ per day. There was a close correlation between the amount of weight lost in the 20 subjects who did not remain weight-stable over this period and the resting metabolic rate ($r=0.68$). Those women who lost the most weight also expended the greatest amount of energy at rest, sitting, post-prandially and estimated over a 24-hour period, using indirect calorimetry.

However, few studies have been able to demonstrate a significant relationship between energy intake, body size and energy expenditure in free-living, weight-stable persons. In 1936, Widdowson and McCance were among the first investigators to quantify food energy intake by the individual, weighed-food method. In 126 men and women, they were unable to demonstrate ny relationship between energy intake and body mass. In fact,

in some cases, weight-stable, sedentary men of similar mass, had a nearly two-fold difference in reported energy intake.

Warwick et al. (1978) showed that 24-hour energy expenditure varied by as much as 40% in persons matched for age, sex, body composition and pattern of physical activity. Booyens and McCance (1959) studied the individual variability in resting energy expenditure and energy expenditure during sedentary tasks such as: sitting, lying and standing in 36 healthy, weight-stable men and women. Again, in age- and weight-matched subjects, resting energy expenditure was found to vary by as much as 25-30%.

Thermic effect of feeding:

No previous studies have described differences in the thermic response to feeding as one possible explanation for "luxuskonsumption" or energy-wasting in large eaters (D'Alessio et al., 1988, Morgan et al., 1982, Rose and Williams, 1961, Segal et al., 1985).

In this study, large eaters demonstrated a more pronounced thermic response to mixed meal feeding after 105 minutes. However, the actual mean difference in terms of energy expenditure over 1 hour was a mere 44 kJ or 2% of the ingested energy. Thus, it is unclear whether the magnitude of differences

in the thermic effect of feeding is a viable explanation for the expenditure of excess energy in large eaters.

It is generally believed that the thermic effect of feeding is determined primarily by the energy content of the meal itself (D'Allessio et al., 1988, Garrow, 1983, Morgan et al., 1982). If the post-feeding increment in oxygen consumption is followed for 8 hours, the thermic effect of feeding is found to account for only 8 to 10% of the energy ingested. A large part of the variation in the measurement of the thermic response to feeding may be attributed to daily variations in the measurement of resting energy expenditure (D'Alessio et al., 1988, Garrow, 1983). However, the thermic effect of feeding appears to adapt readily to changes in energy balance and nutritional status, increasing with overfeeding (Poehlman et al.,1986) and decreasing in response to food restriction (Schutz et al., 1984) or exercise training (Tremblay et al., 1983). In addition, the obese or non-insulin-dependent diabetic also demonstrate a reduction in the thermic effect of feeding (Ravussin et al., 1983, Ravussin et al.,1985), but here a substantial portion of the energy may be lost as glucose in the urine.

It is likely that these responses in the thermic effect of feeding are brought about via changes in sympathetic nervous system activity or hormonal adaptations such as insulin resistance or insulin insufficiency. This may, in part, be the efferent mechanism for the lower thermic response to feeding in

persons with a chronically low food energy intake, although the afferent limb of the homeostat remains unknown.

Thermic effect of exercise:

In this study, the steady-state cost of submaximal cycle ergometer exercise and the post-exercise increment in energy expenditure of matched pairs of small and large eaters were compared. There were no significant differences between groups and in fact, the responses were remarkably similar in magnitude and time course.

In a similar study, Rose and Williams (1961) categorized weight-matched pairs of subjects as large and small eaters with mean group energy intake differing by nearly 50%. They were unable to demonstrate significant differences in absolute oxygen uptake between groups at rest, or in response to feeding, sitting, walking, weight-raising or bench stepping. They concluded that large eaters must be expending more energy in "general restlessness and briskness" of approach to tasks and noted that the large eaters walked at a faster rate in "free" walking than did the small eaters.

Conversely, Ashworth (1968) found that Jamaican farm workers with very low daily energy intakes (between 43 and 98% of the Food and Agriculture Organization recommended requirements, 1957) tended

to have a lower oxygen uptake for a set timed task of moving bricks, when compared to well-nourished, age-matched controls, even after correcting for differences in body mass. Ashworth (1968) also observed that subjects with the lowest energy intake tended to have the lowest increment in energy expenditure when changing position from lying to sitting.

The post-exercise increment in energy expenditure is influenced by the intensity and duration of the exercise bout (Sedlock et al., 1989) and by body composition (Segal et al., 1985). However, these factors were effectively controlled for in the present study. It is unlikely that differences in overall fitness in these sedentary subjects had any effect on the magnitude of the post-exercise energy expenditure. Freedman-Akabas et al. (1985) found that there was little or no measurable increase in oxygen uptake over resting levels after 40 minutes post-exercise. Nor did they find any differences between fit or unfit subjects.

Despite rigid control in the present study for these intervening factors, we were not able to corroborate the findings of Ashworth (1968). Thus, it appears that metabolic efficiency during and following standardized physical activity is not likely to be "regulated" in response to one's habitual energy intake.

However, in the present study, there was no attempt at quantifying spontaneous physical activity or "fidgeting".

Ravussin et al. (1986) found that "fidgeting" could account for as much as 3.4 MJ of energy expenditure per day in some individuals confined to a metabolic respiration chamber.

Errors and assumptions: apparent discrepancies between energy intake and energy expenditure:

The paradox which has been described in this study, as well as many other studies (Ashworth 1968, Miller and Parsonage, 1975, Morgan et al., 1982, Mulligan and Butterfield, 1990, Prentice et al., 1986, Rose and Williams, 1963) in which persons may gain weight while apparently eating very little, while others remain thin despite a larger appetite or comparable food energy intake may be explained, in part, by the following:

1) Dietary surveys are incorrect;
2) Indirect calorimetry does not reflect true metabolic rate.
3) Persons with very low energy intakes adapt by decreasing voluntary energy expenditure.
4) Energy saving or "dumping" may occur primarily at night.

Thus, the question remains as to what degree the apparent differences in metabolic efficiency between groups may be attributed to errors in the estimation of food energy intake.

Previous studies of small and large eaters have demonstrated that small eaters can maintain body weight and remain in apparent energy balance with a total reported metabolizable energy intake which is not different from the resting metabolic rate measured by indirect calorimetry and extrapolated for 24-hours (Miller and Parsonage, 1975, Morgan et al., 1982, Prentice et al., 1986, Rose and Williams, 1961, Warwick et al., 1988).

A similar apparent discrepancy between energy intake and expenditure has been found in studies of energy balance in labourers from developing countries (Ashworth, 1968, James and Shetty, 1982). However, when Ashworth (1968) studied Jamaican farmers and their wives confined to metabolic ward conditions, most lost weight when given the same energy intake as that calculated from the free-living, estimated, weighed food intake. This, therefore, suggests that the weighed-food method for estimating energy intake may be subject to a significant degree of error.

In the present study, small and large eaters were given a set menu and remained weight-stable throughout the trial, which lasted a minimum of 3 days. It is unlikely that subjects were under-reporting food energy intake as the ratio between mean reported nitrogen intake and urinary urea nitrogen excretion was slightly positive and not different between groups.

There are several other factors which must be considered in order to interpret these findings. Firstly, the nutrient content of the diet has been demonstrated to play a significant role in the short-term facultative component of dietary thermogenesis and in the long-term oxidation of dietary fat (Flatt, 1978). In this study, the small eaters had a significantly lower percentage fat in their diet. Thus, any increased efficiency in the utilization of food from a higher percentage dietary fat intake would be expected in the large rather than small eaters.

In addition, it might be argued that large eaters lose more energy in faeces than do small eaters. While this measurement was not taken, a study by Passmore et al. (1955) demonstrated that when healthy normal subjects were "overfed" for 10-14 days, 90% of the excess energy was absorbed.

Differences in daily energy required for body weight maintenance may also be attributed to differences in spontaneous physical activity or the "motor individuality" referred to by Parizkova' (1977). Differences in spontaneous physical activity were not measured in the present study. However, even under controlled conditions, in which subjects were confined to a respiration chamber for 24 hours, differences in spontaneous physical activity were closely correlated to overall differences in 24-h energy expenditure in 180 non-diabetic Pima Indians (Zurlo et al., 1992).

Another possible source of error was highlighted in an energy balance study by Webb et al. (1980b) and has been discussed extensively in Chapter 2 of this dissertation. Briefly, investigators compared free-living energy expenditure using direct and indirect calorimetry in 4 normal-weight individuals previously considered to be in energy balance. There was good agreement between daily energy expenditure measured by direct and indirect calorimetry when these subjects were sedentary. However, when energy expenditure grossly exceeded energy intake, there was as much as a 4.0 MJ difference in daily energy balance determined by direct and indirect calorimetry. Direct calorimetry gave a higher values for energy expenditure than did indirect calorimetry under these conditions. The energy expenditure not accounted for by oxygen uptake measurements was considered "unmeasured energy". This suggests that the assumption that all of the energy released from fuel oxidation can be measured as heat and external work should be examined more closely.

The inability to reconcile energy intake with energy expenditure under extremes of undernutritrion has been clearly highlighted in a study of free-living energy balance in Gambian women (Singh et al., 1989). Energy intake was carefully documented using the weighed inventory technique over 11 days by trained field workers, and energy expenditure was measured using the doubly-labelled water technique. When the energy expenditure associated with the loss of body mass, and fat oxidation were accounted for,

there remained a 5 $MJ \cdot day^{-1}$ unexplained deficit between energy intake and energy expenditure.

Finally, it must be noted that in the present study, the thermic response to feeding expressed relative to the total energy consumed was lower than has been previously reported (Garrow, 1983). However, this may be a result of the shorter duration of the post-ingestion period compared to other studies (3 vs 8 hours). It is unlikely that this influenced the results unless one group demonstrated a peak in response after the 3 hour period because both groups were expending a similar amount of energy at 180 minutes post-ingestion.

Summary:

This study corroborated findings of previous studies by describing a significant relationship between indices of body size (mass, fat-free mass and body fat) and resting energy expenditure (Ravussin et al., 1986).

There was also a significant negative correlation between resting energy expenditure and the post-exercise increment in energy expenditure (Table 9.7). This suggests that the baseline measurement is an important determinant for the response to a specific thermogenic stimuli (D'Alessio et al., 1988)

Many studies have alluded to a "thermogenic" defect in searching for the aetiological factors associated with obesity (Bazelmans et al., 1985, Jequier, 1983, Jung et al., 1979, Segal et al., 1985). Segal and coworkers (1985) found that the thermic effect of exercise and feeding were significantly lower in persons with a higher body fat percentage when compared to weight-matched persons with a lower body fat percentage. In this study, a significant negative association ($r = -0.62$, $P < 0.05$) between the thermic effect of feeding and percentage body fat was described which is consistent with this concept.

However, a significantly positive correlation was found between the thermic effect of feeding and waist-to-hip ratio (r 0.71, $p < 0.05$) or regional adipose tissue distribution. This finding is novel and is particularly relevant with the increasing emphasis on regional adipose distribution and predisposition for disease (Kissebah et al., 1982). Recent studies have found that femorally-obese women (waist-to-hip ratio $\leq$ 0.79) have a lower resting energy expenditure than abdominally-obese and non-obese women (Armellini F. et al., 1992, den Besten et al., 1988). This is consistent with observations made by Bjorntorp (1987) that obese women with a femoral or gynoid distribution of adipose tissue tend to be more difficult to "treat". This study provides additional evidence that these subjects tend to be more energy "thrifty".

Conclusions:

In summary, it may not be correct to assume that because metabolic efficiency in response to certain thermogenic stimuli was not demonstrated unequivocally in the small eaters in this study, that such "efficiency" does not exist. There were many "unaccounted-for" hours which were not characterized and it was impossible to measure the degree of compliance. Resting metabolic rate has been shown to be significantly higher than that measured while sleeping (Flatt, 1978, Sims and Danforth, 1990) and in unpublished work by McBride (1987) small eaters tended to have a lower sleeping metabolic rate.

One can also not exclude the possibility that persons volunteering for this study were "restrained" eaters (Leibel and Hirsch, 1984, Tuschl et al., 1990), had incorrectly reported daily energy intake, or were not in energy balance during the experimental trial.

In this study, the thermic effect of feeding was found to qualitatively reflect some difference in the effiency of food energy utilization, and traditional relationships between energy expenditure, body size and body composition were confirmed. Reported food intake was not associated with any measure of energy expenditure. Finally, these results suggest that regional

adipose distribution in weight-stable women may partially predict individual spontaneous energy intake.

CHAPTER 10

DETERMINANTS OF RESTING ENERGY EXPENDITURE

" It seems,therefore, unjustifiable to apply mathematics to the pooled end-result of the activities of millions of cells each highly differentiated, with different energy potentialities and actuated by different stimuli "

(Benedict FG, *Vital Energetics*, 1938)

Introduction

In previous chapters, this thesis addressed the effects of specific interventions on feeding efficiency, body composition and energy expenditure at rest and in response to feeding and epinephrine infusion, in small, well-defined populations of both animals and humans. However, these investigations do not provide insight into the determinants of energy expenditure in a large and diverse population.

Dietary intervention, in which food energy is restricted, has been shown to result in significant changes in resting energy expenditure (Bray, 1969, Chapter 5, Geissler, 1987, Ravussin et al., 1985). However, studies comparing free-living, weight-stable, "small and large" eaters have been unable to show that "small" eaters have a resting energy expenditure which reflects energy "sparing" on the basis of body size or body composition (Ashworth, 1966, Chapter 9, Morgan and York, 1982, Rose and Williams, 1961).

There is also some evidence from animal studies that the nutrient content of the diet, and the ability to oxidize fat influences energy balance (Flatt, 1987). In human trials, dietary fat intake, and the fat-to-carbohydrate ratio has been shown to be related to body fat content in large group studies (Dreon et al., 1988) and a positive fat balance in both rats and humans (Flatt et al. 1985). However, no previous studies

have examined the relationships between the reported energy and nutrient composition of the diet and the resting energy expenditure in a large population of lean and obese, men and women, both sedentary and exercise-trained.

Evidence has also been profferred to show that exercise training over 14 hours per week results in a significant increase in resting energy expenditure which cannot be explained by the residual effects of a previous exercise bout, and which is not demonstrated in individuals training less than 11 hours per week (Chapter 6). However, there is no evidence to suggest that in a large heterogeneous population of men and women, individuals who participate in regular exercise have an energy requirement, for their body size, which is different to those who do not exercise.

Resting energy expenditure comprises the largest fraction of the total daily energy expenditure, accounting for approximately 60-75% of the total daily energy output (Astrup et al., 1990, Ravussin et al., 1986, Welle et al., 1991). Therefore, much research emphasis has been directed at determining whether a low energy expenditure is a predictor for subsequent weight gain (Ravussin et al., 1988, Roberts et al., 1988) and whether various interventions such as exercise and food restriction influence the resting energy expenditure and result in long-term adaptations in energy balance.

As a result of the well-established positive association between resting metabolic rate and body size and fat-free mass (Ravussin et al., 1986), recent studies have been undertaken to address the problems of standardization of the expression of resting metabolic rate in comparing populations with widely varying body composition (Ravussin et al., 1989), or in which the constituents of fat-free mass may be dissimilar (Cunningham 1992, Luke and Schoeller, 1992, Nelson et al., 1992, Weinsier et al., 1992).

Therefore, the aim of this study was to reexamine those factors which have previously been correlated with resting energy requirements in a large and diverse sample of men and women, both exercising and sedentary, including some individuals with reportedly low food energy intake. This sample represented persons not previously exposed to famine or starvation, with uninterrupted access to food during their adult lives. Specifically, this study addressed the problem of standardization of the expression of metabolic rate and the implications of the way in which resting energy expenditure was expressed on the interpretation of the relationships between metabolic rate and "metabolic body size".

Methods

Subject pool:

Data from 160 subjects participating in one of 6 experimental trials were combined for the purpose of multivariate statistical analysis. The subjects and trials are described below.

Forty-nine women participated in a trial aimed at characterizing menstrual dysfunction in "lean" sedentary and exercising women. This sample included 14 competitive long distance runners, 8 fashion models and 27 ballet dancers (students, professionals and teachers).

Twenty-six male athletes were recruited for two trials in which energy balance during detraining and in the acute post-exercise period were characterized. Eleven "lean" sedentary male subjects served as controls for these trials.

Thirty-eight "overweight" subjects (26 women, 12 men) were recruited for three intervention trials. Baseline data only are included for this analysis (Chapter 5).

Two groups of 18 women were recruited for two trials. In the first trial, sedentary women were matched for body mass and body composition, and for relatively different levels of

reported food energy intake ("large vs small eaters", Chapter 9). In the second trial, women were matched for body mass, body composition, and habitual activity, and were selected as "weight-cyclers" or "weight-stable controls". Criteria for weight cycling included a minimum of 2 weight cycles (loss or gain of > 10% body mass) within the previous 5 years.

Procedures and Data Analysis:

For each subject, the following data were extracted: age, mass, fat mass, fat-free mass, reported food energy intake, dietary carbohydrate-to-fat ratio (w:w) food quotient (FQ), and resting energy expenditure.

Fat mass and fat-free mass (FFM) were determined using the skinfold equations of Durnin and Womersley (1974). Food energy intake was based on 3- or 7-day dietary records, using the weighed-food method. Food records were coded and analyzed using reference food composition and quantity tables (Research Institute for Nutritional Diseases, Parow, South Africa) and a computerized dietary analysis program (Floro Diet Data Program, Durban, South Africa). Carbohydrate-to-fat ratio was calculated as the gram ratio of carbohydrate to fat in the diet. Food quotient was calculated based on the caloric equivalents of the carbohydrate and fat in the diet.

Resting energy expenditure was measured under the same conditions for all subjects, using the ventilated hood system for open-circuit, indirect calorimetry, which has been described in detail (Chapter 5). Subjects reported to the laboratory early in the morning, in the post-absorptive state, and rested in the supine position for a minimum of 30 minutes. Respiratory exchange measures were collected for a 30 minute period and the mean oxygen consumption (VO_2), carbon dioxide production (VCO_2) and the respiratory exchange ratio (VCO_2:VO_2, RER) were determined. Respiratory exchange data were used to calculate energy expenditure from conventional equations for energy equivalents (Weir, 1949). Resting energy expenditure was expressed absolutely, relative to mass, and to FFM.

In addition, each subject was characterized according to their exercise status: regular physical exercise (EX) vs no exercise (NO EX). Subjects who reported a metabolizable food energy intake which was less than 1.3 times the resting energy expenditure (MEI:REE) were characterized as "restrained eaters" (RE) vs "ad libitum eaters" (AD LIB) with a reported metabolizable energy intake greater than or equal to 1.3 times the resting metabolic rate.

Pearson-product-moment correlations were performed to determine simple linear relationships between these data. Stepwise multiple linear regression was performed to determine

the single best model for predicting REE, based on the independent variables which had been identified in the initial analysis. Non-linear regressions using the equations for a rectangular hyperbola and an exponential growth curve were also performed to further describe the relationship between REE and indices of body size.

In previous studies, a measured resting energy expenditure which was lower-than-the average predicted resting metabolic rate has been identified as a marker or "risk factor" for subsequent weight gain (Ravussin et al., 1989). The deviation of the measured resting metabolic rate from the average predicted metabolic rate (delta REE) was compared across gender, and exercise status and for groups of restrained and *ad libitum* eaters.

Analyses of variance were used to examine the effects of gender, exercise status and differences in reported food energy intake (MEI:REE) on resting energy expenditure. In addition, the mean slope and intercept, from the regression of daily resting energy expenditure against FFM were compared between men and women, exercising and non-exercising subjects, and the "restrained eaters" vs "ad libitum eaters", using independent t-tests.

Results

Subject characteristics:

Physical characteristics for the entire sample, and for the various sub-groups, are presented in Table 10.1.

Subjects ranged in age from 16 to 58 years. Body mass ranged from 45.7 kg to 153.2 kg. The lowest measured body fat in the sample was 8.9%, while the highest body fat measured was 53.4%. Fat-free mass ranged from 36.1 kg to 95.4 kg. Of this sample, 112 were women, 48 were men. Sixty-seven subjects participated in regular physical exercise; 72 subjects were non-exercisers. Seventy-two subjects reported a metabolizable food energy intake (MEI) which was greater than or equal to 1.3 times the resting energy expenditure (REE), while 82 subjects had a reported metabolizable food energy intake (MEI) which was less than 1.3 times the REE).

Reported food energy intake ($MJ \cdot d^{-1}$), dietary carbohydrate-to-fat ratio (CHO:FAT, g:g), and resting energy expenditure expressed both absolutely, and relative to mass and FFM are presented in Table 10.2.

Table 10.1 Subject physical characteristics (means ± SEM).

	Age	Mass	Fat(%)	Fat(kg)	FFM(kg)
Total x	29	69.8	24.6	18.0	51.8
SEM	± 1	±1.5	±0.7	±0.9	±0.9
(n)	(160)	(160)	(158)	(158)	(158)
Gender					
Men x	34	81.5[a]	18.2[a]	16.5	65.8[a]
SEM	± 2	±2.9	±1.3	±1.8	±1.4
(n)	(48)	(48)	(46)	(46)	(46)
Women x	27	64.8[b]	27.2[b]	18.7	46.1[b]
SEM	± 1	±1.6	±0.7	±1.0	±0.6
(n)	(112)	(112)	(112)	(112)	(112)
Exstatus					
EX x	27	61.7[a]	18.5[a]	11.2[a]	50.5
SEM	± 1	±1.4	±0.6	±0.4	±1.4
(n)	(66)	(66)	(66)	(66)	(66)
NO EX x	32	78.7[b]	29.8[b]	24.7[b]	54.4
SEM	± 1	±2.6	±1.1	±1.6	±1.5
(n)	(72)	(72)	(72)	(72)	(72)
MEI:REE					
RE x	29	67.8	27.3[a]	19.6	48.2[a]
SEM	± 1	±2.1	±1.0	±1.3	±1.0
(n)	(82)	(82)	(82)	(82)	(82)
AD LIB x	29	70.4	21.6[b]	15.7	54.9[b]
SEM	± 1	±1.9	±1.0	±1.1	±1.4
(n)	(72)	(72)	(70)	(70)	(70)

(means which do not share a common superscript are significantly different, [a] vs [b], $p < 0.001$).

Reported food energy intake was significantly higher in men than in women ($p < 0.05$), and by design, in the AD LIB subjects compared to the RE subjects. The dietary carbohydrate-to-fat ratio was similar in all subgroups.

Table 10.2. Reported energy intake and resting energy expenditure (means ± SEM).

	Food Intake ($MJ \cdot d^{-1}$)	CHO:FAT Intake (g:g)	REE ($MJ \cdot d^{-1}$)	REE ($W \cdot kg^{-1}$)	REE ($W \cdot kg\ FFM^{-1}$)
Total x	8.23	3.2	6.17	1.08	1.43
SEM	±0.30	±0.1	±0.11	±.01	±.01
(n)	(155)	(154)	(160)	(160)	(160)
Gender					
Men x	12.66^{a}	3.1	7.72^{a}	1.12^{a}	1.37^{a}
SEM	±0.50	±0.1	±0.21	±.02	±.02
(n)	(43)	(43)	(48)	(48)	(48)
Women x	6.53^{b}	3.2	5.75^{b}	1.06^{b}	1.46^{b}
SEM	±0.22	±0.1	±0.07	±.02	±.02
(n)	(112)	(111)	(112)	(112)	(112)
Exstatus					
EX x	8.94	3.4	6.30	1.19^{a}	1.46^{a}
SEM	±0.56	±0.2	±0.15	±.02	±.02
(n)	(67)	(67)	(67)	(67)	(67)
NO EX x	7.77	3.1	6.42	0.97^{b}	1.39^{b}
SEM	±0.37	±0.2	±0.20	±.02	±.02
(n)	(70)	(70))	(70)	(70)	(70)
MEI:REE					
RE x	5.62^{a}	3.1	6.51^{a}	1.09	1.40
SEM	±0.20	±0.1	±0.16	±.02	±.02
(n)	(82)	(82)	(82)	(82)	(82)
AD LIB x	11.24^{b}	3.2	6.07^{b}	1.07	1.47
SEM	±0.37	±0.1	±0.07	±.02	±.02
(n)	(72)	(72)	(72)	(72)	(72)

(means which do not share a common superscript are significantly different, ^{a} vs ^{b}, $p < 0.05$).

Absolute resting energy expenditure was higher in men than in women, and in the RE subjects compared to the AD LIB subjects ($p < 0.05$). Resting energy expenditure, expressed per kg body mass, was higher in men than in women ($p < 0.05$), however,

when expressed per kg FFM, REE was higher in women than in men ($p < 0.05$). REE, expressed per kg body mass or per kg FFM, was higher in the exercising subjects compared to their sedentary counterparts ($p < 0.05$).

Fasting respiratory exchange ratio was significantly higher in the "restrained" eaters group compared to the "ad libitum" eaters group (0.81 vs 0.77, $p < 0.005$, for RE and AD LIB, respectively). There were no effects of gender or exercise training on the fasting RER.

There were no interaction effects between gender, exercise status and MEI:REE on resting energy expenditure, relative to FFM .

Correlation analyses and multiple linear regression in the prediction of resting energy expenditure:

The results of simple correlation analyses are presented in Table 10.3. As expected, total body mass, FFM, fat-mass and age were highly interrelated ($p < 0.001$). There were also significant associations between reported food energy intake and FFM ($r=0.62$, $p < 0.001$) and total mass ($r=0.30$, $p < 0.001$). Absolute REE was significantly correlated to body mass ($r=0.73$, $p < 0.001$), FFM ($r=0.85$, $p < 0.001$, Figure

10.1), fat-mass ($r=0.37$, $p < 0.001$),and reported food energy intake ($r=0.54$, $p < 0.001$). On further investigation, however, the correlations between reported food intake and indices of body size were likely caused by the gender-specific differences in these variables. Women tended to have a lower food intake and a lower fat-free mass than men from this sample.

The dietary carbohydrate-to-fat ratio was significantly negatively associated with body mass, and its constituents ($r=-0.22$, $p < 0.01$), as well as, with absolute resting energy expenditure ($r=-0.22$, $p < 0.01$). This correlation with absolute resting energy expenditure is also probably fortuitous, as a result of the covariance with body mass. There was no association between relative nutrient composition of the diet and the fasting respiratory exchange ratio.

There was a significant negative association between resting energy expenditure, expressed relative to FFM, and fat-free-mass ($r=-0.38$, $p < 0.001$ for total sample, $r=-0.48$, $p < 0.001$ for women only, no correlation in sample of men).

There was a significant and negative association between MEI:REE (ratio of reported metabolizable energy intake, $MJ \cdot d^{-1}$, to resting energy expenditure, $MJ \cdot d^{-1}$) and fat mass ($r=-0.23$, $p < 0.005$), and respiratory exchange ratio ($r=-0.28$,

P < 0.005). Fat-free mass was positively associated with MEI:REE (r=0.31, p < 0.001).

Table 10.3 Simple correlation matrix for factors associated with resting energy expenditure (REE= absolute resting energy expenditure, REE(FFM)= resting energy expenditure relative to fat-free mass, $MJ \cdot d^{-1}$= daily reported food energy intake, [a] p < 0.001, [b] p < 0.01, [c] p < 0.05).

	REE (FFM)	MASS	FFM	FAT MASS	$MJ \cdot d^{-1}$	CHO: FAT	AGE	RER	RER: FQ	MEI: REE
REE	.17 (156)	.73[a] (160)	.85[a] (158)	.37[a] (158)	.54[a] (154)	-.22[b] (153)	.35[a] (159)	.38[a] (114)	.39[a] (108)	-.28[b] (108)
REE (FFM)	1.0	.17[c] (156)	-.38[a] (156)	-.16 (156)	-.22[b] (151)	-.09 (150)	-.27[a] (156)	.18 (110)	.20[c] (105)	-.33[a] (151)
MASS	-	1.0	.85[a] (158)	.84[a] (158)	.30[a] (155)	-.22[b] (154)	.61[a] (160)	.30[b] (115)	.30[b] (109)	.05 (154)
FFM	-	-	1.0	.42[a] (158)	.62[a] (152)	-.16[c] (151)	.49[a] (157)	.30[b] (112)	.28[b] (106)	.31[a] (152)
FAT MASS	-	-	-	1.0	-.13 (152)	-.21[c] (151)	.52[a] (157)	.25[b] (112)	.27[b] (106)	-.23[b] (152)
$MJ \cdot d^{-1}$	-	-	-	-	1.0	-.08 (154)	.22[b] (155)	-.08 (109)	-.05 (109)	.89[a] (154)
CHO: FAT	-	-	-	-	-	1.0	-.14 (154)	.03 (109)	-.33[b] (109)	-.02 (153)
AGE	-	-	-	-	-	-	1.0	.28[b] (114)	.28[b] (109)	.12 (154)
RER	-	-	-	-	-	-	-	1.0	.90[a] (109)	-.28[b] (108)
RER: FQ	-	-	-	-	-	-	-	-	1.0	-.27[b] (108)

Stepwise, multiple linear regression was used to predict absolute resting energy expenditure per day (W or $MJ \cdot d^{-1}$).

The following independent variables were entered into the model: age, gender, exercise status, mass, FFM, fat-mass, food energy intake and dietary carbohydrate-to-fat ratio.

The final model selected **only** FFM as a predictor variable. The adjusted R^2 for this regression was 0.69. There was no further improvement in the model, and no additional variance was explained by including any other variables into the prediction equation.

The linear prediction equation for this sample population which best explained the variance in total daily resting energy expenditure expressed as W or $MJ \cdot d^{-1}$, is presented below:

$$REE\ (W) = 17.662 + 1.065\ FFM\ (\pm\ SEE\ 8.010)$$

$$REE\ (MJ \cdot d^{-1}) = 1.526 + 0.092\ FFM\ (\pm\ SEE\ 0.692)$$

For the purposes of comparison to existing equations, the prediction equation for resting energy expenditure expressed as $kcal \cdot d^{-1}$ is also included:

$$REE\ (kcal \cdot d^{-1}) = 355.0 + 21.5\ FFM\ (\pm\ SEE\ 164.8)$$

The linear regression of REE against mass and fat mass did not improve either the R^2 or the standard error of the prediction of resting energy expenditure. Furthermore, none of the

variables which were correlated to REE produced a linear relationship with a zero y-intercept.

Non-linear regression in the prediction of resting energy expenditure:

In the study by Weinsier et al. (1992), it was suggested that the relationship between REE and FFM was best described across a very broad range of FFM by a non-linear relationship or rectangular hyperbola. This function, by definition, passes through the origin. In the present study, REE was regressed against FFM using the equation for a rectangular hyperbola. The R^2 for this relationship was 0.71, and the standard error of the estimate was $\pm$ 8.60. The regression equation is presented below:

$$REE\ (W) = (576FFM)/(354 + FFM)$$

Thus, the relationship between REE and FFM may be described by a rectangular hyperbola, and in this case, has a zero y-intercept.

In the present sample, the relationship between REE and FFM was further characterized by fitting the data to the function for an exponential growth curve. The R^2 for this relationship was 0.75, and the standard error of the estimate was not different than that for the linear regression equation.

The equation for an exponential growth curve which was fitted to the data from the present study is given below:

$$REE\ (W) = 33.629e^{(0.0147*FFM)}\ (SEE \pm 7.952)$$

Based on these results, the relationship between REE and FFM in this population may be described equally well by a linear regression, by a rectangular hyperbola or by an exponential growth curve.

The limitations and assumptions regarding the use of each of these functions to describe this relationship will be discussed in detail.

The linear relationship between resting energy expenditure and FFM: effects of gender, exercise status and food energy intake

Figure 10.1 illustrates the positive and linear relationship between REE and FFM. The correlation coefficient for this relationship was r= 0.85, and the y-intercept was positive (17.662 W).

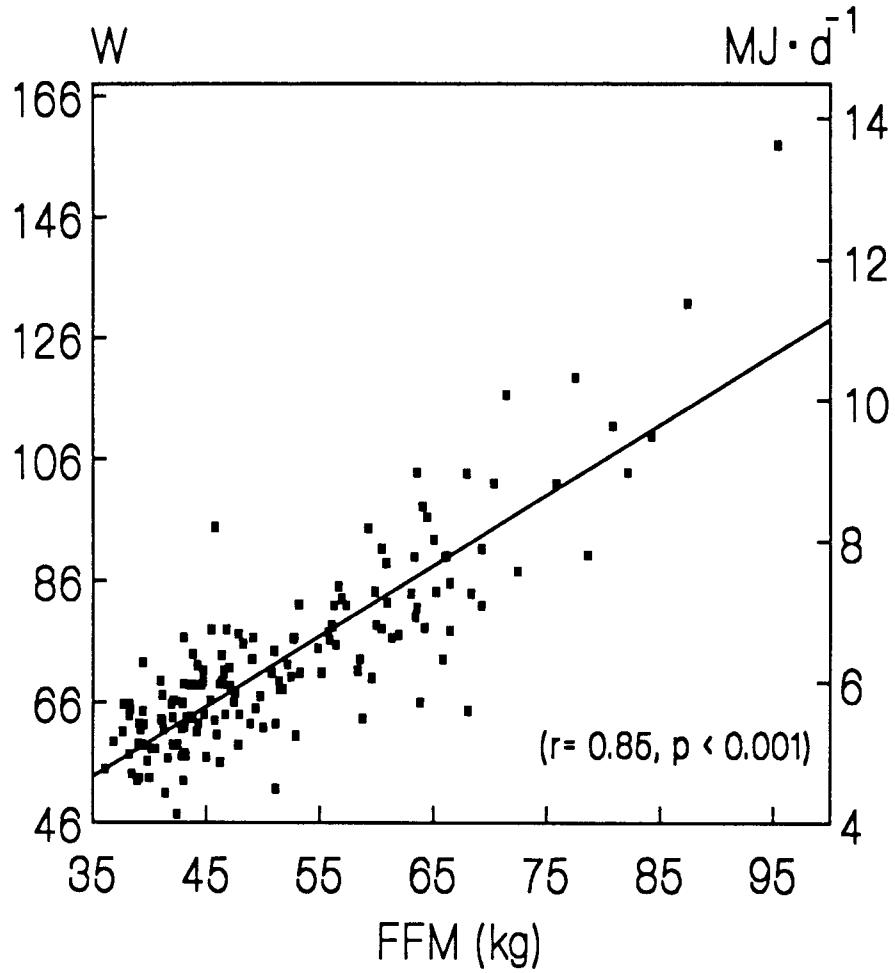

Figure 10.1. The relationship between resting energy expenditure (REE, W or $MJ \cdot d^{-1}$) and fat-free mass (FFM, kg).

The positive y-intercept for the regression of REE against FFM for the entire sample implies that the rate of resting energy expenditure per unit fat-free mass appears to decrease with increasing fat-free mass. If this relationship is, indeed,

linear as suggested in Figure 10.1, and the equation of the regression for REE against FFM was $y = mx + c$, then, the regression of REE per unit of FFM against FFM would yield the equation $y/x = m + c/x$. If c is a positive constant (*y-intercept*), then c/x will decrease as x increases, giving a negative slope ($y/x = m + c/x$).

This is illustrated in Figure 10.2, where REE per unit fat-free mass is regressed against fat-free mass.

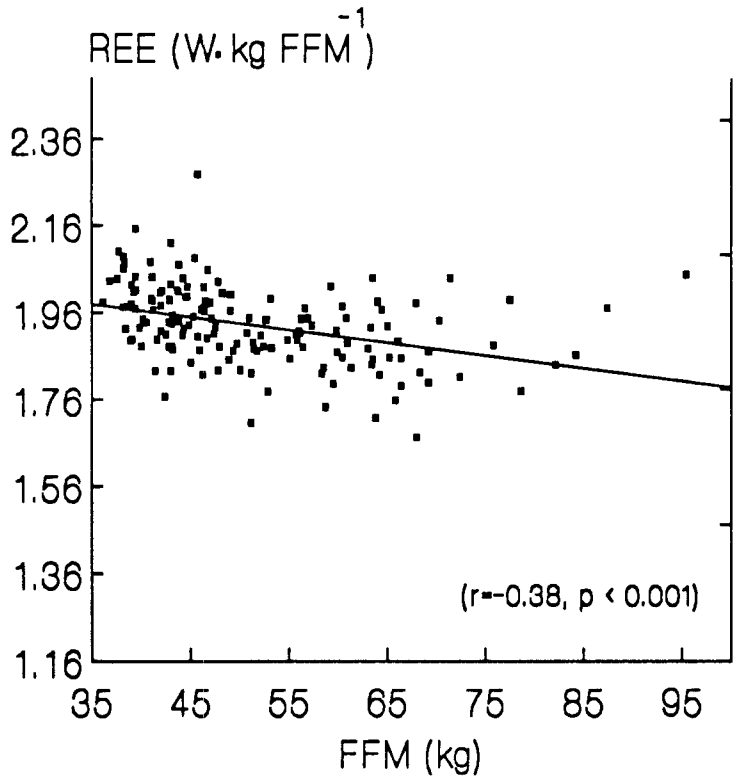

Figure 10.2. The relationship of resting metabolic rate per unit fat-free mass, REE·kg FFM^{-1}, to FFM.

The relationship between REE and FFM for the various subgroups were further examined by comparing the slope and the y-intercept of the linear regression of FFM against absolute resting energy expenditure (Table 10.4, Figure 10.3). The regression between REE and FFM for men in this sample had a significantly greater slope and a lower y-intercept ($p < 0.001$) than that for women ($p < 0.05$).

There was also a significant effect of exercise status on the y-intercept for the regression between REE and FFM. Subjects who exercised had a higher REE for a given FFM than subjects who were sedentary ($p < 0.05$, Table 10.4, Figure 10.3).

Table 10.4. The effects of gender, exercise status and MEI:REE on the slope ($W \cdot kg\ FFM^{-1}$) and intercept (W) of the regression of FFM on resting energy expenditure. (means ± SEM).

Gender	**(n)**	**Slope**	**Intercept**	**r**
Men	(45)	1.5 ± .17[c]	-6.4 ± 11 [a]	0.80
Women	(111)	0.8 ± .09	27.7 ± 4.5	0.64
Exstatus	**(n)**	**Slope**	**Intercept**	**r**
EX	(66)	1.04± .08	19.2± 4.3[a]	0.84
NO EX	(72)	1.27± .08	6.4± 4.8	0.87
MEI:REE	**(n)**	**Slope**	**Intercept**	**r**
AD LIB	(85)	1.16± .08	13.5± 4.3	0.84
RE	(65)	1.04± .10	20.8± 4.7	0.80

([a] $p < 0.001$, [b] $p < 0.01$, [c] $p < 0.05$)

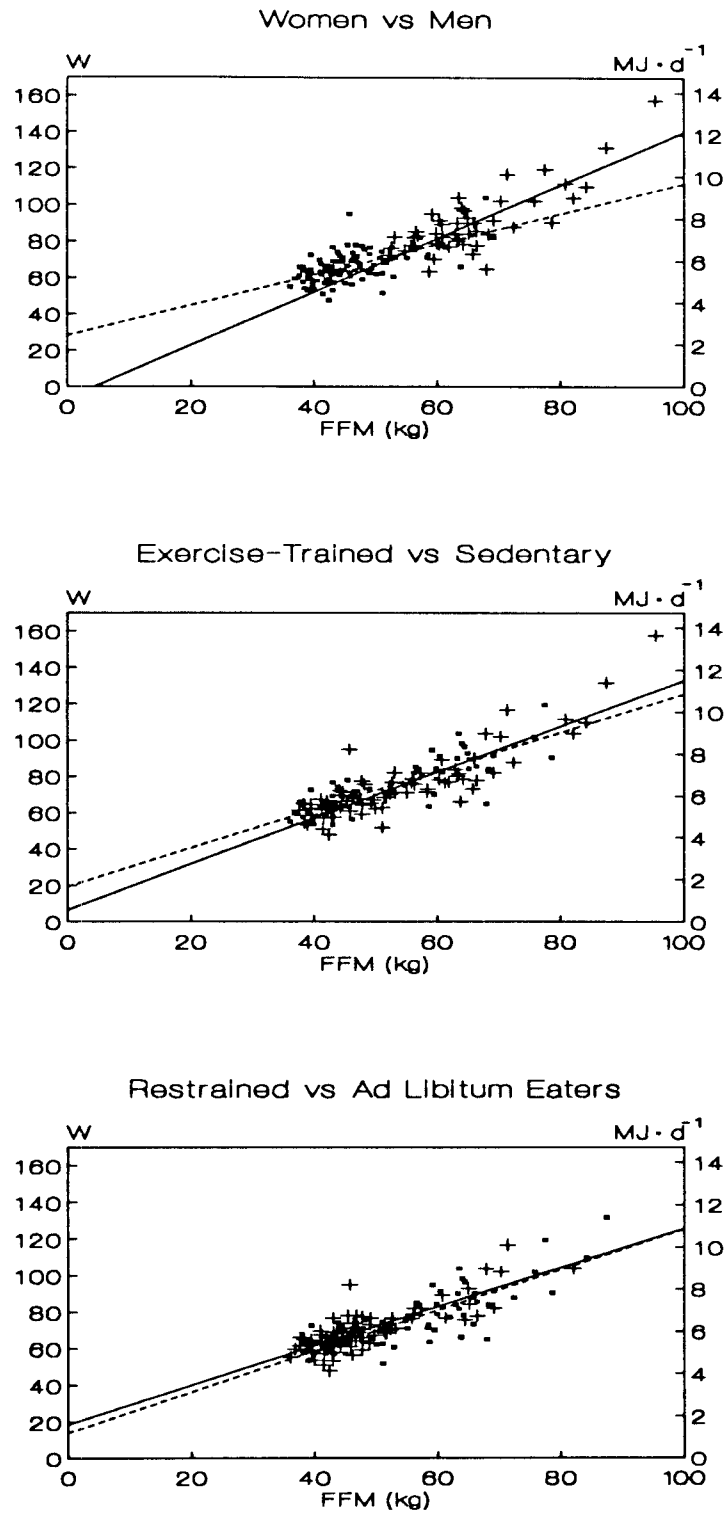

Figure 10.3. Regression of REE (W or $MJ \cdot d^{-1}$) against FFM for subgroups of women ('--) and men (+—), exercising ('--) and sedentary (+_) subjects, and restrained ('--) or *ad libitum* eaters (+_).

There was no difference in the linear relationship between REE and FFM between those subjects characterized as "ad libitum" eaters and those characterized as "restrained eaters" (Table 10.4, Figure 10.3).

The non-linear relationship between REE and FFM: effects of gender exercise and reported food energy intake

The equation which best predicts resting energy expenditure from FFM in the present study was an exponential growth curve, with the highest R^2 and the lowest SEE. However, this function compounds the effect of a positive y-intercept, and may be a function of the difference in slopes of the linear regression between REE and FFM for men and women in the present sample.

The rectangular hyperbola function predicts REE from FFM as well as the linear function in the present study.

No additional variance was explained by using non-linear regression to describe the relationship between REE and FFM for each subgroup.

Measured vs average predicted resting energy expenditure:

There was no relationship between the measured minus the average predicted metabolic rate (delta REE) and body size or body fatness. There was a significant and positive relationship between delta REE and respiratory exchange ratio ($r=0.32$, $p < 0.001$) as well as, resting energy expenditure ($r=0.62$, $p < 0.001$). This suggests that as absolute measured resting energy expenditure increased, there was greater variance in the measured minus the average predicted resting energy expenditure.

There were no differences in delta REE between any of the subgroups from this population (Figure 10.4). This means that the linear prediction equation did not consistently over- or under-estimate the resting metabolic rate of any of the subgroups.

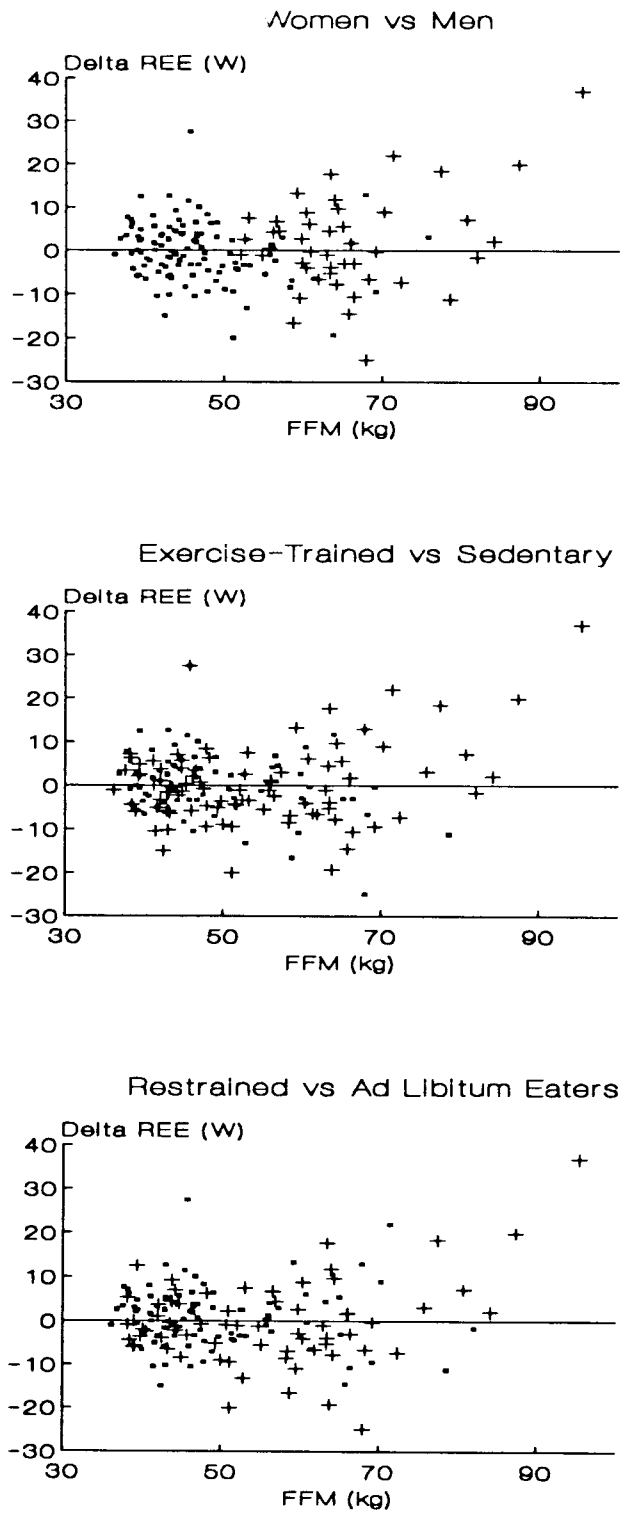

Figure 10.4. The measured minus the average predicted resting energy expenditure (delta REE, W) for subgroups of women (·–) and men (+—), exercising (·--) and sedentary (+__) subjects, and restrained (·--) or *ad libitum* eaters (+__).

Discussion

The results of this study confirmed the now well-established close relationship between "metabolically active" body mass (FFM) and resting energy expenditure in persons who are in energy balance. In the present study, between 69% and 75% of the variance in resting metabolic rate was accounted for by differences in fat-free mass alone. These findings may be compared to those of the study of Pima Indians by Bogardus and coworkers (1986), in which 83% of the variance in resting energy expenditure was explained by fat-free mass. Efforts to standardize resting energy expenditure have been made as it is recognized that for any given body mass, certain subgroups of a population are likely to have a higher percentage of fat-free mass (e.g. men vs women, exercising vs sedentary individuals). The standardization of resting energy expenditure would provide a convenient and uniform expression of metabolic activity, and would provide insight into the determinants of resting energy expenditure, with possible therapeutic implications. As a result of the strong linear relationship between resting energy expenditure and indices of body size and body cell mass, attempts have been made to standardize (or "normalize") resting energy expenditure by expressing energy expenditure relative to kg body weight or fat-free mass in groups with widely varying body size and composition.

Standardization of of resting energy expenditure:

Standardization of resting energy expenditure is only possible if the regression of resting energy expenditure against some index of body size or body cell mass is 1) linear and 2) if the y-intercept of the regression is not significantly different from zero.

In the present study, the regression of REE on total body mass, fat-free mass or fat-mass produces a relatively high correlation coefficient, suggesting that these relationships may be described as linear. However, none of these indices of body size produced a significant relationship with REE that was both linear and had a non-zero y-intercept.

Ravussin and Bogardus (1989) show that if resting energy expenditure is expressed relative to fat-free mass, one would conclude that larger people (e.g. men) generally have a lower metabolic rate per unit fat-free mass than smaller people (e.g. women). For example, an individual with a fat-free mass of 40 kg, would have a average predicted resting energy expenditure of 60.2 W, whereas an individual with a fat-free mass of 80 kg would have a average predicted resting energy expenditure of 103 W. When resting energy expenditure is expressed relative to fat-free mass for these two individuals, the person with the smaller fat-free mass will have a higher metabolic rate per unit fat-free mass than the individual with

a higher fat-free mass (1.51 vs 1.29 W kg FFM^{-1} for small vs large FFM, respectively, Figure 10.5a). Thus, a large person with a metabolic rate which was not different to the average predicted metabolic rate may have a significantly lower metabolic rate per unit fat-free mass, than an equally average smaller person (Figure 10.5a). This is a consequence of the non-zero y-intercept of the regression of REE on FFM, which implies that the total removal of FFM does not lead to a zero metabolic rate.

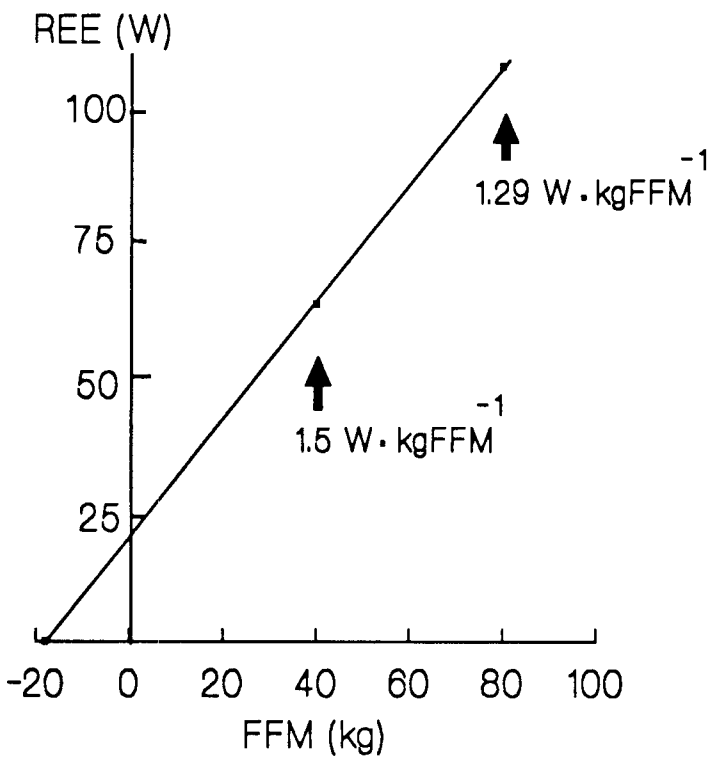

Figure 10.5a. Average predicted resting energy expenditure expressed per unit fat-free mass is higher in individuals with a lower fat-free mass. Note that the x- and y- intercepts do not equal zero.

Ravussin and Bogardus (1989) attempt to circumvent this problem by adjusting the estimate of FFM by adding the absolute value of the x-intercept (from the present study, this value is equal to 16.5 kg). The result of this adjustment is to make the x- and y-intercepts equal to zero. For example, if the absolute value of the x-intercept is added to the FFM of the two individuals used in the previous example, the resting metabolic rate becomes 1.08 $W \cdot kg\ FFM^{\cdot -1}$ vs 1.08 $W \cdot kg\ FFM^{\cdot -1}$ for the small person and large person, respectively (Figure 10.5b).

While this does effectively "standardize" the REE's of persons with different FFM's, the question arises as to the physiological significance of this correction. It is not clear what the extra FFM (16.5 kg) which neither subject possesses represents. The non-zero x- and y- intercept for the regression of REE against FFM suggest that there is a constant or minimum REE which is independent of differences in FFM.

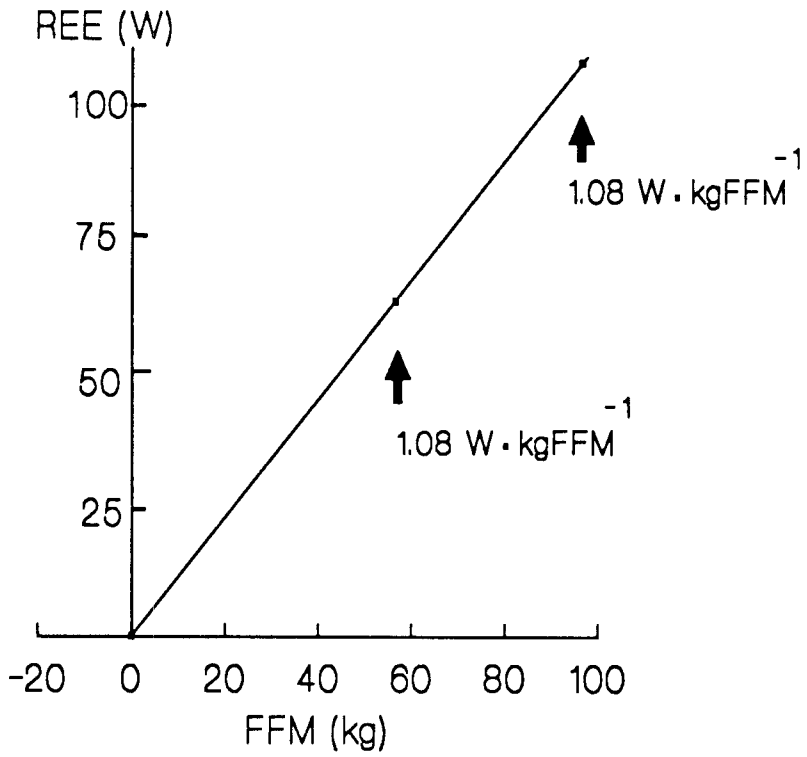

Figure 10.5b. Average predicted resting energy expenditure for the same two individuals in Figure 10.5a after adjusting for the positive y-intercept. Ravussin and Bogardus (1989) suggest that the estimate of "metabolically-active" body mass should equal the fat-free mass + 16.5 kg (x-intercept).

It may, however, be possible to compare the linear relationship between REE and FFM in specific sub-groups of large populations by statistically analyzing the differences in the slope and intercept of the regression of REE and FFM. For example, in the present study the slope of the regression between fat-free mass and metabolic rate was steeper for men than for women. Therefore, one can conclude that for any

given unit increase in fat-free mass, there is a greater increase in resting energy expenditure for men than women. However, it may be difficult to further explain the physiological basis for either a difference in the y-intercept or slope of this regression, unless 1) one can identify the FFM-independent predictor of resting energy expenditure, or 2) unless the "quality" of FFM is not the same between men and women.

Evidence for non-linearity in the relationship between REE and FFM:

As mentioned previously, any attempt to standardize REE for FFM is based on the assumption that the metabolic activity of the FFM is consistent across all ranges of FFM. Because the relationship between REE and FFM may also be described by a non-linear regression, this suggests that the metabolic activity of fat-free mass is not constant across changing fat-free mass.

Weinsier and colleagues (1992) recently addressed this question by reviewing 31 data sets which included infants, preschool-age children, adolescents and adults. They found that the slopes of the linear regression of REE against FFM for adults were lower than those for adolescents, preschool-age children and infants. The y-intercept of the regression

of REE against FFM for infants and children was not different from zero. These data clearly demonstrate that the relationship between REE and FFM is not linear across all ranges of FFM, and that the relationship is probably best described by a curvilinear function.

Weinsier et al. (Figure 1., Weinsier et al., 1992) attributed the non-linearity (or differences in the slopes of the regression of REE on FFM in infants, pre-schoolers, adolescents and adults) to the estimated relative contribution of organ and muscle to the FFM of the various subgroups. For example, they estimated that the FFM of infants was comprised of 30% organ mass and 20% muscle mass, whereas in the adult, the FFM was estimated as 44% muscle and 6% organ mass.

They concluded that because the metabolic rate of resting muscles was lower than organ metabolic rate, the metabolic rate per unit of fat-free mass decreased from infancy to adulthood. Thus, it appears that in comparisons of populations in which the composition or metabolic activity of fat-free mass is likely to be different or under conditions in which FFM is changing, it would be inappropriate to express metabolic rate simply per unit fat-free mass.

In a study by Waki et al. (1991), the constituents of fat-free mass were characterized using whole body counting for ^{40}K and $^{3}H_2O$ for body water determination in middle-aged women with a

mean starting weight of 57 kg compared to age- and height-matched women with a starting mass of 124 kg. This study demonstrated that the ratio between total body water and the body cell mass in the "high-mass" women was greater than the "low-mass" women. A difference in the constituents of the fat-free mass would be expected to result in a lower metabolic activity per unit fat-free mass in the "high-mass" women in this study. This finding would explain a nonlinear relationship between REE and FFM, with REE per unit FFM decreasing with increasing FFM in adults.

In summary, in the present study, the relationship between REE and FFM could be equally well described by a linear function, a rectangular hyperbola or an exponential growth curve. The linear relationship between REE and FFM and the occurrence of a non-zero y-intercept is comparable with many previous studies on the determinants of resting energy expenditure in adults (Cunningham et al., 1980, Jensen et al., 1988, Owen et al., 1987, Ravussin et al., 1986). This is consistent for ranges of fat-free mass of adults from between 39 kg and 73 kg (Weinsier et al., 1992). A rectangular hyperbola best describes the non-linear relationship between REE and FFM when infants and pre-schoolers are included in the regression (Weinsier et al., 1992).

Therefore, it is possible that the non-zero y-intercept of the linear regression of REE on FFM in adults may be a

mathematical artefact resulting from the limited range of FFM which is described by this regression (the "slow rise" portion of the rectangular hyperbola curve). Alternatively, it may be a result of some determinant of resting energy expenditure which is independent of FFM, such as fat metabolism, or gut metabolism. More likely, it is a consequence of both mathematical artefact and physiological processes.

However, in the present study, the best predictor of REE from FFM was an exponential growth curve. No previous study has found a similar relationship. The likely explanation for this exponential growth curve is that the accuracy of estimation of fat-free mass from skinfold equations may deteriorate with increasing fat-free mass. The possible physiological explanations for non-linearity in this relationship may be a failure to obtain measurements when subjects were in energy balance, or differences in the constituents for fat-free mass between men and women (see Table 10.4, differences in the slope and y-intercept of the regression of REE on FFM for men and women). Alternatively, women in this study may not have been in energy balance, or may have been showing the effects of long-term food energy restriction.

Despite the fact that the exponential growth curve was the function which best predicted REE from FFM in the present sample, it is likely spurious as a result of measurement error or differences in the slopes of the linear regression of REE

on FFM in men and women from the present sample. Moreover, it compounds the problem of a positive y-intercept, and providing a physiological explanation for the FFM-independent predictor of REE.

Exercise training and resting energy expenditure:

In Chapter 6 of this dissertation, it was clearly demonstrated that highly-trained triathletes, training more than 14 hours per week, had a higher resting energy expenditure than moderately-trained runners and sedentary young control subjects (Chapter 6). However, there are few studies which have addressed the determinants of resting energy expenditure in a large sample of exercising and non-exercising women and men, and the results of such studies are inconclusive (Broeder et al., 1992a, Poehlman et al., 1990, Ravussin and Bogardus, 1986). The inability to reach consensus on this question may be attributed to the lack of standardization of protocols (i.e. time since last exercise bout) and definitions of training (i.e. state of training, training volume, training intensity, maximal oxygen uptake, etc.).

In the present study, no attempt was made to "quantify" training volume, which varied widely. Nor was any attempt made to quantify "fitness". Those subjects who were regarded as exercise-trained in this sample included: ballet dancers,

competitive runners, triathletes and recreational runners. All trained a minimum of three days per week.

In this study, absolute resting energy expenditure was not different between the exercising and non-exercising subjects, even after covarying for fat-free mass. However, when resting energy expenditure was expressed conventionally, that is, relative to fat-free mass, REE was significantly higher in the exercising subjects. Furthermore, when REE was regressed against fat-free mass for both groups separately, the y-intercept was significantly higher in the trained group (Table 10.4, Figure 10.3). Thus, for any given fat-free mass, metabolic rate was higher in exercising subjects compared to sedentary persons. These results are similar to those of Tremblay et al. (1986).

Broeder et al (1992a) studied 69 men, aged 18-35 years, and compared resting energy expenditure to maximal oxygen uptake as a marker of "aerobic fitness". These investigators found no correlation between absolute resting energy expenditure and maximal oxygen uptake (VO_2max, expressed per kg body weight). Nor did they find a relationship between resting energy expenditure expressed relative to fat-free mass and VO_2max. When they subdivided the group into low, moderate and high fitness levels on the basis of VO_2max, they found no differences in absolute resting energy expenditure, in REE

after covarying for fat-free mass, nor in REE expressed per kg fat-free mass between subgroups.

The differences in interpretation between the study by Broeder et al. (1992a) and the present one may be attributed, in part, to the criteria used for "aerobic fitness", as discussed previously in Chapter 6. Maximal oxygen uptake is not necessarily the best predictor of athletic "fitness" (Noakes et al., 1990), has a large genetic component (Bouchard et al., 1988), and may not actually reflect training status (Lambert and Noakes, 1989).

Secondly, Broeder et al. (1992a) regressed resting energy expenditure per kg fat-free mass against maximal oxygen uptake expressed per kg body mass. The use of two different body size indices in the same regression will make the meaningful interpretation of the relationship between VO_2 max and REE difficult.

In the present study, resting energy expenditure, expressed relative to fat-free mass, in persons regularly engaging in physical activity was higher than those who were sedentary. This was true despite no overall differences in fat-free mass between subgroups (Table 10.1). Furthermore, the higher y-intercept of the regression of REE on FFM for exercising persons vs sedentary persons suggests that this difference in

the relationship in REE for FFM between subgroups, is, in part, independent of FFM.

Food energy and nutrient intake and resting energy expenditure and substrate oxidation:

In this study, there was a significant positive association between reported food energy intake and resting energy expenditure and measures of body size. However, this association was largely accounted for by gender differences in reported food energy intake. When food intake was correlated to body size and to resting energy expenditure in separate samples of men and women, these relationships were no longer statistically significant. In fact, in previous studies of "small and large" eaters (Chapter 9, Edholm et al., 1977, George et al., 1991, Rose and Williams, 1961), individuals reporting nearly two-fold differences in food energy intake have been shown to have similar body mass, "metabolic body size" and, in some studies, similar resting energy expenditure. Indeed, in large population studies, a negative association between food energy intake and body mass has been reported (Kromhout et al., 1983).

Resting metabolic rate in the present study was also not different in the group reporting a daily energy intake which was less than 1.3 times the extrapolated resting energy

expenditure over 24 hours compared to apparently freely-eating subjects. The lack of association between resting metabolic rate and reportedly low food energy intake may be a result of under-reporting. In a recent study of 63 women, in which actual food intake was compared to reported food intake, there was an average under-reporting of food energy intake by 23%. There was, however, no systematic effect of leanness or overweight on the degree of under-reporting (Lissner et al., 1989).

Results from recent studies on the macronutrient composition of the diet and the effects of oxidative and non-oxidative macronutrient disposal on energy balance suggest that the fat content of the diet and the ability to oxidize fat directly influences energy balance (Dreon et al., 1988, Flatt et al., 1985, Flatt, 1987). More recently, studies by Zurlo et al. (1990), Froidevaux et al. (1992) and Seidell et al. (1992) demonstrated that a higher 24-hr respiratory exchange ratio was associated with subsequent weight gain, in both short- and long-term follow-up. In the present study, the dietary carbohydrate-to-fat ratio was inversely correlated to body mass, fat-free mass and fat mass. These findings are similar to those of Dreon et al., (1988), who showed that dietary fat intake was significantly positively associated to body fat and body mass in 155 sedentary men.

However, fasting respiratory exchange ratio was also positively associated with measures of body size. This finding is in contrast to previous studies by Owen et al. (1987) and Schutz et al. (1992), in which RER was found to be lower in the more obese subjects. However, these studies did not include subjects who were exercise-trained. In addition, in the present study, RER was lower in those subjects characterized as "ad libitum" eaters. Discrepancies between these findings and previous studies may be the result of the tendency in over half of the subjects in this study to report a food energy intake which is lower than 1.3 times the resting metabolic rate.

At present, the significance of fasting RER, and dietary carbohydrate-to-fat ratio as determinants of resting energy expenditure in such a diverse sample is not clear, and may be confounded by an underreporting of food energy intake. This is further supported by the poor relationship demonstrated between reported food energy intake and energy expenditure in subjects who were apparently in energy balance.

Measured minus predicted resting energy expenditure:

Ravussin and coworkers (1989) have suggested that persons demonstrating a lower-than-average resting metabolic rate for their FFM are at increased risk for weight gain. This is

based upon a study in which 95 Southwest American Indians underwent measurement for resting energy expenditure and 24-hour energy expenditure after a minimum of 7 days on a metabolic ward ingesting a weight maintenance diet. Ravussin et al. (1988), found that a low measured metabolic rate compared to the average predicted resting metabolic rate based on FFM was a significant predictor of subsequent weight gain during two-year follow-up.

This finding may simply reflect the effects of a familial predisposition for lower rates of energy expenditure in a population which has already demonstrated a high incidence of obesity. It is also possible that those persons with a low resting energy expenditure who subsequently gained weight were initially tested when they were partially food-restricted or at a reduced weight. This interpretation is supported by the fact that resting energy expenditure was "normalized" (based on average predicted resting energy expenditure) after subjects had gained weight. It is also supported by the fact that not all persons with a low energy expenditure relative to their average predicted energy expenditure gained weight during the 2 year follow-up.

In the present study, the measured minus average predicted energy expenditure was not different in any of the subgroups of the population, between men and women, exercising and

sedentary individuals, nor for those individuals characterized as "restrained eaters" vs "ad libitum eaters" (Figure 10.4).

Thus, this suggests that the measured minus the average predicted resting energy expenditure may be a more sensitive indicator of **acute** changes in energy balance rather than energy "thriftiness", and is not a meaningful basis of comparison in cross-sectional samples. The exception to this would be in the case of a genotype-specific "thriftiness" reflected by a low resting energy expenditure (Ravussin et al., 1988).

Summary:

In this study, fat-free mass accounted for between 69% and 75% of the variance in resting energy expenditure in a large sample of men and women 1) who were either exercise-trained or sedentary, 2) whose body composition ranged from 9% fat to 53% fat, and 3) whose reported food energy intake varied between 0.4 and 2.9 times the resting energy expenditure. This was true, whether this relationship was described by a linear regression or by a non-linear regression.

The diversity of this sample provided an opportunity for illustrating the problems of standardization of the expression of resting energy expenditure, and in comparing various groups

with regard to resting energy requirements. It is evident that any "correction" of the expression of resting energy expenditure for metabolic body size involves the assumption that the REE is linearly related to the FFM throughout the entire range of FFM. Based on the findings of this study, and that of Weinsier et al. (1992), this is clearly an invalid assumption.

Therefore, resting energy expenditure can be standardized for a limited range of FFM. However, the interpretation of this standardization may be misleading unless one can characterize the FFM-independent predictor of resting energy expenditure. The physiological significance of the non-zero y-intercept of the regression of REE on FFM remains to be elucidated, and may simply be the result of mathematical artefact.

Thus, in samples of men and women, or exercise-trained vs sedentary persons, comparisons between groups should be made on the basis of the slopes and intercepts of the regression of resting energy expenditure against fat-free mass. Further studies are required, partitioning the fat-free mass component into its various constituents and studying *in vivo* tissue-specific metabolic rate, before there can be a full understanding of the metabolic adaptations which occur to resting energy expenditure with chronic exposure to factors such as exercise and food energy restriction.

CHAPTER 11

SUMMARY AND CONCLUSIONS

Summary

The first aim of this dissertation was to monitor both rat and human responses to short-term perturbations in energy balance brought about through food energy restriction and refeeding, exercise training and the cessation of exercise training or surgical lipectomy. The second aim of this dissertation was to identify factors which might explain differences in food energy intake in weight-matched, weight-stable "large and small eaters". The final aim of this dissertation was to identify factors which might explain differences in resting energy expenditure in a large sample of weight-stable men and women, including exercising and non-exercising persons, and including persons who may be regarded as "restrained eaters".

In the first study, Long-Evans rats underwent pre-weaning litter size manipulation and were exposed during the initial 18 weeks following weaning, to either a standard chow diet or a diet comparatively high in fat. Additionally, some of the rats were housed as pairs, and others were housed singly during the post-weaning period. Changes in metabolic efficiency were described by quantifying differences in feeding efficiency, changes in body size, and body fat accretion.

The first important finding of this dissertation was that unlike previous models of pre-weaning undernutrition or

more-than-adequate nutrition, there was no persistent effect of pre-weaning litter size on post-weaning growth, feeding effiency and fat accretion. These results may differ from those of previous studies because of the second novel finding of this dissertation. After covarying for litter size, gender and diet group, mean food energy intake was significantly higher in the rats which were housed as pairs (440 ± 14 $kJ \cdot d^{-1}$ and 403 ± 14 $kJ \cdot d^{-1}$, $p<0.05$, for rats housed as pairs or singly, respectively).

Thus, whereas it was previously believed that early undernutrition during previously-defined "critical periods" of growth resulted in stunting and a relatively lower spontaneous food intake, the results of this dissertation suggest that relative undernutrition during so-called "critical periods" of growth are reversible. Moreover, post-weaning development following pre-weaning undernutrition may be influenced by something as straightforward as the method in which the animals are housed.

In the second study of this dissertation, Long-Evans rats, habituated to spontaneous running activity in specially designed wheel cages trained for 8 weeks, after which randomly-selected rats were placed in ordinary cages without wheels for 2 weeks. The metabolic responses to short-term detraining were quantified by measuring feeding efficiency, body mass, body fat

accretion, and changes in adipose tissue lipogenic enzyme activity in trained, detrained and untrained rats.

The major findings from this study were (i) that rats which were previously trained and had stopped training had a higher rate of growth and fat accretion even than untrained controls, (ii) and that this effect appears to be dose-response related to training "load" or weekly spontaneous activity (km). These differences in feeding efficiency and rate of growth occurred despite the absence of measurable differences in food energy intake between groups.

The metabolic responses to stopping training were studied further in human athletes. Body composition, resting and glucose-stimulated energy expenditure were measured before and one and two weeks after stopping training in highly-trained triathletes (>14 hours per week training),and moderately-trained runners (6-10 hours per week training). These results were compared to untrained control subjects.

The major finding of this study was that highly-trained athletes demonstrated a significant and progressive reduction in resting energy expenditure during two weeks of stopping training, despite a relatively constant reported food energy intake. In contrast, there was little or no change in resting energy expenditure in moderately-trained runners who had also stopped training,

and occurred despite insignificant changes in body mass and body composition during detraining in any of the athletes.

The differences in the response of trained rats and highly-trained triathletes to stopping training suggest that growing rats accomodate the change in energy expenditure with stopping training by accelerating growth, whereas previously-weight-stable humans accomodate this change in energy output without measurable changes in body mass, and despite a progressive reduction in resting energy expenditure. Thus, the triathletes may accomodate this change in energy output by a decreased metabolic efficiency not measured in this study. Alternatively, food energy intake may have altered insensibly during the detraining period. It is also possible that spontaneous physical activity increased in the detraining athletes.

In the next study of this dissertation, free-living, high-mass adults underwent moderate food energy restriction with or without exercise training for 12 weeks. Another group underwent very-low energy dieting for 4 weeks.

All subjects were asked to undergo voluntary "partial" refeeding after the period of food energy restriction. During refeeding, subjects were instructed to increase their voluntary food energy intake, so that it was not

different from their food energy intake per unit fat-free mass during the pre-trial period. The refeeding period was 3-4 weeks in duration. The mean reported food energy intake per unit fat-free mass prior to the trial for all groups was 140 ± (SEM) 10 kJ·kg $FFM^{-1}\cdot d^{-1}$ and not different to that reported during the refeeding period, 135 ± 14 kJ·kg $FFM^{-1}\cdot d^{-1}$.

Resting and glucose-induced energy expenditure, and body composition were measured before and after voluntary food energy restriction, food energy restriction plus exercise training, or very-low energy dieting. The most important finding of this study was that the relationship between resting energy expenditure and fat-free mass did not change as a result of food energy restriction and the consequent loss of mass. Moreover, neither the rate of weight loss, nor the mode by which the energy deficit was achieved influenced the relationship between resting energy expenditure and fat-free mass. The second important finding from this study was that the thermic effect of glucose feeding was attenuated during active food energy restriction, but returned to normal during refeeding.

These findings suggest that the changes in resting energy expenditure and the thermic effect of feeding which result from food energy restriction in previously weight-stable, high-mass persons are reversible, and do not

support the popular notion that "reduced-obese" persons have an enhanced metabolic efficiency.

In addition, persons starting with a higher-than-average-predicted resting energy expenditure lost weight more successfully than those starting with a lower-than-average-predicted resting energy expenditure. This suggests that either certain individuals are pre-disposed to energy "thriftiness" or that the individuals with lower-than-average-predicted resting energy expenditure were already food-restricted at the start of the study.

The metabolic response to energy deficit was further investigated by characterizing the metabolic rate and thermic response to feeding in women before and after undergoing surgical lipectomy. This study demonstrated that surgical reduction of adipose tissue accounting for nearly 2% of total mass did not result in any compensatory changes in energy expenditure. These data provide indirect evidence that fat cell size, and not adipose tissue mass, may be the involved effector in regulating the metabolic response to energy deficit.

In order to address the second aim of this dissertation, resting energy expenditure, and the thermic response to feeding and exercise were compared in matched, weight-stable, body-composition-stable women, who reported very large differences in food energy intake. This study demonstrated that apparent differences in metabolism, as

evidenced by differences in food energy intake required to maintain weight-stability, cannot be explained by differences in resting energy expenditure, but may be related to an increased efficency of food energy utilization. Recent studies suggest that differences in spontaneous physical activity may account for apparent differences in the energy required for body weight maintenance in "large and small eaters".

The final aim of this dissertation was addressed by studying the relationship between indices of body size, food intake, age and gender on resting energy expenditure in a large and diverse population of men and women, which included exercising and sedentary persons, lean and obese persons and "large and small" eaters. These investigations confirmed the now well-established relationship between indices of body size, including fat-free mass, and resting energy expenditure. Furthermore, this study provided evidence that the metabolic "activity" of fat-free mass ($W \cdot kg^{-1}$ FFM) per unit of fat-free mass decreases with increasing fat-free mass. In addition, the resting energy expenditure per unit fat-free mass was found to be higher in exercising persons compared to non-exercising persons.

Thus, in samples of men and women, or exercise-trained vs sedentary persons, comparisons of resting energy expenditure between groups should be made on the basis of the slopes and intercepts of the regression of resting

energy expenditure against fat-free mass. Evidence from this study suggests that in comparative studies of populations in which the constituents of fat-free mass are likely to be different, attempts should be made to further partition the fat-free mass component into its various constituents.

Conclusions

The first 6 experimental trials presented in this dissertation examined the metabolic response to short-term perturbations in energy balance in rats and humans. When energy balance is perturbed in an organism, there are several possible "responses". These include: (i) a change in body energy stores, (ii) a change in the efficiency of food energy utilization (energy expenditure for food energy storage), (iii) a change in the rate of cellular metabolism, or (iv) a change in spontaneous food energy intake or physical activity. These studies have highlighted the complexity of responses and the importance of considering the antecedent energy balance status of the organism.

For example, physically-trained, growing rats respond in a very different way to stopping training than weight-stable, trained humans. Growing, detrained rats demonstrate an enhanced feeding efficiency and a consequent increase in body energy stores. Highly-

trained athletes, however, already have a resting energy expenditure which is higher per unit fat-free mass than their moderately-trained and untrained counterparts. Stopping training in these athletes does not result in significant changes in body energy stores, and suggests that athletes are accomodating the change in energy output with stopping training by an insensible change in food energy intake or spontaneous physical activity.

Thus, interpretations of responses to perturbations in energy balance are influenced by the sensitivity of measures of energy expenditure, food intake and body energy stores in detecting change. For example, fat-free mass assessed by anthropometric means cannot provide information regarding the nature of change in the various constituents of fat-free mass after perturbing energy balance.

Finally, the results of these studies suggest that "weight stability" may describe an organism in energy balance, however, this information does not provide insight into energy "flux" in this organism. For example, chronic undereating or overeating will eventually result in asymptotic changes in body weight and body energy stores. Thus, this would no longer be considered a response to a perturbation in energy balance, but would be considered an adaptation. Future studies should consider the effects of perturbing energy balance in relation to the energy "flux" in an organism.

These studies have highlighted the plasticity of energy balance in biological systems, and have provided insight into possible factors which may influence responses to short-term perturbations in energy balance. Moreover, they have provided indirect evidence for the nature of the signals which result in these responses to perturbations in energy balance.

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