EFFECT OF ENERGY RESTRICTION ON SUBSTRATE UTILIZATION, MUSCLE FUNCTION AND MYOFIBRILLAR PROTEIN DEGRADATION IN SEDENTARY MALES AND ATHLETIC FEMALES

By

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ABSTRACT

An oxidative preference for fat or carbohydrate substrates has an impact on muscle protein metabolism. This study investigated how changes in substrate oxidation (through dieting) influences muscle function and contractile protein profiles.

Eight sedentary males (Age:22 ± 1 years; Height:175.9 ± 1.1 cm; Weight: 84.2 ± 2.9 kg) were placed on a diet and exercise program for 14 days where total energy deficit was 33% (22% from diet; 11% from exercise). All meals were consumed at the study center. Subjects jogged for 0.5 hours/day. Muscle biopsies and quadriceps muscle group function tests (Kin-Com isokinetic dynamometer) were completed pre- and post-treatment.

Body weight decreased (1.5 ± 0.3 kg; $p < 0.01$) and fat oxidation increased ($p < 0.05$). Concentric average torque was lower ($p < 0.05$) at a movement velocity of 30 degrees/second. Myofibrillar proteins treated with a non-lysosomal protease, calpain, showed no increase in calpain activity within myofibrils.

To test the hypotheses in an active population, this study was repeated in athletic females. Fourteen endurance athletes (Age:27 ± 2 years; Height: 167.2 ± 1.6 cm; Weight: 61.8 ± 1.9 kg; VO_2 max: 49.0 ± 0.9 ml/min/kg) were randomly assigned to maintenance energy (100% kcal) or energy restricted (75% kcal) diet groups. Subjects exercised for 100 minutes/day (within their normal exercise routine).

Among the 75% diet group, body weight decreased (1.7 ± 0.3 kg; $p < 0.05$) and fat oxidation increased ($p < 0.05$). Peak concentric torque was significantly lower ($p < 0.05$) for the 75% diet group at 120 degrees/second, while average eccentric torque was significantly larger ($p < 0.05$) at 180 degrees/second. There were no significant changes in myofibrillar calpain activity in both diet groups.

Results obtained from both groups suggest that increased fat oxidation induced by energy restriction was not conclusively related to changes in myofibrillar calpain activity. There were also no changes in quadriceps muscle function due to inconsistent trends within

both sets of data. Indirectly, elevated calpain activity may be related to decreased muscle function since lack of changes in calpain activity was associated with no changes in muscle function. However, a direct effect of calpain activity on muscle function remains to be demonstrated.

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Chapter I

INTRODUCTION:

1. RATIONALE

In 1988 the Canada's Health Promotion Survey:Technical report by H. Neilson stated that more than 50% of Canadians want to lose weight. Because there is an increasing amount of concern about weight control among Canadians, Neilson concluded that Canadians participating in weight control programs need information regarding the degree of risk and types of problems associated with a combined weight reduction and fitness program. This has implications for the performance capability of individuals involved in a weight loss program who engage in physical activity (through work and an exercise program). The projects outlined in this study were designed to test whether a combined energy deficit (restricted diet and exercise) selectively alters the protein composition of muscle and whether this results in a decrease in overall muscle function. This information may have significant physiological impact on diet therapy and/or intervention programs for individuals who wish to lose weight but must continue to perform muscular work at prescribed work rates.

Weight is an especially important concern in the female athlete population in that a lower body weight has often been equated with "better" performance. For example, there is an increased demand to be thin when: an athlete is judged on the basis of how she looked during a performance, or an athlete is involved in endurance sports and a lower body weight is equated with a faster time for a given distance (Wilmore, 1991). The fact that a low calorie intake may be a problem in the athletic population is demonstrated by the fact that as many as 25 % of athletes have an eating disorder (Wilmore, 1991). Knowledge regarding the effect of a low calorie intake in an athletic population may provide useful

information for athletes, coaches and parents regarding healthy dietary/exercise regimens that will optimize muscle performance.

Thus, the main objective of this study was to assess whether changes in fat oxidation (induced by an energy restriction plus elevated energy expenditure) causes selective breakdown of myofibrillar proteins. Previous studies (Belcastro et al, 1988; Parkes et al, 1993) have shown that myofibrillar protein degradation is elevated when fat oxidation is increased as a result of an energy restriction in the form of diet. These changes in muscle proteolysis may there have an effect on muscle function.

In summary, Chapter II will provide background information on changes in fat oxidation with diet and exercise, myofibrillar proteolysis, and muscle function. Chapter III discusses the methods used in the study. Chapter IV describes the results of the study, while chapter 5 discusses the results.

2. PURPOSE OF THE STUDY

OBJECTIVES (MALES)

1) To assess rates of fat oxidation with a 33% energy deficit (i.e. moderate diet restriction plus moderate aerobic exercise).

2) To measure alterations in muscle proteolysis by evaluating calcium stimulated protease activity (calpain-like activity).

3) To measure muscle function by assessing quadricep/hamstring capabilities with a maximal knee extension and flexion as well as an endurance test using a Kin-com apparatus.

OBJECTIVES (FEMALES)

1) To assess rates of fat oxidation in female endurance athletes who are/are not receiving 25% dietary energy restriction.

2) To measure alterations in muscle calcium stimulated proteolysis by evaluating calpain-like activity in female endurance athletes who are/are not receiving a 25% dietary energy restriction.

3) To measure quadriceps muscle group function by assessing quadricep capabilities performing a knee extension and flexion at maximal effort, and at submaximal effort as an endurance test, using the Kin-com apparatus in female endurance athletes who are/are not receiving a 25% dietary energy restriction.

3. HYPOTHESES

The null hypotheses for this study were:

Hypothesis 1:

Changes in fat oxidation, induced by dietary restriction and aerobic exercise, result in no difference in proteolysis of skeletal muscle.

Hypothesis 2:

Changes in fat oxidation, induced by dietary restriction and aerobic exercise, result in no difference in quadriceps muscle group function.

Chapter II

REVIEW OF LITERATURE

1. INTRODUCTION

The maintenance of sufficient ATP levels, which is essential for survival and function of mammalian cells, is coupled tightly to the rates of substrate oxidation (Flatt, 1977). Because the rates of substrate oxidation are determined by the availability of substrate, the amount of energy provided should directly influence the viability and function of mammalian tissue. In the past, these issues in human nutrition and performance led to research which concentrated on energy balance, where maintenance of physiological function, tissue growth, and survival were believed to be adequate provided that enough energy was available. The nitrogen balance method was also used as a research tool in this area when it was identified that amino acids are essential for directed tissue growth and maintenance.

Nitrogen and energy balance methods have gained support in nutrition studies from a practical perspective. However, from a theoretical perspective, they offer limited information regarding the mechanism(s) underlying the role of energy restricted diets on substrate oxidation or on the potential metabolic repercussions for specific tissue/organ function. It has been reported that energy restriction may cause changes in substrate oxidation, which may subsequently alter muscle composition and function (Butterfield, 1987). A selective degradation of functional muscle proteins may occur as a result of the reduced energy state (Dahlmann et al, 1986; Frayn and Maycock, 1979). This may have

implications for people who are energy restricted and are required to maintain specific physical performance levels.

Athletes are one such group of people where specific performance levels may be compromised by a low energy intake. The combined effects of high energy expenditure and low energy intake may increase fat utilization, which in turn may have compounding effects on muscle protein degradation and therefore, muscle performance.

Whether the effect of an increase in fat oxidation induced by an energy deficit (in the form of an energy restriction or energy restriction plus increased energy expenditure) causes selective degradation of identifiable functional proteins in muscle has not been examined in humans. This is the primary focus of this study.

The literature review examines this question. Parts two and three of the literature review deal with changes in substrate oxidation as a result of an energy restriction in the form of diet and exercise, respectively. The impact that this increase in fat oxidation has on myofibrillar protein degradation is discussed in Part 4. The possible mechanism(s) responsible for the increase in muscle proteolysis as a result of an energy restriction is discussed in section 5. Part 6 describes the effect of an energy restriction and an increase in calpain activity on muscle function. Finally, Part 7 examines the prevalence of a low energy intake in the athletic population.

2. DIET INDUCED CHANGES IN SUBSTRATE OXIDATION

The purpose of this section is to assess changes in substrate oxidation induced by changes in diet.

The quantity and source of macronutrients influences the type of substrate utilized and the rates of substrate oxidation (McCargar et al, 1989). For example, protein ingestion activates both protein degradation and protein synthesis. In fact, protein and non-protein energy may stimulate protein synthesis and/or degradation through different mechanisms

and therefore may have an additive effect on protein turnover (McCargar et al, 1989). Thus, non protein energy, while affecting nitrogen retention, affects protein turnover in a different manner than protein energy (Reeds and Fuller, 1983).

Ingestion of non-protein energy in the diet influences nitrogen balance. For example, Richardson et al (1979) reported that when carbohydrate, as opposed to fat, was the major energy source in a liquid diet (daily energy intake was less than 50 kcal/kg) fed to healthy male subjects, the most efficient nitrogen utilization was achieved. At higher energy intakes (above or equal to 50 kcal/kg body weight, for example), the opposite was observed (fat, as the major energy source, achieved the most efficient nitrogen utilization).

McCargar et al, (1989), found that when increasing the fat content in the diet (and thus decreasing the CHO:fat ratio) there was an increase in nitrogen retention and fat oxidation and a slight decrease in energy expenditure. Acheson et al (1984) also reported a blunted thermogenic effect of a meal following exposure to a high fat (75% of total energy) diet.

The thermic effect of feeding is one method used to quantify rates of substrate oxidation. It is the energy expended in excess of resting metabolic rate for the digestion, absorption, transport, metabolism, and storage of food and it accounts for approximately 10% of daily energy expenditure (Van Zant, 1992).

Findings of McCargar et al (1989) were supported by findings of Phinney et al (1983) who showed that there was a significant increase in fat oxidation after feeding healthy lean subjects a high fat (84% of total energy) diet at maintenance energy levels for 4 weeks. This preferential shift to fat oxidation for energy spares nitrogen losses as the reliance on glucose and/or amino acid oxidation for energy is decreased.

McCargar et al (1989) also found that when subjects were fed a high carbohydrate diet, there was an increase in oxygen consumption and carbon dioxide production as compared to subjects ingesting a high fat diet. These findings in combination with the facts that the thermogenic response to feeding and lipogenesis are both increased with a high

carbohydrate diet (CHO), contribute to the elevated energy expenditure seen with a high carbohydrate diet. This increase in metabolism associated with a high carbohydrate diet promotes an increase in amino acid oxidation and subsequent nitrogen excretion.

The amount of energy consumed also affects the rate of substrates oxidized. For example, both starvation (Mole', 1989) and semistarvation (Mole', 1990) have been shown to decrease the resting metabolic rate (RMR: the energy necessary to maintain the physiological systems at rest (Van Zant, 1992) between 15 to 30%.

This decrease in resting metabolic rate with energy restriction is not immediate. Stock (1986) did not find a change in RMR after 32-36 hours of fasting. Bray (1969) found that restricting energy intake from 3500 kcal/day down to 1900 kcal/day decreased RMR by 15% after 3 weeks of dieting, but RMR was unchanged during the first 4 days of restriction. Similarly, Mole' et al (1989) showed that RMR did not change relative to a 10-day control period until day 5 of a calorie-restricted diet (500 kcal/day). Thus, unlike overfeeding which enhances RMR after 24 hours, underfeeding may require several days before RMR is reduced significantly (Mole', 1990).

A low energy intake also affects the types of substrates which are oxidized. For example, Nelson et al (1992) found that a larger percent of resting energy expenditure was derived from lipid after a 5 month diet restriction in obese females. Furthermore, Froidevaux et al (1993) found that the resting RQ value decreased in moderately obese women as a result of an energy restriction involving diet and exercise. Thus, the results obtained from these studies show that an energy restriction promotes the utilization of fats as an energy source under resting conditions.

A low energy intake may also affect what fuels are metabolized after the ingestion of food. For example, Froidevaux et al (1993) found that the post-absorptive RQ decreased in 10 moderately obese women who lost weight due to a restrictive energy intake. They also found that the rate of lipid oxidation measured over a 24 hour time period, increased significantly during dieting.

Although it is well established that an energy restriction in obese individuals results in an increased fat utilization under resting conditions and after the ingestion of food, the effect of an energy restriction on the types of substrates utilized under resting conditions and after the ingestion of food in endurance trained athletes and normal weight individuals remains to be determined.

3. EXERCISE INDUCED CHANGES IN SUBSTRATE OXIDATION

This section examines the effect of prolonged low intensity exercise and a trained state on the substrates utilized during exercise.

Glucose (stored as glycogen), and fatty acids (stored as triglycerides) are the only quantitatively important energy sources during exercise. Of these, glycogen is the more important fuel for strenuous exercise that requires 65% to 100% VO_2 max (Vranic and Lickley, 1990). Thus, as exercise intensity increases, a shift in substrate utilization towards carbohydrate occurs, even in the trained state (Brooks and Mercier, 1994). Liver glycogen is important because it is the major source of blood glucose, which is the primary substrate for the central nervous system (Vranic and Lickley, 1990).

Endurance exercise training increases the capacity to use all classes of energy substrates (Brooks and Mercier, 1994). For example, it has been suggested that athletes are better able to regulate the balance between glucose mobilization and utilization via the hormonally induced extraction of glucose from liver glycogen (Vranic and Lickley, 1990). Near the end of prolonged exercise, athletes do not show a continuous fall in arterial glucose as seen in untrained persons, but rather a slight rise has been observed (Keul et al, 1972). It is also possible that the increased free fatty acid (FFA) levels (Vranic and Lickley, 1990), produced as a result of triglyceride lipolysis during exercise, may inhibit the extraction of glucose from liver glycogen and its subsequent oxidation; this constitutes the glucose-fatty acid cycle (Brooks and Mercier, 1994).

Glycogen content is also thought to be increased after a training program (Vranic and Lickley, 1990). Short et al (1969) found that after 6 weeks of training there was a 40% increase in muscle glycogen in the working leg. Thus, due to the increased substrate utilization occurring during athletic training, a higher storage capacity of the endogenous substrate, glycogen, may occur in the muscle cell (Vranic and Lickley, 1990).

During prolonged strenuous exercise, for which muscle glycogen utilization is essential, fatty acids serve as an important secondary fuel. This results in a sparing of glycogen and prolongs the exercise time before glycogen depletion and exhaustion occur (Vranic and Lickley, 1990). Overall, the proportion of fat contributing to oxidative metabolism during prolonged exercise has been reported to be between 40 and 80% (Young et al, 1967).

During rest and mild- to moderate-intensity exercise, lipids predominate as energy sources, especially in the endurance trained state. The supply of free fatty acids to the working muscle is promoted by physical training (Cobb and Johnson, 1963). Endurance exercise training increases the subject's capacity to oxidize lipids during exercise. For example, Keul et al (1963) found that trained animals had a significantly increased oxidation of palmitic acid (Keul et al, 1972). It was also found that the plasma FFA concentration was generally higher in the trained, compared to the untrained state during submaximal exercise at the same absolute intensity (Costill et al, 1973; Winder et al, 1979). Thus, during prolonged exercise, trained muscle utilizes FFAs for energy production to a greater extent than untrained muscle. This is reflected in a lower respiratory quotient (Hermansen et al, 1967).

It was found that an increased capacity of trained muscle to oxidize blood borne FFAs and triglyceride along with an increased capacity to access intramuscular triglycerides results in glycogen sparing (Hermansen et al, 1967) and increased exercise endurance (Brooks and Mercier, 1994; Randle and Garland, 1963) in humans (Hermansen et al, 1967; Rennie et al, 1976) and rats (Randle and Garland, 1963; Randle et al, 1964). This

increase in endurance in trained subjects appears to be a result of the increased mitochondrial reticulum mass (Brooks and Mercier, 1994). Specifically, training increases the enzymes required for: FFA translocation, the TCA cycle, the beta-oxidation path (Brooks and Mercier, 1994). Training also increases the components of the electron transport chain needed to oxidize FFAs (Brooks and Mercier, 1994). There is also a simultaneous inhibition of glucose uptake, glycolysis, glycogenolysis, and pyruvate oxidation (Randle and Garland, 1963; Randle et al, 1964) as a result of down regulation (through substrate inhibition; Brooks and Mercier, 1994) of cytoplasmic phosphofructokinase and pyruvate dehydrogenase (Havel et al, 1967; Phinney et al, 1984). Thus, use of anaerobic energy systems such as anaerobic glycolysis and phosphocreatine in providing energy for endurance exercise is not significant or facilitated (Fox, 1984).

The source of additional triglycerides utilized in the trained state is thought to originate from intramuscular triglyceride stores as it has been proposed that the trained muscle has an increased capacity to access intramuscular triglycerides (Brooks and Mercier, 1994). Furthermore, additional utilization of triglycerides in trained muscle may be due to metabolic adaptations in adipose tissue. Savard et al (1986) did biopsies of supra-iliac adipose tissue in male marathon runners and sedentary controls and found that the runners had greater lipoprotein lipase activity in the adipose tissue along with greater basal and insulin-stimulation of conversion of glucose to triglycerides.

The time course of these adaptive changes in substrate utilization as a result of becoming trained has not been defined. For example, it is unknown whether described enzymatic changes in lipid and carbohydrate metabolism may occur after 1 week of training in previously sedentary males. Therefore, the changes in fat oxidation occurring as an individual becomes more trained needs to be established.

It is unknown as to whether the increased utilization of fats in the trained state offers any physiological advantage. It may be that it is only a compensatory mechanism for the decreased beta-adrenergic stimulation of lipolysis in adipose tissue (Vranic and Lickley,

1990). It has also been suggested that a reduced sympathetic nerve stimulation (resulting in a decreased beta-adrenergic response) after training results in a decrease in glycogenolysis and attendant decreases in carbohydrate utilization during exercise (Brooks and Mercier, 1994). This in turn would promote the utilization of FFAs and triglycerides as fuels for the working muscle (Brooks and Mercier, 1994).

The amount of FFA utilized during exercise is determined by the energy requirement and the level of substrate (Vranic and Lickley, 1990). For example, when glycogen stores are low, the substrate supply is no longer sufficient to cover energy cost, the triglycerides are hydrolyzed to fulfil that purpose.

When there is a lack of energy substrates in the nutritive medium, triglycerides are broken down and utilized. Havel et al (1967) found that in fasted subjects, fat accounted for 40-50% of total energy supply (low glucose levels occurring during prolonged exercise may promote the increase in FFAs in the plasma). Thus, fatty acids can also function as a primary fuel (Havel et al, 1967). This makes it possible for exercise to be continued for longer duration, even in the fasting state (Costill et al, 1973). When subjects were placed on a high carbohydrate diet, fat accounted for only 10% of total energy supply (Cobb and Johnson, 1963).

Shifts in the choice of fuel oxidized during exercise can be modified by the type of food consumed (Gollnick and Bayly, 1986; Wagenmakers et al, 1991). A shift towards a greater use of fat during exercise can be induced simply by reducing the concentration of glycogen stored in muscle (Gollnick et al, 1981). In these experiments the low glycogen concentrations were produced in one leg by a previous day's one-legged exercise and the consumption of a low carbohydrate diet. During two-legged exercise on the second day, the respiratory quotient, obtained through indirect calorimetry,was lower in the subjects whose leg had low glycogen as compared to the subjects whose leg had normal glycogen levels. The uptake of FFAs was also greater in the glycogen depleted leg. Therefore, it was suggested that chronic consumption of a low carbohydrate diet can produce alterations

in the concentrations of key enzymes in muscle that would promote the use of fat during exercise (Gollnick and Bayly, 1986).

Overall, substrate utilization patterns during exercise of graded intensities are determined by a number of factors. These include the following (Brooks and Mercier, 1994): sympathetic nervous system initiated events along with those initiated by actions of insulin and glucagon; blood lactate level; dietary and exercise histories; size of glycogen depots; cardiac output (determined by exercise intensity and duration); blood flow distribution; muscle recruitment; muscle mitochondrial mass.

The exercise bout itself, and the training status of the individual may affect the rate at which substrates are utilized. For instance, it is well known that oxygen consumption and energy expenditure remain elevated above resting values for a period of time after a single bout of exercise. The magnitude and duration of the elevation of postexercise energy expenditure has been the focus of considerable research, although the findings are inconsistent. Several studies (Brehm and Gutin, 1986; Freedman-Akabas et al, 1985; Pacy et al, 1985) suggest that metabolic rate returns to pre-exercise/resting conditions within the first hour after exercise, whereas other studies have indicated that metabolic rate may remain elevated for a much longer period of time after exercise (Bahr et al, 1987; Bielinski et al,1985; Maehlum et al, 1986; Tremblay et al, 1988).

For example, Poehlman et al, (1989) examined the effect of acute exercise on metabolic rate. Six untrained men performed a 90-minute bout of exercise on a cycle ergometer at 50% of VO_2 max. Metabolic rate was measured at rest (control period), and 24, and 48 hours after exercise. There was no difference in RMR 24 and 48 hours after exercise compared to the control condition implying that low intensity acute exercise of 90 minutes duration has no sustained effect on RMR in the late postexercise recovery period.

In contrast, Maehlum et al (1986) demonstrated that metabolic rate in young men and women was significantly higher during the 12 hours following a 65-90 minute bout of bicycle exercise at 70% VO_2 max compared to the 12 hours following a 90 minute supine

rest. Metabolic rate was also higher 24 hours after the exercise bout compared to 24 hours after the controlled resting condition.

Wolfe et al (1990) described the contribution of the energy cost of the triglyceride-fatty acid cycle to energy expenditure during and after exercise. This substrate cycle consists of cleaving the triglyceride molecule into the glycerol and fatty acids in the adipocyte, followed by re-esterification of fatty acids to triglyceride which may occur in the adipocyte (intracellular) or the liver (extracellular). Five healthy males were studied during 4 hours of walking at 40% VO_2 max and for 2 hours postexercise. At the end of the 2 hour recovery period energy expenditure remained elevated above pre-exercise level by 20%. The energy cost of the triglyceride cycling was 0.8 kcal/h at rest, 1.7 kcal/h during exercise, and 4.7 kcal/h during recovery. The energy cost of the triglyceride-fatty acid cycle therefore became significant during the recovery period, contributing 13.7% of the postexercise metabolic rate. This study suggests an important role of the triglyceride-fatty acid cycle in postexercise energy metabolism in healthy subjects. It can only be speculated that the same response would occur in trained individuals.

The amount of time in which fat utilization remains elevated after an exercise bout has not been characterized in athletes or previously sedentary individuals. Additionally, the type of substrates utilized in trained individuals and untrained individuals (put on an exercise program) during daily activities and after consumption of food remains to be established.

The subjects' fitness level may affect the change in the RMR measured post-exercise. Several studies have examined differences in RMR between trained and untrained individuals and have obtained conflicting results. LeBlanc et al (1984) measured resting oxygen consumption in 30 females separated into untrained (n=10), moderately trained (n=10) and highly trained females (n=10). All women were tested during the postovulatory period of their menstrual cycle and resting oxygen consumption was measured using indirect calorimetry. Although the authors reported no differences between

the groups, there was a trend for resting oxygen consumption (adjusted for body weight) to increase with the level of training.

Poehlman et al (1988) found that RMR, expressed per kilogram of fat-free weight, was higher in aerobically trained men compared to untrained men. Using a ventilated hood system the RMR of 9 trained and 9 untrained weight stable men was compared 24 hours after the last exercise bout. Resting metabolic rate adjusted per kilogram of fat-free weight was 11% higher in trained men than untrained men.

Although the mechanism for the higher RMR in highly trained people remains to be established, Poehlman et al (1991) hypothesized that RMR may be affected due to the high caloric turnover concurrent with the maintenance of energy balance. This 'high energy flux state' would be accomplished by matching a high level of food intake with a high level of exercise-induced energy expenditure, which is characteristic of weight stable endurance-trained athletes (Poehlman et al, 1991).

The cross-sectional studies do not provide a clear picture regarding the effects of the 'trained state' on RMR due to many confounding variables. The factors which contribute to discrepant findings may include (Poehlman et al, 1991): insufficient sample size; the timing of indirect calorimetry measurements relative to the last exercise bout; technical or methodological errors in determining energy expenditure; or within subject variability due to preceding dietary practices.

The effect of a diet and exercise program on the rate at which energy is utilized yields conflicting results. Belko et al (1987) found that the RMR was not altered by an exercise and energy restriction program compared to an energy restriction only regimen in moderately overweight women confined to a metabolic unit. In both groups, energy intake and expenditure were manipulated to result in an energy deficit of 50% for 6 weeks. The authors concluded that despite a 50% energy deficit achieved by diet alone or diet in combination with exercise, no energy conservation mechanisms were evident.

Nieman et al (1988) however found that 21 mildly obese women during a 5 week exercise program and a 1300 kcal/day diet showed a 6% increase in RMR (kcal/day) compared to a control group who were consuming the same diet with no exercise. These results suggest that RMR may be enhanced when a caloric restriction resulting in moderate weight loss is combined with exercise training of sufficient intensity. The effect of a combined diet and exercise program in a trained and untrained normal weight population on RMR has not been characterized.

In summary, it has been shown that prolonged low intensity exercise and a trained state promote the utilization of fat as an energy source during exercise. However, the effect of this on the type of substrates utilized after the ingestion of a meal requires further investigation. Furthermore, the combined effect of a diet and exercise program in trained and untrained individuals on substrates utilized after consumption of food also needs to be established.

4. SUBSTRATE OXIDATION AND PROTEIN DEGRADATION

The increases in fat oxidation induced by an energy restriction may be associated with increases in muscle proteolysis. A concrete relationship between these two variables has not been established. This section investigates the impact of an increase in fat oxidation, induced by an energy restriction (in the form of a low energy intake, elevated energy expenditure, or a combination of both), on muscle proteolysis.

The equilibrium of the rate of protein synthesis and protein degradation determines protein metabolism. Any one- sided change in these rates will favor either synthesis or degradation. For example, when animals and humans are subjected to adverse dietary conditions such as food deprivation, the result is a negative nitrogen balance (Young et al, 1967) originating from striated muscle. In this situation the energy requirement is not being met, nitrogen is lost from the body, and the protein lost from the body is more than

the protein intake. This implies that greater than normal amounts of protein degradation are occurring in muscle under the condition of a low energy intake.

Pathological states associated with altered metabolic status have been found to cause a one-sided change in reaction rates. For example, increases in fat oxidation induced by diabetes may result in increases in the activity of calpain (calcium activated neutral protease: a non-lysosomal protease capable of degradating myofibrillar proteins) (Kettelhut et al, 1994) and calpain degradation rates in the Z-line area of a myofibril. It was suggested that the 'more oxidized' (lower sulfhydryl group reactivity and content) diabetic cardiac myofibrils produced a more susceptible substrate for calpain (Belcastro et al, 1994). Thus, the altered oxidation-reduction status induced by diabetes has been suggested to be a targeting mechanism for selected protein degradation (Belcastro et al, 1994). There is also indirect evidence that the Z-line region of the muscle is selectively affected when fat oxidation is increased as a result of fasting (Dahlman and Reinauer, 1981) and exercise (Belcastro, 1993).

Although hormone activity has been shown to directly control protein synthetic and degradative processes and therefore total amount of protein in muscle (Vranic and Lickley, 1990), it is becoming increasingly apparent that the oxidation of fat or carbohydrate substrates occurring, as a result of diet manipulation, impacts protein metabolism independent of the hormonal changes (Goldberg et al, 1980; Short et al, 1969). The mechanism(s) underlying the changes in protein metabolism occurring as a result of changes in fat and/or carbohydrate metabolism are not known. Lowell and Goodman (1987) investigated this question by inhibiting lipid metabolism using nicotinic acid (which is an antilipolytic agent). Nicotinic acid administration resulted in increased protein degradation and decreased protein synthesis in muscle. Because protein degradation was elevated when lipid metabolism was inhibited, they concluded that under uninhibited conditions lipid fuels directly modulate protein metabolism in muscle by attenuating the breakdown of myofibrillar protein independent of their oxidation as a fuel source for

muscle. However, nicotinic acid administration was also accompanied by a decrease in plasma insulin levels (Garza et al, 1976) and an increase in plasma corticosteroid levels (Lowell and Goodman, 1987). Thus, a role of insulin and/or glucocorticoids in causing the protein changes seen with nicotinic acid treatment could not be ruled out.

It was postulated that free fatty acids may specifically modulate the myofibrillar protein degradation in two ways (Lowell and Goodman, 1987). Free fatty acids may directly attenuate the breakdown of protein in skeletal muscle. Free fatty acids may regulate the levels of insulin and/or glucocorticoids in plasma, resulting in an indirect regulation of the breakdown of proteins in skeletal muscle. It is also possible that the change in hormone levels themselves cause the changes in FFA levels, which may then regulate protein breakdown in skeletal muscle in some way. Although it cannot be shown that lipid fuels directly attenuate the degradation of protein in skeletal muscle independent of hormonal changes, Lowell and Goodman (1987) strongly suggest a direct role.

A) Elevated Energy Expenditure

The increased utilization of fat promoted by prolonged endurance training and a trained state may result in increases in myofibrillar protein degradation during and/or after exercise. This section examines the effect of exercise training (associated with an increased fat oxidation) on myofibrillar protein degradation.

It is difficult to state whether muscle proteins, under well defined conditions, can be used as a fuel or rather as a precursor of muscle fuel during exercise (Keul et al, 1972). The energy demands of skeletal muscle during exercise can be almost entirely met by the degradation of glucose, glycogen and FFA. The extent to which proteins are used is dependent on duration and intensity of exercise, levels of glycogen stores, and the levels of energy intake (Randle et al, 1963).

One way in which muscle protein degradation can be assessed is by looking at the branched chain keto acid dehydrogenase (BCKAD) activity in muscle. Branched chain keto-acid dehydrogenase is the rate limiting enzyme for the oxidative decarboxylation of

branched chain amino acids (BCAA) in muscle (Randle et al, 1964). Branched chain keto acid dehydrogenase is found on the inner mitochondrial membrane and is regulated by phosphorylation and dephosphorylation, where the active form is the dephosphorylated form (Kasperek and Snider, 1987). It catalyzes the irreversible oxidative decarboxylation of branched chain keto-acids like alpha-ketoisocaproate, alpha-keto-beta-methylvalerate, and alpha-ketoisovalerate - all formed from BCAA transamination (Kasperek and Snider, 1987). Branched chain amino acids comprise about 35% of the indispensable amino acids in muscle proteins (Shimomura et al, 1990).

Energy deficits can result in significant increases in rates of muscle protein degradation during or after exercise. Kasperek and Snider (1987), investigated the effects of an energy deficit and found that there was a 482% increase in BCKAD activity in the muscle of starved rats who exercised and only a 142% increase in enzyme activity in fed rats who exercised compared to rested rats. It was found that starvation itself does not increase BCKAD activity - but enhances the exercise induced activation. The activation of BCKAD in muscle during exercise means that the transamination of branched chain amino acids is elevated. This may occur as a result of the increased breakdown of myofibrillar proteins to branched chain amino acids. Thus, the amino acids produced by protein degradation could supply a significant fraction of the increased energy expenditure during exercise.

The effects of low energy intake on increased muscle protein degradation may occur due to glycogen depletion which is often associated with low energy intakes. When human subjects cycled for 1 hour at 61 % VO_2 max in a glycogen depleted state, the amount of amino acids and protein utilized increased and accounted for a higher percent of total energy utilization (Winder et al, 1979). Thus, when carbohydrate is not readily available as an energy substrate, muscle protein may be degraded so that the carbon-chains can be utilized for energy through the oxidation of BCAA (Kasperek and Snider, 1987). Alternatively, the carbon-chains may be used for glucose formation via gluconeogenesis, and subsequent

energy use. For example, in glycogen depleted subjects (resulting from exercise and starvation), it was found that the activity of BCKAD in muscle increased significantly (Kasperek and Snider, 1987).

Brouns et al (1989) investigated the carbohydrate (CHO) requirement to adequately glycogen load an athlete so that muscle protein degradation and muscle protein oxidation could be prevented. They fed cyclists who performed 2 days of exhaustive cycling a diet consisting of: a fructose-maltodextrin beverage equal amounts of each component in addition to their normal CHO diet (abbreviated the FM diet); or a normal CHO diet (abbreviated the N diet) in which CHO accounted for 60% of the total amount of energy ingested. The N and FM diets resulted in a negative CHO balance. They found that protein oxidation (as measured from daily nitrogen losses in urine and sweat) significantly increased in the N diet during exercise and recovery and in the FM diet on the second day of exercise, while there was no significant change in protein oxidation in the FM diet on the first day. They suggested that the N and FM diets resulted in a CHO depleted state, which through biochemical changes, lead to increased protein degradation in muscle. On day 1 of the FM diet, the high CHO intake may have counterregulated the biochemical factors inducing protein degradation resulting in no changes in protein degradation. However on day 2, where energy balance remained negative for longer periods of time, protein catabolism may have been initiated. The results of this experiment, however, may be questioned, as the investigators did not measure the amount of protein lost in feces.

Duration of exercise may also affect the quantity of muscle protein degradation. It has been suggested that increased exercise duration is associated with increased protein catabolism (Lemon and Mullin, 1980). In fact, it may be that prolonged exercise has the same effects on protein catabolism as starvation (Lemon and Mullin, 1980).

Kasperek and Snider (1987) also confirmed the fact that increased exercise duration is associated with increased protein catabolism, especially in muscle. The activation of BCKAD in muscle was elevated as duration of exercise increased resulting in a subsequent

increase in oxidation of BCAA in muscle. Thus, BCKAD is activated in response to increasing energy demands.

The variability of muscle protein degradation associated with exercise intensity has recently been documented, where the calpain activity (an enzyme which breaks down Z-line proteins in the myofibril; Johnson, 1990) increased with increasing intensity in the plantaris muscle (which contains fast muscle fibers) of rats (Belcastro et al, 1995). This intensity-dependent increase in calpain activity was dependent on the duration of exercise and fiber type, where the intensity effect was not seen when exercise duration was short or when assessed in slow muscle fiber types (the soleus). The effect of exercise intensity and duration on muscle proteolysis has not been documented in humans.

Conclusions made about myofibrillar protein degradation during exercise are conflicting. Several investigators have found that muscle protein degradation remained unchanged during exercise, but increased significantly during the recovery period. For example, Dohm et al (1982) found no significant change in the 3-methylhistidine excretion on the first day of exercise involving a 2 hour run. However, during the day after the experiment when no further exercise was performed, the excretion rate of 3-methylhistidine (3-MH) in urine was significantly increased suggesting that contractile protein degradation increased in recovery rather than during the exercise bout.

Kasperek et al (1992) suggested that the rate of myofibrillar protein degradation continued into the recovery phase as the urinary excretion of 3-MH was increased for several days after exercise consisting of downgrade running. The fact that myofibrillar protein degradation was unchanged during exercise may represent adaptation of the contracting muscle to prevent degradation of the proteins in the motor units responsible for the continuation of the exercise (Kasperek et al, 1992).

Rennie et al (1981) also supported the finding that contractile protein degradation rate is decreased during exercise but is increased during the recovery period if exercise is of high enough intensity and long enough duration. Rennie et al (1981) investigated 3-MH

excretion in urine and 3-MH concentration in plasma of human subjects after running on a treadmill for 2-3.75 hours. There was a fall in 3-MH urine excretion as well as a decrease in intramuscular free 3-MH concentration during exercise. Decombaz et al (1979) also found that 3-MH excretion was not increased during a 100 km running race. Immediately after exercise Dohm et al (1987) found that the production rate of 3-MH increased again. Thus it was suggested that during exercise there was a decrease in the fractional rate of myofibrillar protein degradation and a subsequent increase in myofibrillar protein degradation during the recovery period.

However, Kasperek et al (1992) suggested that the large increase in the urinary excretion of 3-MH during recovery from exercise may be due to a decreased food intake. They indirectly observed this by noting that myofibrillar protein degradation was significantly lower in rats that had been starved for 24 hours, were exercised and were re-fed compared to rats who exercised but were fed at their energy requirement. This demonstrates the profound impact that energy intake may have on muscle protein degradation in exercised animals.

Wong and Booth (1990) suggested that protein degradation was increased in the tibialis anterior in rats undergoing stimulatory chronic eccentric exercise. Protein degradation, however, was not measured directly and this suggestion arose from the observation that protein synthesis decreased with repetitive chronic stimulation in the tibialis anterior.

Lemon and Mullen (1980) found that protein degradation increased during exercise. They found that urea nitrogen excretion was increased during exercise. However, they stated that the source of protein catabolism could not be determined. Whether one finds an increase, no change, or a decrease in muscle protein degradation depends on the timing and type of measurement made, and the intensity and duration of exercise. Thus, more sophisticated methods are needed before the effects of exercise on muscle protein degradation can be completely understood.

It has been suggested that the increase in muscle protein degradation seen in exercise is related to the loss of structural protein, which subsequently may cause myofibrillar ultrastructural abnormalities (Heyliger et al, 1985). For example, a dramatic decrease in a 58 kDa and 95 kDa protein as well as changes in the ratios of several plantaris myofibrillar proteins were associated with an extensively disrupted sarcomeric structure (Belcastro et al, 1988). It was suggested that these 58 and 95 kDa proteins were probably desmin and actinin (myofibrillar proteins involved with inter and/or intra- Z line structure) (Belcastro et al, 1988). Thus, the loss of protein occurring as a result of increased muscle protein degradation resulted in dramatic ultrastructural changes in the muscle.

This increase in muscle protein degradation occurring as a result of exercise may actually represent adaptive changes in protein composition of skeletal muscle occurring with endurance training (Zaidi and Narahara, 1989). For example, increased protein degradation may be needed to clear tissue debris for deposition of newly synthesized muscle protein during remodeling (Reddy et al, 1983). Recent evidence suggests that protein changes from one isoform to another and that the control site is at the level of transcription (Babif and Booth, 1988).

Conclusions made about protein degradation during and after exercise may be conflicting due to the differing exercise protocols and methods of assessing protein degradation. The type, frequency, intensity and duration of exercise was different among studies and may have contributed to the differing results obtained from studies looking at muscle proteolysis and exercise. Additionally, methods of assessing muscle protein degradation may be difficult to interpret and perform (especially during exercise). Furthermore, methods of evaluating muscle proteolysis make it very difficult to determine which myofibrillar proteins are broken down.

In summary, a direct relationship between an increase in fat oxidation, induced by an elevated energy expenditure, and an elevated muscle proteolysis has been implied but not established. Some studies suggest that there is an increase in myofibrillar protein

degradation during exercise, while others suggest that it increases after exercise. Most of the studies looking at muscle protein breakdown and exercise used athletes as the study population. Therefore, more research is needed to quantify myofibrillar protein degradation in a non-athletic population who is trained.

B) Low Energy Intake

A low energy intake in extreme forms (ie starvation) and less extreme forms (ie dieting) may result in increases in myofibrillar protein degradation as a result of the changes in substrate oxidation. For example, prolonged starvation is associated with increased lipolysis (Costill et al, 1977), which in turn may promote changes in myofibrillar protein breakdown, generating glycerol and non-esterified fatty acids. Glycerol acts as a gluconeogenic precursor and non-esterified fatty acids act as an energy source such that there is an increased amount of fatty acid beta-oxidation in this state (Sugden et al, 1989). Therefore the lipid status associated with starvation may promote changes in muscle metabolism.

Hickson et al (1977) investigated the effects of starvation on muscle metabolism by using isotopic and 3-methyl histidine (3-MH) excretion measurements. They found that during starvation of rats, muscle protein was not degraded but progressively conserved, until the later stages of starvation. However, Dahlman et al (1986) found that muscle taken from rats during the early days of fasting showed protein degradation. This was evident by a 2-3 fold increase in the amount of easily releasable myofilaments, which are intermediates in the degradative pathway of myofibrillar proteins. Kasperek et al (1992) also found that muscle protein degradation was elevated in rats fasted for 24 hours. This was evident by a significant increase in 3-MH release, measured from skinned hindlimb muscles to eliminate the release of 3-MH from skin into the perfusate.

Three-methyl histidine is used as an index of contractile protein degradation because it is not utilized by the muscle (Dohm et al, 1982). Once released, this amino acid can

neither be degraded or re-utilized in de novo protein synthesis and is rapidly removed from the plasma by the kidney and excreted in the urine (Gibson, 1990).

There are various limitations associated with using 3-MH to quantify myofibrillar protein degradation. For example, 3-MH release is an actin marker of myofibrillar degradation (Furuno et al, 1990), which represents about 20% of myofibrillar proteins (Vander et al, 1990). As a result, no information can be gained regarding the degradation of other regulatory proteins such as troponin, tropomyosin, or Z-line proteins. Thus, it may not be possible to address issues regarding loss of function since the only structural component accounted for by this method is actin (which does not solely determine muscle function).

Furthermore, non-muscle sources of 3-MH have been identified. A small amount of contractile proteins are found in the gut and in the skin and contribute to the urinary loss of 3-MH (Gibson, 1990). Although they represent a small portion of the total 3-MH pool they have a high turnover rate which is faster than in skeletal muscle. The result may be a falsely estimated myofibrillar protein degradation due to the non-muscle contributions of 3-MH (Gibson, 1990).

Finally, there can also be a problem of interpretation of 3-MH results in situations where muscle wasting occurs (as might be seen with diet restriction). When muscle wasting occurs, the proportion of skeletal muscle diminishes, resulting in a decreased contribution to total 3-MH excretion. Thus, the inability to determine the 3-MH in relation to muscle mass may produce results that may be misleading.

The problems associated with the methods of assessing myofibrillar protein degradation make it difficult to make conclusions about muscle protein degradation. More research is needed to compare methods of assessing protein degradation and develop a standardized method of assessing this.

Frayn and Maycock (1979) also confirmed early protein degradation with fasting by observing that, in vitro, the extensor digitorum longus of starving rats had a significantly higher rate of protein degradation (3.36 ± 0.21 umol tyrosine released/gram protein, $P <$

.001) compared to control rats (2.54 ± 0.1 umol tyrosine released/gram protein) as measured with [^{3}H]tyrosine. These observations, however were dependent on muscle fiber type. There was a significantly higher protein degradation in the extensor digitorum longus, which contains a high number of fast-twitch fibers compared to the soleus, which contains more slow-twitch muscle fibers. These findings suggest that striated muscle protein degradation may play a role in the early response to food deprivation when fat oxidation is enhanced. However, this method of assessing myofibrillar protein degradation does not address the mechanism by which myofibrillar proteins are broken down, or the source from which the released tyrosine came from.

Moreover, Li and Wassner (1984) found that contractile proteins and non contractile proteins showed different rates of protein degradation and a different response to fasting. The rates of total protein and actomyosin degradation (representing the contractile protein degradation) were calculated by measuring the release of two non-metabolizable amino acids from the hemicorpus of rats (phenylalanine and N'-methylhistidine). When rats were deprived of food for 48 hours, the rate of total protein degradation increased to 148% of the fed controls while the rate of actomyosin degradation was 47% of that of fed controls. It was found that the contribution of actomyosin breakdown to total muscle protein breakdown was small in the fed state (11%) and increased threefold after food deprivation. In addition, when food deprived rats were refed, there was a decrease in rate of actomyosin degradation to that of the fed controls. These results suggest that a restriction in energy intake may cause a substantial amount of weight loss originating from lean tissues, primarily in the form of water and protein.

The change in substrate oxidation occurring as a result of dietary restriction may cause specific proteins, such as contractile proteins to be lost, perhaps by causing a change in the rate of protein degradation and/or synthesis. However, in humans the specific or selective degradation of identifiable, functional protein occurring as a result of a calorie restricted diet has not been examined. Shifts in substrate turnover of resting muscle such

as fasting, unbalanced diets, nervous/hormonal effects, may result in the inclusion of muscle protein/amino acid utilization as an energy source. More research is required to establish a direct cause-effect relationship between fat oxidation and muscle proteolysis as a result of a low energy intake.

5. PROTEOLYTIC SYSTEMS INVOLVED IN PROTEIN DEGRADATION

The proteolytic system(s) that may be involved in the increase in myofibrillar protein degradation is discussed in this section.

Proteins in eukaryotic cells are continually degraded and replaced under precise control mechanisms. Although this continual proteolysis may seem wasteful, it serves several important functions: cells selectively degrade proteins with abnormal sequences or conformations, the accumulation of which could be harmful; rapid degradation of regulatory peptides and enzymes is essential for the control of metabolic pathways and the cell cycle; breakdown of proteins in starvation provides amino acids for gluconeogenesis and energy metabolism; post-translational processing of newly synthesized proteins occurs (Kettelhut et al, 1994).

Under conditions of nutritional deprivation and/or hormonal or amino acid deficiency there is a basal rate of proteolysis as well as an additional accelerated rate of proteolysis (Garza et al, 1976). These intracellular proteolytic events are controlled by multiple pathways within mammalian striated muscle cells.

Myofibrillar protein breakdown in eukaryotic cells may occur through distinct pathways: lysosomal enzymes; calcium-dependent proteases; ATP-dependent proteases; ATP-independent proteases. The proteases involved in the ATP-independent degradative system is not known (Kettelhut et al, 1994). Only some of these proteases are well characterized, and it was found that they range in size from 20 to 800 kDa (Sugden et al, 1989).

Lysosomal proteases are small monomeric units that are compartmentalized within striated muscle (Bond and Bulter, 1987) and include enzymes such as cathespins B, H, and L. They serve in a digestive capacity, reducing peptides into individual amino acid components. They are not in contact with the cellular environment and prefer an acidic pH for optimal activity.

Adenosine triphosphate-dependent proteases are high-molecular weight, oligomeric proteins with alkaline pH optimum (Bond and Butler, 1987). The enzymes fall into two distinct classes (Bond and Butler, 1987): one group is found in the cytosol and consists of cysteine proteases; the other group is found in the mitochondria or bacteria and consists of serine proteases. The major differences between the groups lie in the active-site catalytic residue and the mechanism of action of ATP. The cysteine proteases, which do not require magnesium for activity, are allosterically stimulated by ATP (Bond and Butler, 1987). By contrast, serine proteases, which require magnesium for activity, require ATP hydrolysis for proteolytic activity (Bond and Butler, 1987).

Protein degradation involving ubiquitin is an ATP-dependent cysteine protease where ubiquitin is first activated in an ATP-dependent fashion and then covalently bound to protein substrates (Attaix et al, 1994). Ubiquitination of proteins always requires the ubiquitin-activating enzyme and one of the ubiquitin carrier proteins to generate the first attachment of a single ubiquitin moiety to a substrate. Substrates can be multiubiquitinated by the attachment of ubiquitin chains or trees (Attaix et al, 1994).

Polyubiquitinated proteins are preferentially degraded by a very large 1500 kDa (26 S) proteolytic complex that requires ATP for activation and substrate hydrolysis (Attaix et al, 1994). The 26 S complex contains the 20 S proteasome with proteolytic function and multiple other components. Although the 20 S proteasome has been shown to be an essential component of the ATP-ubiquitin-dependent proteolytic pathway, skeletal muscle proteasome can degrade proteins in an ATP-dependent process that does not require ubiquitin (Attaix et al, 1994).

Non-lysosomal proteases are optimally active at neutral pH. These enzymes, also found in skeletal muscle, are under direct influence of intracellular events (Bond and Bulter, 1987). These enzymes appear to act in more of a regulatory role, instead of a digestive role, as they form restricted fragments from their specific protein substrates in vivo (Bond and Bulter, 1987).

The best characterized of the non-lysosomal proteases is a calcium activated neutral protease, calpain (Murachi, 1989). Two active forms of this enzyme can be distinguished by their requirement for calcium: calpain I is half maximally activated by 20-50 uM calcium, while calpain II requires .3-.7 mM calcium for half maximal activity (Li and Wassner, 1984). Calpain is ubiquitous to the cell and selectively degrades myofibrillar proteins, (especially at the level of the Z-line in muscle cells (Brooks,1987), cytoskeletal proteins, and membrane proteins (Block et al, 1986).

Calpains are normally intracellular enzymes (Johnson, 1990). Most intracellular calpain is cytosolic, with between 7-30% of the calpain being associated with membrane structures (Johnson, 1990). Within the membrane, it seems likely that the distribution of calpain is not uniform, but is associated with various membrane components such as membrane phospholipid, structural proteins, transport systems and receptors (Johnson, 1990). Within the sarcomere, calpain I has been found to be associated with the I-band and Z-band region of the sarcomere (Johnson, 1990). Calpain activity in striated muscle is controlled by the following factors (Belcastro et al, 1994): the calcium concentration; autolyzed/unautolyzed state of the enzyme; the level of inhibitor, calpastatin; the availability of digestible substrates.

The calpains do not cleave undenatured actin or myosin (Goll et al, 1991). Because these two proteins are the principal sources of 3-MH released during muscle protein degradation, release of 3-MH cannot be used to indicate whether the calpain system has a role in myofibrillar protein degradation (Goll et al, 1991).

The calpains do cleave skeletal muscle plasma membrane proteins and phospholipids, as well as cytoskeletal and contractile proteins (Johnson, 1990). The degradation of membrane proteins by calpain (such as band 3 protein, fodrins, and band 4.1 protein) seems to be restricted to the cytoplasmic side of the membrane (Johnson, 1990; Zaidi and Narahara, 1989). Cytoskeletal proteins that are substrates for calpain include (Johnson, 1990): alpha-actinin, tropomyosin, lamins, vimentin, neurofilament protein, tubulin, microtubule-associated protein, C-protein, and desmin.

Proteins indirectly involved in myofibril cross-bridge formation can also be degraded by calpain. These proteins include myosin light chain, tropomyosin, troponin-T-like protein (in smooth muscle), caldesmon, calponin, and alpha-actinin (Johnson, 1990). Their functions involve the maintenance of a myofibrillar conformation needed for cross-bridge formation, and the regulation of cross-bridge cycling. For example, alpha-actinin is a protein in the Z-line to which the ends of the F-actin molecule of the thin filaments attach (Murray et al, 1990). Tropomyosin is more of a regulatory protein in that it covers the myosin-binding site on each actin, thereby preventing the cross bridges from making contact with actin (Belcastro, 1988). The binding of calcium produces a change in the shape of troponin such that it pulls the tropomyosin, to which it is bound, out of its blocking position, uncovering the cross-bridge binding sites on actin.

It has been suggested that calpain's role in muscle protein degradation is directed exclusively at the myofibrillar or cytoskeletal proteins, where their effects result in disassembly of the myofibril and release of large polypeptide fragments, but not in the liberation of free amino acids (Goll et al, 1991). Thus, it has been suggested that calpain is an ideal enzyme for initiating disassembly of myofibrils (Goll et al, 1991).

Zaidi and Narahara (1989) suggest that calpain's role in myofibrillar protein degradation is through the plasma membrane proteins. They found that calpain preparations consisting of plasma membranes from frog skeletal muscle caused a striking loss of a major membrane protein of molecular weight 97 kDa, designated band c.

Overall, the proteolytic systems involved in muscle protein breakdown as a result of an energy restriction may be due to the non-lysosomal enzyme, calpain. Lysosomal enzymes (such as cathepsins) and ATP-dependent proteases (such as those involved in the ubiquitin system) may also be involved. It is difficult to isolate the proteolytic system responsible for the increased myofibrillar protein degradation due to the possible involvement of more than one system, lack of knowledge about the proteolytic substrates, and the lack of methodological procedures quantitating one specific system.

A) Proteolytic Systems Involved in Protein Degradation Occurring with Exercise

This section investigates the proteolytic systems responsible for the proteolytic changes in muscle proteins occurring with exercise.

Mechanically induced changes in myofibril ultrastructure occurring as a result of prolonged exercise may be an initiating factor for muscle protein degradation during exercise. For example, Friden et al (1983) found that there was extensive myofibrillar Z band streaming and broadening up to 3 days after intense eccentric exercise in human males. In this type of exercise there is a high degree of mechanical stress. Kasperek et al (1985) also found that muscle damage induced by downgrade running was associated with an increased rate of myofibrillar protein degradation (as was shown by an increase in urinary 3-MH release).

Warhol et al (1984) also observed post-race ultrastructural changes in runners. They found that all runners showed some degree of myofibrillar lysis, of which damage was confined to individual sarcomeric units. Belcastro et al (1988) found similar results where dissolution of specific Z-line myofibrillar proteins (desmin and actinin as determined by SDS gel electrophoresis) occurred in rats as a result of a running protocol.

Belcastro et al (1984) suggested that the alteration in neuromuscular transmission induced by ultrastructural changes causes an uncoupling of excitation-contraction which may provide a favorable cellular environment for the initiation of myofilament degradation.

Although the proteolytic system responsible for this is not clear, it has been suggested that the increase in calcium concentration in the cytosol reported following exercise (Dohm et al, 1981) may activate both lysosomal and non-lysosomal proteases. This postulation was based on the observation that the number of lysosomes and the total activity of lysosomal acid hydrolases increases a few days after prolonged exercise.

Belcastro (1993) found that level running increased the activity of the non-lysosomal enzyme, calpain, in rats. It was postulated that this was due to an enhanced calcium sensitivity of the enzyme as well as an enhanced susceptibility of myofibrillar substrate protein to calpain action with prolonged level running. The lowered calcium requirement for half maximal calpain activity was thought to be due to an increased ratio of autolyzed to unautolyzed enzyme (the autolyzed forms of u and m calpain were speculated to have a reduced calcium requirement, and an elevated phospholipid concentration (Belcastro et al, 1988).

The increase in calcium concentration seen with exercise may activate calpain, which consequently results in changes in skeletal muscle plasma membrane proteins and phospholipids, cytoskeletal and contractile proteins. Disruption of these proteins by calpain during exercise may prevent cross-bridge formation and may decrease the force production during exercise through alterations in plasma membrane proteins (Zaidi and Narahara, 1989) and/or alterations in cytoskeletal proteins (Belcastro, 1988).

Friden et al (1983) also suggested that metabolic disturbances during work may have caused lysosomal enzymes to be liberated into the damaged fibers to initiate degradation of myofibrillar material. Beaulaton et al (1977) and Fox (1975) also postulate that the soluble alpha-actinin or Z-line proteinase (activated by the non-lysosomal enzyme calpain) may also be involved in initiating myofibrillar protein breakdown.

Other researchers have also concluded that lysosomal enzymes may be responsible for initiating muscle protein breakdown. For example, Dohm et al (1979) found that free cathepsin D activity increased during exercise. They suggested that this enzyme could have

leaked out of the lysosome into the cytoplasm and caused an elevated level of muscle protein degradation to occur as a result of exercise. Pilstrom et al (1978) supported this finding by observing that exercise does in fact increase the activity of cathepsin D, as well as the activity of other lysosomal enzymes.

Most lysosomal degradation takes place in secondary lysosomes because lysosomal enzymes are largely inactive at the pH values in the cell cytosol. Myofibrils are so large that they could not be assimilated by lysosomes. Therefore, striated muscle myofibrils would have to be disassembled to individual filaments and possibly to filament fragments before the lysosomal cathepsins could have a role in their degradation (Goll et al, 1991). Furthermore, it appears unlikely that large proteolytic complexes such as macropain (the multicatalytic lysosomal protease) could effectively degrade intact myofibrils before they were disassembled into filaments (Goll et al, 1991).

It has further been suggested that lysosomal enzymes may not be involved in the increased rate of degradation of contractile muscle proteins but rather that of noncontractile muscle proteins. Kasperek et al (1992) found that chloroquine (an inhibitor of lysosomal fusion) failed to bring the rate of tyrosine release from the isolated soleus muscles of exercised rats to control levels. Thus, it was suggested that the increase in "free" cathepsin D activity observed with exercise caused an increase in the rate of degradation of noncontractile muscle protein (Kasperek et al, 1992).

Therefore, evidence suggests that the non-lysosomal system may be responsible for the increase in muscle protein breakdown seen with exercise because the sensitivity of this system is increased and the activity of this system is higher. The effect of exercise intensity, duration, frequency and type on the proteolytic system(s) involved needs to be quantified.

B) Proteolytic System(s) Involved in Protein Degradation Occurring with a Low Energy Intake

Both non-lysosomal enzymes and lysosomal enzymes have been implicated in causing proteolytic changes in skeletal muscle with a low energy intake. For example, in one study it was found that calpain activity (a non-lysosomal enzyme) was increased in rats that were calorically restricted in the following manner: 50 % dietary restriction for 2 days; 75% dietary restriction for 2 days; fasted over 2 days (Murray et al, 1991). It was also found that the activity of calpain increased relative to the extent of the dietary restriction: there was a 13% increase in calpain activity with a 50% dietary restriction; there was a 22% increase in activity with a 75% dietary restriction; there was a 29% increase in activity in fasted rats. Thus, calpain may play a pivotal role in muscle protein turnover during a dietary restricted state by initiating the process of myofibrillar disassembly (Murray et al, 1991).

The involvement of calpain in increasing myofibrillar protein degradation as a result of fasting comes from a study administering E-64 (a cysteine protease inhibitor that inhibits the calpains). The increase in the amount of easily releasable myofilaments (intermediates in the degradation of myofibrillar proteins (Dahlmann et al, 1986; Van der Westhuyzen et al, 1981) as a result of fasting was reduced by treatment of the animals with E-64.

Lysosomal enzymes, such as cathepsin D, have also been implicated in initiating myofibrillar disassembly in a dietary restricted state. For example, in the study by Murray et al (1991) as previously described, it was found that the cathepsin D activity was also significantly increased in dietary restricted groups compared to controls. The extent of this increase, however, was significantly lower than the increase in calpain activity.

It has been suggested that calpains, and not the cathepsins, appear to play a role in the enhanced degradation of proteins in energy-depleted muscles (Fagan et al, 1992). Weak bases were administered to ATP-depleted chick skeletal muscles in order to prevent the digestion of proteins with the lysosome by increasing intralysosomal pH. However,

the weak bases did not block the increase in the degradation of proteins in energy-depleted muscles, suggesting that these proteins were degraded by a nonlysosomal proteolytic pathway, such as one involving calpain (Fagen et al, 1992).

This was further supported by the fact that 3-MH release did not differ between energy-depleted and control rats when calpain activity was inhibited with E64 (Fagen et al, 1992). This meant that actin and myosin, which are not calpain substrates, were not being broken down (as was indicated by the absence of a change in 3-MH release between energy-depleted and control rats). This suggested that the myofibrillar proteolysis was due to calpain action.

Therefore, the proteolytic enzyme, calpain, may have a pivotal role in myofibrillar protein degradation occurring with a low energy intake. More research is required to determine the involvement of other systems as a result of a low energy intake.

6. MUSCLE FUNCTION

The purpose of this section is to describe how muscle function changes as a result of increased myofibrillar protein degradation and an energy restriction.

A decrease in strength may result in the inability to sustain a required power output. Strength is frequently defined as the peak force or torque developed during a maximal voluntary contraction (Alexander, 1990). Peak strength generation is determined by an optimal and dynamic interaction between the muscle (excitation-contraction processes), neural recruitment (type of motor units, size of motor unit pool, frequency and pattern of discharge), and the metabolic source of ATP production (anaerobic and aerobic; Edgerton et al, 1986).

Alterations in muscle protein structure occurring as a result of increased myofibrillar protein degradation may decrease the force generating capabilities of skeletal muscle associated with prolonged repetitive low force producing contractions due to the disruption

in sarcomere structure (Edgerton et al, 1986). Specifically, it has been suggested that calpain action on the myofibril produces a loss of muscle function.

Goll et al (1991) suggested that the formation of a myofibril with a smaller diameter produced as a result of calpain action, would have less strength and a diminished force production. Belcastro et al (1988) suggest that the changes in relative protein composition of the myofibrillar structure may implicate decrements in force transmission in situations where calpain activity may be enhanced due to the disruption in sarcomere structure (Edgerton et al,1986). Specifically, calpain action on cytoskeletal elements may compromise force generation due to an uncoupling of excitation-contraction (Belcastro et al, 1984) as a result of the impairment in muscle cell endocytosis, exocytosis and intercellular transport (Belcastro, 1988).

Zaidi and Narahara (1989) suggest that calpain may compromise skeletal cell muscle function through alterations in sarcolemma structure (for example, changes in membrane fluidity). As a result of the difficulty associated with impulse transmission across the neuromuscular junction, an uncoupling of the excitation-contraction coupling, induced by calpain action, may occur, subsequently resulting in a decreased force transmission. The force transmission failure may be responsible for the presence of fatigue and a decrease in muscle performance during exercise or during continuous training.

Associations between myofibrillar protein degradation and muscle function as a result of an energy restriction have not been established. However, energy restriction and athletic performance has been investigated where it has previously been found that an energy restriction combined with excessive exercise may result in a decrease or no change in athletic performance (Beals and Manore, 1994).

Ingjer and Sundgot-Borgen (1991) studied the effects of dieting in athletes on VO_2 max and running speed. The dieting athletes did show a significant decrease in VO_2 max and running speed relative to control during and after 2 months of weight reduction. Thus, there appeared to be an immediate reduction in aerobic capacity and performance. Possible

mechanisms for these decreases in muscle function included electrolyte imbalances and cardiovascular changes, glycogen depletion and delayed healing and recovery from injury (Beals and Manore, 1994).

The results obtained by Ingjer and Sundgot-Borgen (1991) are in contrast with those found by Scott et al (1992) who found that there was no change in maximal physical performance as a result of an imposed energy restriction. Thirty-six mildly obese premenopausal females ingested 1000 kcal/day for 8 weeks. The strength values (assessed using a Cybex 340 isokinetic device) did not differ pre- and post-diet.

In summary, the effect of an energy restriction on athletic performance yields conflicting results. This may be due to differing diet protocols between studies, differing athletic testing regimens, and differing subject populations. Moreover, an association between myofibrillar proteolysis and muscle function remains to be established.

7. DIETING AND ATHLETES

Female endurance athletes provide a special population for investigation of these issues as they are known to habitually restrict food intake and exercise excessively. Also, although eating disorders have been documented in athletes, relatively little work has been done to examine the physiological effects of a restricted energy intake in athletes. Dieting is known to mean that energy intake is restricted in an attempt to lose weight (Reeds and Fuller, 1983).

The fact that female endurance runners ingest a low energy intake has been documented. For example, various studies reported an intake range from 5860 kj/day to 8330 kj/day (1400-1991 kcal/day/) for female endurance runners (Davis and Cowles, 1989). Thus, some female endurance athletes have surprisingly low energy intakes whereas the reported physical performances suggest a high level of energy expenditure.

For example, Mulligan and Butterfield (1990) found that female distance runners, averaging 50 km/week, had energy intakes similar to those of female nonrunners (1745 versus 1786 kcal/day for runners), yet the energy expenditure of the runners was significantly higher than those of non-runners. Moreover, among nonrunners, calculated mean daily energy intake was equal to the calculated energy expenditure but for the runners, calculated mean energy expenditure exceeded mean energy intake by more than 645 kcal/day. Yet all groups were reported as being weight stable over the two month study period.

The discrepancy between reported energy intake and energy expenditure may be explained in part by underreporting of food intake (Reeds and Fuller, 1983). None the less, it has been suggested that athletes who are restricting their energy intake may predispose themselves to the development of an eating disorder (Davis and Cowles, 1989).

The fact that a low caloric intake may be a problem in the athletic population is demonstrated by the fact that as many as 25 % of athletes may have an eating disorder (Wilmore, 1991). Various factors may predispose an athlete to an eating disorder, or a

restricted energy intake. There is a high demand to be thin when an athlete is judged on the basis of how he/she looked during a performance (gymnastics, and diving; Beals and Manore, 1994). There is an increased demand to be thin in endurance sports when a low body weight is equated with a more efficient performance, and therefore a faster time for a given distance (i.e., long distance running) (Wilmore, 1991). It was found that in sports where a thin build was perceived advantageous, there were greater weight and diet concerns, higher emotional lability, and greater dissatisfaction amongst athletes involved in these sports (Davis and Cowles, 1989). Specific psychological characteristics such as perfectionism, goal oriented, and perhaps being under tight control of a parent or coach, may also increase the risk of an athlete becoming involved in restricting energy intake (Wilmore, 1991).

The perceived advantage to performance in reducing body weight, as well as the sociocultural demands placed on females to achieve and maintain an ideal body shape, may put intense pressure on the female athlete to diet. In fact, the relationship between dieting and activity may be a vicious cycle that perpetuates itself: activity may promote dieting, which in turn induces activity (especially during the early stages of food restriction) (Davis and Cowles, 1989). For example, strenuous exercise may suppress appetite as a result of changes in endorphin levels (Katz, 1986), which serves to decrease the value of food reinforcement. As a result, food intake decreases and body weight is lost. As body weight decreases, the motivation for more exercise increases. Thus, it has been suggested that the increased training load may have induced a caloric deprivation in endurance athletes, which in turn has elicited certain biological and social reinforcements leading to the development of an eating disorder (Sundgot-Borgen, 1994).

Weight preoccupation in the form of dieting can represent one of the progressive steps to a more severe form of weight preoccupation such as anorexia nervosa and/or bulimia in some female athletes (Beals and Manore, 1994). Weight preoccupation often

coincides with restrictive eating behaviour. Thus, the physiological effects of dieting in athletes was studied.

The effects of a low energy intake in athletes may be enhanced due to possible additive effects of low calorie intake and increased energy expenditure on muscle protein degradation. Changes in muscle protein degradation, induced by increased fat utilization, may decrease total muscle mass and fat mass, and therefore decrease performance (Beals and Manore, 1994).

8. SUMMARY

Overall there has been extensive research on exercise as it is related to myofibrillar protein degradation, or a low energy intake as it is related to myofibrillar protein degradation. However, few studies have actually looked at the combined effects of an exercise program and a low energy intake on myofibrillar protein degradation. Furthermore, most studies have looked at the combined effects of diet and exercise on myofibrillar protein degradation in obese subjects. Information about the mechanism by which myofibrils are broken down as a result of an energy restriction is limited.

In conclusion, it is evident that more research is required to determine if the increase in fat oxidation is responsible for the proteolytic changes in muscle proteins seen with an energy restriction. Additionally, the activation of calpain as a result of this change in substrate oxidation remains to be established. Subsequently, the changes in myofibrillar protein breakdown as a result of calpain action may have detrimental effects on muscle function. A relationship between muscle function and calpain activity has not been previously determined. Therefore, more information regarding the effect of an energy restriction in the form of diet and exercise is needed to further understand the relationship between substrate oxidation, calpain activity, and muscle function.

Chapter III

EXPERIMENTAL DESIGN

1. STUDY DESIGN

The basic design of the study was that of an intervention study extending over approximately a two week period. The hypotheses were tested in male and female subjects. Male and female subjects were provided with all of their meals for the study duration, and all exercise was supervised and/or monitored in the male subjects. Exercise was performed as usual in the female subjects.

Experimental male subjects received nutrition and exercise intervention (i.e. 2/3 of the energy deficit arose from dietary restriction, 1/3 of the energy deficit came from increased energy expenditure). Female subjects received nutrition intervention only; the imposed deficit in these subjects arose solely from a dietary restriction.

All male and female subjects ingested breakfast and lunch at the Family and Nutritional Sciences Building, University of British Columbia. Dinner and a snack were prepared and packaged and were taken home by the subjects. All food was weighed and measured to provide accurate amounts to meet the diet protocol and individual requirements. The three-day rotating menu followed for male and female subjects is shown in Appendix 1. All individual menus were calculated from this master menu. For example, to determine the amounts of food for a person who required 3000 kcal/day, values in the master menu were multiplied by a proportional factor to obtain the correct dietary intake amounts. The dietary analysis of this menu is shown in Appendix 2.

Thus, the average percent protein, carbohydrate and fat for all three days was 15 ± 2, 56 ± 3, and 28 ± 2% respectively, which were within the recommended limits suggested for a healthy diet (Hunt and Groff, 1990).

Energy intakes for male and female subjects were determined by estimating resting energy expenditure (REE) through the Harris-Benedict equation (Harris and Benedict, 1919), and multiplying REE by an activity factor to determine total energy expenditure (TEE):

For men:

REE = (kcal/day) = 66 + (13.8 X weight in kg) +(5.0 X height in cm) - (6.8 X age in yrs)

For women:

REE (kcal/day) = 665+ (9.6 X weight in kg) + (1.8 X height in cm) - (4.7 X age in years)

TEE (kcal/day) = REE x activity factor.

The thermic effect of food was not accounted for in these equations.

For the male subjects, total energy expenditure was calculated by multiplying the REE by an activity factor (1.55, for light activity; Harris and Benedict, 1919).

For the female subjects, total energy expenditure was calculated by multiplying the REE by an activity factor (1.82, for heavy activity; Harris and Benedict, 1919). The activity level of each female subject was determined in the initial interview by discussing with the subject how much and what type of exercise she did on a regular basis.

A) MALES

Young healthy male subjects were recruited. The energy requirement of the experimental subjects was cross-checked with the aid of a four day diet record, after which they received nutrition and exercise intervention resulting in a 33% energy deficit.

A series of baseline measurements were taken 3 days prior to the period of diet and exercise manipulation, as well as 1 to 3 days after the period of diet and exercise manipulation. These included anthropometric measures of height (ht), weight (wt), and skinfolds at four sites (triceps, biceps, subscapular, suprailiac). Indirect calorimetry was used to assess substrate oxidation. To evaluate muscle function and assessment of quadricep muscle group strength, a Kin-com isokinetic dynamometer was performed. To assess alterations in muscle protein degradation, human myofibrillar preparations (obtained from a muscle biopsy) were subjected to calpain digestion. Exercise prescription involved daily walking/running on a track until 11% of the total energy requirement had been expended (about 30 minutes of walking/jogging). Measurement of VO_2 max, implemented with a cycle ergometer, and heart rate response to a known workload, were used to determine the amount of energy expended during exercise. A male control group, originating from a previous study (Parkes et al, 1994), were placed on a 100% and 67% of maintenance energy requirement for a time period identical to this study. The control males, however, were completely sedentary. Thus, the 33% deficit originated solely from a dietary deficit.

B) FEMALES

Female long distance runners were recruited for both the control and experimental groups. The energy requirement of the subjects was assessed with the aid of a four-day diet record and the Harris-Benedict equation, after which the experimental group received nutrition intervention resulting in a 25% caloric deficit for a 10 day period. All subjects ingested a caloric intake at their energy requirement two to four days prior to the 10 day diet

period and weight was monitored to determine if the energy level was appropriate. If a subject lost or gained weight while ingesting their calculated maintenance energy requirement, the caloric intake was adjusted before the initiation of the dietary protocol.

Before candidates began the diet protocol they completed: a VO_2 max test; an anthropometric assessment (which included height, weight, and 7 skinfolds); a thorough description of their present exercise status and medication history. If the candidate met the criteria of the study the following initial measurements were done: Kin-Com muscle function test, muscle biopsy, substrate oxidation measurement. Subjects were then randomly assigned to the control or experimental group, after which they ate their respective diets for 10 days. During the dietary period, all subjects were asked to keep a precise exercise diary. An example of a daily exercise recording form can be seen in Appendix 3. At the end of the dietary period the following final measurements were made: Kin-Com muscle function test; muscle biopsy; anthropometric indices; substrate oxidation.

2. SUBJECTS

A) MALES

Eight male subjects were recruited from the University area. Subjects were recruited using posted notices describing the study's objective and the benefits it would provide for the participants. An example of a recruitment advertisement can be see in Appendix 4.

Subject criteria included:

- males
- non-smokers
- free from illness and requiring no medication
- body fat content between 10% and 20%
- previously sedentary (previous exercise should have been no longer than one hour per week)

- no consumption of extreme diets such as very high or very low fat diets (greater than 45% and less than 20% of energy intake respectively).

- no food allergies.

B) FEMALES

Fourteen women were recruited to participate in the study. Subjects were recruited using posted notices describing the study's objective and the benefits it would provide for the participants. An example of a recruitment advertisement can be see in Appendix 5.

The total sample size recruited was 14 women, of which 7 were in the experimental group, and 7 were in the control group. This sample size was chosen based on the subject number (n = 8) used in a different study of similar design, where significant differences were found in muscle function as a result of an imposed energy restriction in male subjects (Parkes et al, 1994). A sample size was not calculated because the variability associated with calpain activity in the subject types studied has not previously been documented.

The fourteen elite female athletes who were recruited were defined as those who possessed the following criteria:

- taking oral contraceptives and/or having a regular menstrual cycle (every month for the past two years)*
- not taking anti-inflammatories
- body fat between 16-26%
- long distance runners
- distance run per week at least 50 km
- VO_2 max greater than 42 ml/min/kg on a cycle ergometer**

*This criteria was specified because recruitment of subjects with similar hormonal profiles was desired, as hormones have been shown to have an effect on muscle protein degradation (Goldfarb and Kendrick, 1981).

**This VO_2 max value was chosen because it is considered to be an excellent value for women between the ages of 20-29 years (Canadian Standardized Test of Fitness Operations Manual, 1986), and therefore would reflect women who are well-trained.

Exclusion criteria for the study included those candidates who possessed food allergies and/or intolerances. These subjects were omitted from the study due to the necessity to keep the menu similar among subjects.

All male and female subjects provided written informed consent prior to participating in the study (see Appendices 6 and 7 respectively).

3. STUDY PROTOCOL

Figure 2: Study protocol.

	DAYS														
	A			B				C							
Measurement	**-3**	**-2**	**-1**	**0**	**to**	**4**		**5**	**to**	**11**	**12**	**13**	**14**		
Anthropo-metrics	X											X			
Substrate Oxidation	X											X			
Muscle Biopsy			X										X		
Muscle Function		X											X		
VO_2 Max		X													

A: Pre-diet phase
B: Maintenance diet phase
C: Experimental diet phase

METHODS

1. DIETARY INTAKE

To assess "usual intake" subjects were instructed to record the type and amount of all foods, beverages (including snacks), and dietary supplements consumed on a dietary intake form for a four day time period (which included at least one weekend day). Subjects were encouraged to maintain their normal eating patterns as much as possible because it has been suggested that recording the amount of food consumed may affect the actual intake in an attempt to circumvent inconvenient or complex entries (Recker et al, 1992).

Food intake studies have suggested that women may ingest extra energy in the luteal phase of the menstrual cycle as compared to the follicular phase (Dalvit, 1982; Lyons et al, 1989). Thus, to maintain some consistency among women regarding the ingestion of food during different phases of the menstrual cycle, the female subjects were asked to record their food ingested for four days during the follicular phase of their menstrual cycle. The male subjects were simply asked to submit one 4-day diet record before the 10 day diet and exercise manipulation period.

The subjects were asked to give thorough descriptions, including brand names (Gibson, 1987), of all foods and beverages ingested. Measurement of the amount of food eaten was encouraged by using weights, standard household measuring cups, spoons, and rulers for foods such as meat and cake, and counts for other food like eggs and slices of bread (Gibson, 1987).

A copy of dietary record instructions provided to subjects is available in Appendix 8. The food intake record form (Appendix 9) was modified from the UBC Hospital, UBC Site, Dietary Department, Dietary History Food Intake Form and the food intake form developed for a previous clinical nutrition study (Mertz and Kelsay, 1984). For a small group, a 4-day food record has been shown to determine usual protein intake within 10%

with 95% confidence (Basiotis et al, 1987). A minimum of three days of food intake records are required for estimating usual energy intakes (Basiotis et al, 1987). Completed food records were reviewed with the subjects.

Portion size measures were analyzed for energy and nutrient composition using the computer program Food Processor II (enhanced version 3.14, ESHA Research, Salem OR). Use of the Canadian Nutrient File database allowed for a more accurate representation of the subjects food composition intake (ESHA Research, enhanced version 3.14, ESHA Research, Salem OR). Information regarding foods not available in Canada were obtained from the ESHA database. The Canadian Nutrient File has been shown to be deficient for certain nutrients that have been shown to be underestimated. The American (ESHA) database is substantially more thorough in this respect (Barr et al, 1994).

The average daily values were determined from the four day records. The intake of the following dietary components were calculated for foods ingested in this study: calories, carbohydrate, protein, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, cholesterol, dietary fibre, calcium, copper, iron, magnesium, potassium, phosphorus, zinc, sodium, pantothenic acid, thiamin (B_1), riboflavin (B_2), niacin (B_3), vitamin B_6, vitamin B_{12}, vitamin A, and vitamin C.

All dietary intakes were analyzed by one investigator to reduce variability in translation of intakes.

2. ANTHROPOMETRICS

A) WEIGHT

All subjects were weighed on a medical balance scale, which is accurate to the nearest 0.1 kg. The scale was calibrated to zero between subject weighings (Dikovics, 1987). All subjects wore minimal clothing (no shoes, shorts and t-shirts) for each measurement to minimize variability (Dikovics, 1987). Subjects were asked to stand in the center of the platform, with their arms at their side. All subjects were weighed in the

morning, in a fasted state. However, it was not always possible to weigh subjects before they had consumed fluids and/or emptied their bladder (Dikovics, 1987).

Body weight of all subjects was measured before the test period, daily during the test period, and after the test period. Resulting body weights were compared to ideal body weight according to Metropolitan Height and Weight Tables 1983 (Metropolitan Insurance Company; refer to Appendix 10).

B) HEIGHT

Height was measured using a stadiometer, which was read to the nearest 0.1 cm (Health Promotion Directorate, 1988). Height was measured twice throughout the duration of each study phase: at the beginning and at the end. For each measurement, heights were measured twice. If the heights were significantly inconsistent, a third measurement was made, and the two most similar heights averaged (Dikovics, 1987).

The measurement was done with the subject barefoot in the center of the platform with feet parallel and heels together. Their back was kept as straight as possible with their shoulders, buttocks, and heels in contact with the vertical surface. The arms were left hanging comfortably at their sides, and the head was kept erect, with the Frankfort plane horizontal (the line from the lower orbit to the upper external auditory meatus). The horizontal bar was then lowered at an angle of 90 degrees making contact with the head (Dikovics, 1987). See Appendix 11.

C) BODY MASS INDEX (BMI)

Body mass index was calculated from the averaged weight and height measurements using the following formula:

$$BMI = \frac{\text{Weight (kg)}}{\text{Height}^2 \text{ (m)}}$$

D) SKINFOLDS

All measurements were made with Lange calipers (Cambridge Scientific Industries Inc., Cambridge MD) to indirectly assess body fat. All precision calipers are designed to have a standard contact surface area of 20 to 40 millimeters squared and exert a defined and constant pressure of 10 grams per square millimeter throughout the range of measured skinfolds. Skinfold measurements were made in triplicate. Actual skinfold values were recorded to 0.5 mm. An average of the three values was used, and if one value varied by more than 10% of the others, it was disregarded and a fourth measure taken (Dikovics, 1987).

The specific location of each skinfold thickness measurement for male and female subjects was kept consistent to ensure reliable results (Dikovics, 1987; Gibson, 1987) by marking the site with a pen. For all measurements, the caliper was applied for three seconds before the reading was made. To eliminate inter-examiner error, all of the skinfold measurements were taken by the same examiner throughout the study. All measurements were made on the right side of the body (Lohman et al, 1988).

i) MALES

Skinfold measurements were taken at four sites (triceps, biceps, subscapular, suprailiac). Percent body fat was estimated from the sum of the four skinfolds using tables published by Durnin and Womersley (1974; refer to Appendix 12).

The triceps skinfold was taken 1 cm from the midpoint of the upper left arm, between the tip of the olecranon and the acromion process, with the arm hanging relaxed as seen in Appendix 11 (Lohman et al, 1988). A vertical pinch of skin and subcutaneous fat was pulled away using the thumb and index finger directed inferiorly. In order to make certain that no muscle was included in the skinfold, the subjects flexed their triceps.

For the measurement of the biceps skinfold thickness, a vertical fold was raised on the anterior aspect of the arm, over the belly of the biceps muscle. This was done 1 cm

superior to the line marked for the measurement of the triceps skinfold thickness on a vertical line joining the anterior border of the acromion and the center of the antecubital fossa. The subject stood facing the examiner, with the arms relaxed at the side, and the palm directed anteriorly. A vertical skinfold was then raised with the left hand and its thickness measured to the nearest 0.5 mm. The calipers were applied for three seconds before the reading was made, followed by the release of the calipers and of the fingers, and the immediate recording of the values (Dikovics, 1987; Lohman et al, 1987).

The suprailiac skinfold was measured in the midaxillary line superior to the iliac crest. The subjects were asked to stand in an erect position and to place their arms at their sides with their feet together. An oblique skinfold (aligned inferomedially at 45 degrees to the horizontal) was grasped just posteriorly to the midaxillary line following the natural cleavage lines of the skin. The caliper jaws were applied about 1 cm from the fingers holding the skinfold, and the thickness was recorded to the nearest 0.5 mm (refer to Appendix 11).

The measurement of the subscapular skinfold was taken at a locale that was just beneath the inferior angle of the scapula. The subscapular skinfold was grasped on a diagonal, inclined infero-laterally approximately 45 degrees to the horizontal plane in the natural cleavage lines of the skin. The subject was asked to stand erect, with the upper extremities relaxed at the sides of the body. To locate the site, the examiner palpated the scapula with her fingers along the vertebral border of the scapula until the inferior angle of the scapula was identified. For some subjects, the inferior angle was identified by gently placing the subject's arm behind the back. The caliper jaws were applied 1 cm infero-lateral to the thumb and finger raising the skinfold, and the thickness was recorded to the nearest 0.5 mm (refer to Appendix 11). Refer to Appendix 13 for data collection sheet used for the male subjects.

ii) FEMALES

Skinfold measurements were made at seven sites to utilize the female specific equation to determine body fat. Seven skinfold measurements were completed at the beginning and end of the dietary period for all subjects and included: abdominal, chest, axilla, triceps, subscapular, suprailiac, front thigh. The sites for the triceps, subscapular, and suprailiac skinfold measurements were the same as those described for male subjects.

The abdominal skinfold was taken one centimeter inferior to and three centimeters lateral to the midpoint of the umbilicus. All measures were made to the right of the umbilicus so that consistency could be sustained. For the measurement, subjects were encouraged to breathe normally and relax their abdominal muscles as much as possible. A horizontal skinfold was then taken with the left hand and its thickness measured to the nearest 0.5 mm.

The axilla skinfold was measured at the level of the xiphi-sternal junction, in the midaxillary line, with the skinfold horizontal. The subject was asked to stand erect and slightly abduct and flex the arm at the shoulder joint. The skinfold was taken horizontally with the left hand and measured to the nearest 0.5 mm (refer to Appendix 11).

The chest skinfold was measured using a skinfold with its long axis directed to the nipple. The thickness was measured 1 cm inferior to the anterior axillary fold. The measurement was made to the nearest 0.5 mm while the subject stood with her arms hanging relaxed at their side (refer to Appendix 11).

The thigh skinfold was measured in the midline of the anterior aspect of the thigh, midway between the inguinal crease and the proximal border of the patella. In order to locate the inguinal crease, the subject was asked to flex the hip. The subject then extended the leg so that the distal reference point, located on the proximal border of the patella, could be located. A vertical skinfold thickness was measured while the subject was in a standing position. For the measurement, the subject was asked to shift body weight to the other foot and to relax the leg on which the measurement was made while keeping the right knee

slightly flexed and the right foot flat on the floor. If maintaining balance was a problem, the subject was asked to hold on to a counter top. The caliper jaws were applied about 1 cm distal to the fingers holding the fold and the skinfold was measured and recorded to the nearest 0.5 mm (Lohman et al, 1987; refer to Appendix 11). Refer to Appendix 14 for the data collection sheet used for female subjects.

Total body fat was determined from skinfold measurements by first calculating body density (using a population specific regression equation), which was then used to determine percent body fat (using an empirical equation). Subsequently, total body fat was determined. Body density (BD) was calculated using a generalized regression equation derived for women varying in age and body composition (Jackson et al, 1980; see below). This equation showed that multiple correlations for body density equations and the sum of seven skinfolds was 0.852 with a standard error of 3.8% body fat (Jackson et al, 1980).

$$BD = 1.0907 - 0.00046971(X_1) + 0.00000056(X_1)^2 - 0.00012828(age)$$

where BD is in units of kg/m^3; X_1 = the sum of all seven skinfolds in millimeters (Jackson et al, 1980).

$$\% \text{ Body Fat } (\%BF) = (495/BD) - 450 \qquad \text{(Jackson et al, 1980)}$$

$$\text{Total body fat} = (\text{Body Weight (kg)} \times \%BF)/100 \qquad \text{(Gibson, 1990))}$$

The fat-free mass (FFM) for both male and female subjects before and after the study period was also determined:

$$\text{Fat-free mass (kg)} = \text{Body weight (kg)} - \text{Fat mass (kg)}$$

The FFM is the mass of the body excluding all extractable lipid (Mitchell and Truswell, 1987). Thus, FFM does not include the "essential" lipid component of the body.

3. EXERCISE INTERVENTION

A) EXERCISE PROTOCOL

i) MALES

After completion of baseline measurements, the male subjects participated in a 10 day moderate intensity endurance exercise regimen, in which all subjects exercised for 9-10 days. Exercise sessions were organized so that all subjects were able to participate for at least 9 days. However, if a subject was not able to make the exercise session, he was asked to make up for the missed session on his own time, and make the appropriate heart rate recordings.

The exercise protocol involved a 5 minute period of warm-up stretching led by the exercise supervisor, followed by a 25 minute run/walk at 60-80% of each individual's maximum heart rate (as determined by the VO_2 max test on a cycle ergometer). This was followed by a 5 minute cool down, consisting of walking and stretching.

Subjects were educated about their heart rate range and the recording of their heart rates throughout the exercise period. They were instructed to keep their heart rate in a certain range during the 20-25 minute run/walk for the dual purpose of ensuring that the subjects were exercising at a high enough intensity and to calculate the amount of energy expended during the exercise period. Heart rates were measured by the subjects themselves as called out by the exercise supervisor and recorded by the exercise supervisor every 5 minutes.

ii) FEMALES

The female subjects, who received no exercise intervention, were instructed to exercise during the 10 day testing period as they regularly would. The female subjects recorded all exercise sessions on an exercise recording form shown in Appendix 3. The time spent running and the intensity at which the subjects ran at was specified. For all other activities, subjects were instructed to specify the amount of time spent exercising, and the intenisty of the workout.

B) ENERGY EXPENDITURE DETERMINATION

i) MALES

Before starting the exercise intervention period, a VO_2 max test was done for male subjects. During this test, subjects cycled on an electronically braked cycle ergometer (Mijnhardt, Mode KEM-3, Bunnik, Holland, 1987) for 15-20 minutes at the Allan McGavin Sports Medicine Clinic at UBC, with a 5 minute warm-up, a 10-12 minute period of increasing work load, and a 5 minute cool-down. Heart rate was monitored every 15 seconds throughout the duration of this test.

Based on each subjects performance, an equation relating oxygen consumption (dependent variable) and heart rate (independent variable) was obtained, as the oxygen consumption and heart rate are directly proportional to one another (Sharkey, 1990). The regression equation provided a value for oxygen consumption for each 5 minute period. For equations of all subjects, refer to Appendix 15. The total oxygen consumption occurring during an exercise session was determined from the sum of all of the 5 minute oxygen consumption values. This value was then multiplied by the caloric equivalent of oxygen, which is assumed to be 5 kcal/L oxygen during exercise exceeding 70% of the aerobic capacity (Fox et al, 1993), to obtain the amount of energy expended during the exercise session (see equation below). It was assumed that a heart rate obtained at the end of each 5 minute period was a representative value for the entire 5 minutes of exercise.

Energy Expenditure (Kcal) = Oxygen consumed (L/30 min) x 5 Kcal/L oxygen

ii) FEMALES

The amount of energy expended by the female subjects was calculated using the Weight Loss Programmer (Version 3.1, Ohio Distinctive Software, Cleveland, Ohio, 1992). In order to calculate the amount of energy expended by running, the time spent running and the intensity of the workout was entered into the computer, and the program computed the energy expended. Determination of the energy expended for activites other than running was calculated in the same manner. However, two subjects forgot to specify the time spent exercising for activities other than running for two and three days respectively when recording their exercise time, and simply checked off the time period in which they were active. Thus, in order to calculate the energy expenditure from these subjects, the lowest time point in the range of time checked off was appointed as the time spent exercising. For example, if a subject checked off the 31-60 minute time range, then the exercise time entered into the computer was 31 minutes.

The time spent exercising was also computed from the exercise recording forms by addition of the time spent exercising for each different activity.

4. SUBSTRATE OXIDATION

Substrate oxidation was evaluated by determining the resting energy expenditure (REE), the resting respiratory quotient (RQ), and post-prandial RQs using indirect calorimetry. Carbon dioxide production (VCO_2), oxygen consumption (VO_2), REE, and RQ were determined by using the Sensormedics MMC Delta Trac metabolic cart (Summit Technologies, Anaheim, Ca) located in the Nutrition Laboratory, School of Family and Nutritional Sciences.

Subjects reported to the Family and Nutritional Sciences Building early in the morning. They were instructed not to consume any food or beverages or engage in any physical activity before the testing period. Upon arrival, they were asked to lay in a supine position for 30 minutes and rest, after which REE and RQ were measured.

Measurements were done in a quiet, dimly lit room at a constant temperature. While lying supine on a bed, subjects were asked to relax and close their eyes without sleeping. If the subject became cold, a blanket was available. Relaxation tapes were also available to the subjects to help them relax.

Resting energy expenditure and RQ were measured for 20-30 minutes (determined by the consistency of the values) at the beginning and the end of the experimental manipulation period for both male and female subjects. Values were considered consistent: when they did not change from one measurement to the next (the values were within 35 kcal/day of one another). The equipment that was used is summarized in Appendix 16. The subject's head was enclosed in a plastic canopy with a flexible seal at the neck. A canopy system is useful in preventing discomfort associated with nose clips and mouth pieces. The subject inhaled room air through an opening in the canopy. Exhaled air was drawn by a slight suction at a constant low rate to the metabolic monitor mixing chamber where it was analyzed for oxygen and carbon dioxide.

Resting energy expenditure and RQ were monitored for approximately two and a half hours after a test breakfast was ingested by the subject. The test breakfast provided 30% of the maintenance energy intake for all subjects pre-diet, and 30% of the daily energy intake of the respective diet group post-diet. The breakfast consisted of 12% protein, 60% carbohydrate, and 28% fat (refer to breakfast ingested on day two of the menu in Appendix 1).

Thirty minutes after the breakfast was consumed, the first five minute measurement was taken, after which a five minute measurement was made every thirty minutes four

more times. Results from McCargar et al (1989) suggest that this schedule of data collection was appropriate for the proposed REE and RQ in this experiment.

An attempt was made to mease the substrate oxidation measurements for the female subjects during the follicular phase of the menstrual cycle, as it has been shown that resting energy expenditure is elevated in the periovulatory phase of the menstrual cycle (Herring et al, 1992). The follicular phase of the menstrual cycle was defined as the 15 days following the initiation of menstruation (Fox et al, 1988). However, due to difficulties associated with scheduling subjects for testing times, substrate oxidation measurements were not made in the follicular phase for three subjects.

A) RESTING ENERGY EXPENDITURE

Resting energy expenditure (REE) is the energy necessary to maintain the physiological systems at rest (Van Zant, 1992).

The following equation was used to determine REE:

$REE = [(3.941 \times VO_2) + (1.106 \times VCO_2)] \times 1.44$ (Weir, 1949)

Where VO_2 and VCO_2 represent the volume with respect to the milliliters of oxygen inspired and carbon dioxide expired, respectively; 1.44 is the multiplier required to extrapolate the values over 24 hours.

REE may account for 60-75% of the daily energy expenditure and is closely correlated to the fat-free body mass (Van Zant, 1992). Therefore, in addition to presenting REE in terms of its absolute value, it was also expressed in terms of FFM and body weight. The thermic effect of food may also contribute to the resting energy expenditure as the thermic effect of food has been shown to last many hours (Mole', 1990).

B) RESPIRATORY QUOTIENT

The respiratory quotient (RQ) is equal to the volume of carbon dioxide produced divided by the volume of oxygen consumed (Hunt and Groff, 1990). The RQ can provide information regarding the type of fuel utilized for energy. For example, when carbohydrate

is being oxidized the RQ is 1.0. When fat is being oxidized, the RQ is 0.70 (Hunt and Groff, 1990). Determination of the RQ for protein oxidation is more complicated because metabolic oxidation of amino acids involves removal of the nitrogen, some oxygen, and carbon as urea. Thus, the RQ for protein varies according to the type of protein being oxidized. The RQ for a typical mixed diet is considered to be about 0.82 (Hunt and Groff, 1990).

A relatively accurate non-protein RQ was obtained even though urinary nitrogen losses were not measured due to the fact that the Delta Trac indirect calorimeter assumed that 13 grams of nitrogen were lost each day. Thus, although an exact value for individual nitrogen losses was not available, an attempt was made to account for oxidation of proteins.

5. MUSCLE FIBER ANALYSIS:

A muscle biopsy was taken at the beginning and end of each experiment. The site chosen for the biopsy was the vastus lateralis muscle because this muscle is used significantly during running. The skin around this area of the thigh was first sterilized, after which the skin and subcutaneous tissue was infiltrated with 7 to 10 ml of 2% lidocane without epinephrine. A 5 to 7 mm stab incision was made on the anterolateral surface of the thigh at the junction of its midal and distal thirds. This position was selected because it is least likely to result in significant complications (Kirby et al, 1982).

The biopsy needle was inserted perpendicular to the skin surface and advanced 2 to 5 cm into the vastus lateralis to the femur with the needle window closed facing posterolaterally. Withdrawing the inner cutting needle 1 to 2 cm opened the window. This procedure provided sufficient tissue to perform the required protein isolations and resulted in minimal complications (Kirby et al, 1982).

The muscle sample was then removed and blotted to remove surface blood after the needle was withdrawn. Adjacent fat and connective tissue was then dissected free and the remaining tissue weighed and frozen in pre-cooled isopentane stored in liquid nitrogen.

Pressure was applied to the wound until bleeding stopped. Sterile adhesive strips and an anchor dressing were then applied to the edges of the wound.

Male and female subjects had 2 muscle biopsies performed: one occurred prior to the testing period, and another occurred on day 9 or 10 of the testing period. After the muscle biopsy was taken, the calpain activity of the resulting muscle fibers was assessed.

In preparation for these assays, the muscle fibers were homogenized in a borate-KCl buffer (pH 7.0) containing 39 mM sodium borate, 25 mM KCl and 5 mM EGTA (Belcastro et al, 1991). The homogenate was then centrifuged at 13,000 rpm for 15 minutes (Hermle 360Z, rotor VO2805) and the supernatant, which represented the soluble particulate fraction, was transferred to an eppendorf tube and placed on ice.

The soluble and particulate fractions were then separated. This involved washing the pelleted material with 0.33% Triton-X 100 for removal of membrane bound proteins (while maintaining calcium-dependent proteolytic activity). The pellet was homogenized and centrifuged again under the same conditions as before. The supernatant, which represented the particulate bound fraction (membranous calpain), was placed on ice.

The samples were then prepared for the assay:

Table 1: Table of reagents to add in determining myofibrillar calpain activity.

Eppendorf Tube	Buffer 1 (ul)	Sample (ul)	Casein* (ul)	$CaCl_2$§ (ul)	DDH_2O (ul)
Blank	200	0	100	0	75
Soluble unactivated	0	200	100	0	75
Soluble activated	0	200	100	75	0
Bound unactivated	0	200	100	0	75
Bound activated	0	200	100	75	0

*Concentration of stock solution = 50 mM.
§Concentration of stock solution = 10 mg/ml.

As soon as the sample was added, the eppendorf tubes were vortexed and incubated at 30 degrees Celsius in a drybath for 30 minutes.

After 30 minutes of incubation, the protein assay was performed. This involved putting 100 ul of sample solution in the appropriate wells on the microplate, after which the sample solutions and the blank were read in the microplate reader. Each sample/fraction was done in triplicate.

6. MUSCLE FUNCTION ASSESSMENT

Muscle function assessment was accomplished by determination of quadriceps muscular strength. This was done with four knee extensions and flexions using the Kin-Com isokinetic dynamometer (Kin-Com: computerized exercise, testing, and research

system, Model 5030, Med-ex Diagnostics of Canada Inc., Port Coquitlam, B.C., 1985). This method has previously demonstrated a high correlation between strength (determined on the isokinetic dynamometer) and athletic performance. For example, a correlation coefficient of 0.87 was found between peak total leg strength per unit body weight and the 100-yd sprint times of a women's track team (Komi, 1986).

Strength is frequently defined as the peak force or torque developed during a maximal voluntary contraction (Alexander, 1990). Isokinetic dynamometers measure the muscle moment, which is the muscle force application times the length of the radius of the lever arm from the axis to the line of muscle pull, which is usually labelled the "muscle torque". Thus, torque scores are actually the moments of force produced by muscles contracting to produce rotation around a joint (Alexander, 1990). In order to ensure that the radius of the lever from the axis to the line of muscle pull was consistent between subjects, the bottom of the calf pad of the lever arm was placed at a distance that was 75% of the distance between the head of the fibula and the lateral malleolus for each subject. In order to calculate torque, the distance between the point of application of the generated force and the axis of rotation of the exercise arm was entered into the computer (which quantified the amount of force exerted on the calf pad of the Kin-Com).

Recruitment of a homogenous sample of male and female subjects suggested that the neural recruitment required for peak strength generation did not differ significantly between subjects of each gender. Furthermore, the fact that the female athletes were endurance athletes and the male subjects had not been involved in regular exercise for the previous two years suggests that the energy pathways required to reach peak strength (phosphocreatine, glycolytic) using the Kin-Com are not compromised. In the female athletes, these pathways were replete because they are minor pathways used in the type of training in which the female subjects were involved (Fox, 1984) and therefore are unlikely to be a confounding variable. The energy stores were also replete in the male subject group due to their inactivity pre-treatment, and utilization of aerobic training during the study.

Thus, the effect of an imposed energy restriction on strength production measured with the Kin-Com was not limited by neural recruitment or inadequate energy production, but by the processes involved in excitation-contraction coupling. Therefore, the ultimate purpose of determining strength using the Kin-Com isokinetic dynamometer involved investigation of the effect of changes in muscle structure (as a result of the imposed energy restriction) on muscle strength.

The Kin-Com measures strength in both a concentric and eccentric mode, which is useful in that both of these modes are used in running. The torque obtained from the concentric contractions was obtained from the upward phase of the lever arm, where the quadriceps muscle group shortened (the net muscle moment was in the same direction as the change in joint angle, and mechanical work was positive; Komi, 1986). The eccentric torque value was obtained from the downward phase of the lever arm (where the subjects were resisting the movement of the lever arm as it moved down) and involved a lengthening contraction of the quadriceps muscle group (net muscle moment was in the opposite direction to the change in joint angle, and mechanical work was negative; Komi, 1986).

It is in the eccentric mode that the force and power capacities of the skeletal muscle are greatest (Komi, 1986). Also, to attain a certain force level requires much less motor unit activation in eccentric than concentric contraction (Komi, 1986). Additionally, oxygen consumption is much lower during eccentric exercise than in comparable concentric exercise (Komi, 1986).

Peak and average torque value for each contraction type were used to describe muscle strength. Peak torque is the highest torque value obtained throughout the range of motion (65 degrees in this case), while average torque is the torque values averaged throughout the range of motion. Although both the peak and average torque values represent a measure of strength, peak torque attained in a single, high speed, velocity-

specific test is often used as a relative index of maximum power and strength (Komi, 1986).

The functional capacity of the quadriceps muscles in the right leg were determined by a series of maximal knee extension and flexion movements with the subject sitting upright in the attached chair and the trunk stabilized by supporting straps. Muscle function was tested for both male and female subjects at the beginning, and on day 9 or 10 of the experimental testing period.

Before every test session, the Kin-Com load cell was calibrated. To ensure gravity-compensated torque values for the data analysis, the right leg was weighed by the Kin-Com force transducer with the exercise arm positioned 15 degrees from the horizontal plane. This allowed the computer to adjust all subsequent force measurements for the calculated effect of gravity at each angle in the test range.

The axis of the right knee was aligned with the axis of the Kin-Com exercise arm. Accuracy of this alignment was checked by allowing the subject to extend the leg while pushing against the shin pad which was positioned over the lower third of the leg.

The peak and average torques generated for isotonic contractions and angular velocities of 30, 90, 120, and 180 degrees/second were compared across all conditions. Velocities of 30 and 180 degrees/second were chosen specifically because these speeds are most often selected in other studies to measure peak torque as an indication of maximal strength, especially during the concentric phase of contraction (Alexander, 1990). This range of joint angular velocities also ensured a wide range of muscular contraction velocities and therefore a wide range of fiber recruitment. The data collection form can be seen in Appendix 17.

The subjects performed four consecutive extension and flexion movements with maximal intensity at each movement velocity with encouragement from the investigator. This was preceded by 4 practice submaximal extension-flexion movements at the specified movement velocity so that subjects were able to familiarize themselves with the equipment.

The order of movement velocities tested was randomized for each subject by having the subjects choose a movement velocity (written on a piece of paper) out of a jar. This ensured that there was no subject or investigator bias on the results of the test. The mean of the final three trials was used for statistical analysis because the first trial at each movement velocity has a variable torque value, as a result of the subject becoming accustomed to that movement velocity (Alexander, 1990).

For the actual test, each subject was told to grasp the side of the table, lean against the backrest, and pull their right leg down against the calf pad as hard as possible, during the four flexion-extension cycles. During each test, frequent verbal encouragement was given.

Concentric and eccentric torque scores were then compared to literature values. The concentric peak torques obtained at movement velocities of 30 and 180 degrees/second were given special attention as these indices are most often reported in other studies (Alexander, 1990).

Frequently, torque values are divided by lean body mass, muscle mass, or fat-free mass (FFM) for the purpose of expressing strength values in relation to muscle mass. Muscle mass has been shown to be a determinant of muscle strength (Pronk et al, 1992). Therefore, in order to correct for muscle mass, the torque values were divided by the FFM.

STATISTICAL ANALYSIS OF THE DATA

To determine if nutrition and exercise intervention in male subjects and to determine if nutrition intervention in female subjects had a significant impact on muscle protein degradation and the quadriceps muscle group function, the following statistical analyses were performed using BMDP 9D statistical program (BMDP Statistical Software, PC 90, Los Angeles, CA, 1994). For the male subjects, baseline and post-study parameters for the anthropometry, REE, RQ, calpain activity, and muscle function parameters were analyzed by performing the Hotelling t-squared statistical test. If there was a difference between the parameters, they were then compared with a correlated t-test.

Similarly, the baseline and post-study parameters for the same variables as listed above were analyzed for the female subjects by performing the Hotelling t-squared statistical test. If there was a difference between parameters, they were then compared with a (2 x 2) (group x time) repeated measures ANOVA.

Correlation analyses were performed between: substrate oxidation data and calpain activity: calpain activity and muscle function data. Correlations were calculated with the pre- and post-diet data, as well as the change in each parameter.

Multiple stepwise regression was performed between the substrate oxidation data and calpain activity for pre- and post-diet data as well as the change in both variables. This was performed to determine: if calpain activity was determined by substrate oxidation parameters; and which substrate oxidation parameters were most important in determining calpain activity.

Simple linear regression was performed on the muscle function data (dependent variables) and calpain activity (independent variable) for pre- and post-diet data as well as the change in both variables. This was done to determine: the extent to which muscle function was determined by calpain activity; which muscle function parameters were strongly determined by calpain activity. Simple linear regression was used to determine

relationships between muscle function and calpain activity because there was one independent variable and several dependent variables.

Comparisons were considered significant at a probability of $p < 0.05$. All results are presented as mean ± SEM.

SIGNIFICANCE OF THE STUDY

Because there is an increasing concern about body weight among Canadians, more information associated with the functional implications of a combined weight reduction and fitness program is required. Furthermore, knowledge regarding the effect of a low calorie intake in an athletic population, a practice which is commonly reported, may provide useful information for optimization of muscle performance. Thus, an energy restriction may have implications for performance capability of individuals (both sedentary and athletically trained) involved in various types of physical activity.

Reports in the literature have indicated that an energy restriction results in an increase in fat oxidation. In turn, this was found to be associated with an increase in muscle proteolysis. A mechanism linking the above finding was speculated to include calpain because of the changes in the Z-line area of the myofibril (proteins in this area are substrates for calpain) occurring when fat oxidation is increased as a result of fasting and prolonged exercise. Disruption of sarcomere structure may result in a failure of force transmission, subsequently resulting in a decrease in muscle function.

This study ascertained if there were links between substrate oxidation, calpain activity, and muscle function. Specifically, the relationship between substrate oxidation and calpain activity was assessed, to evaluate if substrate oxidation determined calpain activity. Also examined was the relationship between calpain activity and muscle function to evaluate if calpain activity determined muscle function.

HUMAN STUDIES

This study received ethical approval from the University of British Columbia Clinical Screening Committee for Research and Other Studies Involving Human Subjects. See Appendix 18 for a copy of the acceptance form.

CHAPTER IV

RESULTS

1. SUBJECTS

Eight men and fourteen women completed the study representing an attrition of 11% and 0% respectively. The ethnic profile of the male subjects who completed the study was Caucasian. The ethnic profile of the female subjects were Caucasian except one subject of mixed heritage (Chinese/Caucasian).

A. MALES

Table 2 displays the male subject characteristics. All of the subjects were university students, and only one of the subjects was employed part-time. The types of activities that the males had previously participated in included: soccer (n=1), hockey (n=6), weight lifting (n=4), skiing (n=2), and running (n=1). The previous activities in which the male subjects were involved in are listed in Appendix 19. All of the males had been sedentary for the past two years as none of them were involved in more than 1 hour of activity per week. Their lack of activity was evident by their VO_2 max value (39.9 ± 2.9 ml/min/kg), which, for men, is not indicative of a trained person (Canadian Standardized Test of Fitness Operations Manual, 1986).

The male subjects had body weights on average 20.0% higher than ideal body weight (refer to Appendix 20 for equation used for calculation of ideal body weight). The pre-study body weights ranged from 67.2 - 104.8 kg, of which 2 subjects were within the ideal body weight range for their height, and 6 subjects had weights which were larger than the ideal body weight for height . The mean Body Mass Index (BMI: 27.1 ± 1.4 kg/m^2)

was outside of the healthy limit of 18 to 25 kg/m^2 (Hunt and Groff, 1990). The pre-study BMI values ranged from 22.7 - 32.9 kg/m^2, where 2 of the subjects had BMI values within healthy limits, and 6 had BMI values that were larger than 25 kg/m^2.

The percent body fat (19.9 ± 2.1%), which corresponded to a fat mass of 17.8 ± 3.7 kg, was within the desired subject criteria range of 10-20%. The pre-study percent body fat ranged from 12.9 - 28.2 % of which 4 subjects had body fat values that were outside of this range.

Table 2: Baseline characteristics of male subjects.

Variable	**Pre-diet (n = 8)**
Age (years)	22 ± 1**
Height (cm)	175.9 ± 1.1
Weight (kg)	84.2 ± 4.9
Body Mass Index (kg/m^2)	27.1 ± 1.3
Triceps Skinfold (mm)	11.2 ± 3.5
Subscapular Skinfold (mm)	15.8 ± 3.7
Suprailiac Skinfold (mm)	18.1 ± 5.8
Biceps Skinfold (mm)	7.6 ± 1.8
Sum of Skinfolds (mm)	59.2 ± 13.3
Body Fat (%)	19.9 ± 2.1
Total Body fat (kg)	17.8 ± 3.7
Fat Free Mass (kg)	68.5 ± 4.6
VO_2 Max (ml/min/kg)	39.9 ± 2.9

**Mean ± SEM.

B. FEMALES

Tables 3 displays the subject characteristics of all of the women as a group, and the women in each diet group at baseline. None of the female subjects were vegetarian. All of the female subjects had regular menstrual cycles and/or were taking oral contraceptives.

Twelve of the female participants were university students. Six of the female subjects held part-time jobs, while one subject had the full time occupation of running her own business. One subject was unemployed throughout the duration of the study.

Some of the subjects finished the study on day 9 of the study due to the timing of the measurements of the post-diet variables. The sample size on days 10 and 11 of the study was 6 and 3 respectively.

The type of exercise the women participated in consisted of: running (n=14); biking (n=13); swimming (n=9); weight lifting (n=5); walking (n=2); field hockey (n=1); aerobics (n=3); downhill skiing (n=1); karate (n=1); lacrosse (n=1); rowing (n=2) (refer to Appendix 22 for a description of the activities in which the female subjects were involved). All of the women were involved in at least 3 different types of exercise throughout the duration of the study. All of the women had previously been involved in running, and had been exercising regularly and/or competitively for the last 9.4 ± 1.0 years.

Evidence that these athletes were of the same caliber is shown by the VO_2 max test results, where the 100% diet group (VO_2 max = 49.4 ± 1.1 ml/min/kg) had a similar VO_2 max value to the 75% diet group (VO_2 max = 48.5 ± 1.4 ml/min/kg) at the initiation of the study. Note that the average VO_2 max value of 49.0 ± 0.9 for all subjects is considered to be an excellent value for women between the ages of 20-29 years based on (Canadian Standardized Test of Fitness Operations Manual, 1987), and therefore indicated that the women were well-trained.

Table 4 displays the baseline anthropometric measurements of the female subjects. Overall, the female subjects were well matched, as the two study groups did not differ

significantly with respect to all of the characteristics listed in Table 3 or 4. The average body weight was within the desirable weight range (assuming a medium frame size; Metropolitan Height and Weight Tables, 1983). The pre-study body weights ranged from 51.2 - 75.6 kg, where 3 subjects had weights that were lower than their desirable weight, and 11 subjects had weights that were within the range of their desirable weight ranges.

The female subjects had a mean BMI (22.1 ± 0.6 kg/m^2)which was within the range set for subject inclusion. All female subjects except one, met the inclusion criteria of having a BMI within the healthy limits of 18 to 25 kg/m^2 to participate in the study. The pre-study BMI values ranged from 18.4 - 25.6 kg/m^2, where one subject had a BMI of 25.6 kg/m^2. Because this value closely approached the healthy BMI range, she was accepted into the study.

Table 3: Baseline characteristics of the female athletes according to the dietary subgroup.

Variable	All (n = 14)	100% diet (n = 7)	75% diet (n = 7)
age (years)	27 ± 2	27 ± 2	27 ± 4**
height (cm)	167.2 ± 1.6	164.5 ± 2.2	170 ± 1.7
weight (kg)	61.8 ± 1.9	58.5 ± 2.4	65.1 ± 2.4
BMI (kg/m^2)	22.1 ± 0.6	21.7 ± 0.9	22.6 ± 0.8
VO_2 max (ml/min/kg)	49.0 ± 0.9	49.4 ± 1.1	48.5 ± 1.4
years spent exercising	9.4 ± 1.0	10.0 ± 1.9	8.9 ± 0.9

**Mean ± SEM.
No significant differences between diet groups.
Comparisons made by student's unpaired t-tests.

The average percent body fat for all of the subjects (20.0 ± 1.3 %), which corresponded to a fat mass of 12.6 ± 1.0 kg, fell within the desired subject criteria range of 16-26%. The pre-study percent body fat ranged from 12.6 - 26.9 %, of which 3 subjects had a percent body fat that was lower than 16% and 11 subjects had a percent body fat that was within the range of 16-26%.

Table 4: Baseline anthropometric measurements of female athletes according to the dietary subgroup.

Variable	All, Pre-diet (n=14)	100% Diet (n=7)	75% Diet (n=7)
Triceps Skinfold (mm)	13.7 ± 1.3	11.6 ± 1.7	16.1 ± 1.5**
Subscapular Skinfold (mm)	14.7 ± 1.7	13.7 ± 2.7	15.8 ± 2.2
Suprailiac Skinfold (mm)	17.1 ± 2.1	114.2 ± 3.1	20.4 ± 2.1
Abdominal Skinfold (mm)	18.0 ± 1.4	15.7 ± 2.0	20.6 ± 1.5
Axilla Skinfold (mm)	113.6 ± 1.6	12.4 ± 2.8	15.0 ± 1.5
Chest Skinfold (mm)	6.2 ± 0.9	4.9 ± 1.1	7.8 ± 1.2
Thigh Skinfold (mm)	15.4 ± 1.1	14.2 ± 1.3	16.8 ± 2.0
Sum of Skinfolds (mm)	97.2 ± 7.5	86.6 ± 12.6	107.8 ± 6.8
Body Density (kg/m^3)	1.055 ± 0.003	1.060 ± 0.010	1.050 ± 0.002
Body Fat (%)	20.0 ± 1.3	18.2 ± 2.2	21.8 ± 1.2
Total Body Fat (kg)	12.6 ± 1.0	10.9 ± 1.6	14.2 ± 1.1
Fat-free Mass (kg)	49.3 ± 1.3	47.7 ± 1.7	50.9 ± 1.8

**Mean ± SEM.
No significant differences between diet groups.
Comparisons made by student's unpaired t-tests.

2. DIETARY INTAKE:

A. MALES

Table 5 shows the males' mean nutrient intakes based on selected nutrients for the four days of diet records. The average energy intake per day (4321 ± 1160 kcal) supplied 175% of the calories recommended for a sedentary 22 year old male of height and weight of 176 cm and 84 kg respectively (averages for male subjects in this study; Food Processor II Nutrient Analysis Software, ESHA Research, Salem, OR). Dietary carbohydrate, protein and fat (as a percent of total energy) were respectively 60.7 ± 4.7%, 14.3 ± 2.4%, and 25.3 ± 2.9. Data was not obtained from one subject as he failed to return the four-day diet record.

Of the 27 nutrients analyzed for which Recommended Nutrient Intakes (RNI) were available, the males exceeded the RNI for all of the nutrients which included: total vitamin A, thiamin, riboflavin, niacin, vitamin B_6, vitamin B_{12}, folacin, pantothenic acid, vitamin C, vitamin E, calcium, copper, iron, magnesium, potassium, phosphorus, sodium, selenium, and zinc. The subjects ingested at least double the RNI of the following nutrients: thiamin, riboflavin, niacin, vitamin B_{12}, folacin, vitamin C, iron, potassium, phosphorus, sodium, and selenium. However, use of the Canadian database for assessing the micronutrient intake may be inappropriate due to the large percentage of missing values for these nutrients in the Canadian database (Barr et al, 1994). Therefore, assessment of micronutrient intake through the Canadian database should be approached with caution.

Table 5: Mean nutrient intakes of male subjects for four days*.

Daily Dietary Intakes	Mean ± SEM (n = 6)
Calories (kcal)	4321 ± 116**
Protein (g)	147.3 ± 33.2
Carbohydrates (g)	665.7 ± 221.7
Fiber (g)	29.6 ± 6.8
Total Fat (g)	115.0 ± 21.63
Saturated Fat (g)	39.8 ± 9.8
Monounsaturated Fat (g)	40.4 ± 9.2
Polyunsaturated Fat (g)	27.6 ± 3.9
Cholesterol (mg)	325.0 ± 72.5
Vitamin A - Carotene (RE)	450.0 ± 187.4
Vitamin A - Preformed (RE)	687.7 ± 43.9
Vitamin A - Total (RE)	1137.7 ± 164.3
Thiamin (mg)	4.45 ± 1.31
Riboflavin (mg)	4.10 ± 1.19
Niacin (mg)	45.1 ± 10.9
Vitamin B_6 (ug)	3.02 ± 0.37
Vitamin B_{12} (ug)	7.20 ± 2.71
Folacin (ug)	531 ± 141
Pantothenic Acid (mg)	8.27 ± 1.44
Vitamin C (mg)	183 ± 82
Vitamin E (mg)	15.77 ± 2.69
Calcium (mg)	1841 ± 466
Copper (mg)	2.50 ± 0.58
Iron (mg)	28.3 ± 9.2
Magnesium (mg)	506 ± 104
Potassium (mg)	4971 ± 598
Phosphorus (mg)	2427 ± 600
Sodium (mg)	6680 ± 1746
Selenium (ug)	235.7 ± 75.4
Zinc (mg)	18.93 ± 5.21
Protein (%)	14.3 ± 2.4
Carbohydrates (%)	60.7 ± 4.7
Fat (%)	25.3 ± 2.9
Polyunsaturated: saturated fat	0.73 ± 0.23
Sodium:Potassium	1.30 ± 0.21
Calcium:Phosphorus	0.73 ± 0.09

*Determined by Food Processor II Nutrient Analysis Software (ESHA Research, Salem, OR) prior to the study.
**Mean ± SEM.

B. FEMALES

Table 6 shows the females' mean nutrient intakes based on selected nutrients for the four day diet records. The average caloric intake per day was 2052 ± 153 kcal which is 17% lower than that recommended for a very active 27 year old female of height and weight of 167 cm and 62 kg respectively (averages found for females in this study; Food Processor II Nutrient Analysis Software, ESHA Research, Salem, OR). The 100% diet group satisfied 77 ± 10% of the caloric requirement, while the 75% group satisfied 90 ± 7% of the caloric requirement at baseline.

Dietary carbohydrate, protein, and fat (as a percent of total energy) were respectively 54.6 ± 3.7 %, 14.6 ± 1.1%, and 30.8 ± 4.2% respectively. The two diet groups ingested similar proportions of macronutrients in their diets. The only significant difference for the nutrient intakes between the 75% and 100% diet group was found with total vitamin A intake, where the 100% diet group had a larger total vitamin A intake than the 75% diet group ($p < 0.01$).

Of the 27 nutrients analyzed for which Recommended Nutrient Intakes (RNI) were available, the female subjects as a whole exceeded the RNI for 23 of the nutrients: total vitamin A, thiamin, riboflavin, niacin, vitamin B_6, vitamin B_{12}, folacin, vitamin C, vitamin E, calcium, copper, iron, magnesium, phosphorus, sodium, potassium, selenium, and zinc. They had lower than recommended intakes for pantothenic acid and copper. Of these nutrients, the 100% diet group had lower intakes for all of these nutrients, while the 75% diet group had lower than recommended intakes for all nutrients in addition to the monounsaturated fat intake. Both diet groups ingested more than the recommended intakes by a large margin for vitamin C, potassium, and sodium. As mentioned before, assessment of micronutrient intake through the Canadian database should be approached with caution.

Table 6: Mean nutrient intakes of the female athletes for four days, according to the dietary subgroup.*

Daily Dietary Intakes	All Subjects (n = 14)	100% Diet Group (n = 7)	75% Diet Group (n = 7)
Calories (kcal)	2052 ± 153	1928 ± 253	2209 ± 140
Protein (g)	72.2 ± 5.4	68.8 ± 7.7	76.4 ± 16.2
Carbohydrates (g)	284.2 ± 19.76	260.2 ± 29.6	314.2 ± 18.50
Fiber (g)	23.7 ± 2.5	21.0 ± 2.5	27.0 ± 4.6
Total Fat (g)	73.5 ± 10.4	70.3 ± 17.8	77.5 ± 10.4
Saturated Fat (g)	25.5 ± 4.2	23.9 ± 17.8	27.5 ± 4.7
Monounsaturated Fat (g)	28.0 ± 4.1	29.0 ± 6.9	26.8 ± 4.8
Polyunsaturated Fat (g)	18.2 ± 2.1	15.6 ± 3.4	21.5 ± 4.7
Cholesterol (mg)	196.3 ± 45.92	221.4 ± 78.9	165.0 ± 40.9
Vitamin A - Carotene (RE)	516.8 ± 121.8	630.7 ± 201.8	374.3 ± 94.3
Vitamin A - Preformed (RE)	387.6 ± 56.1	390.6 ± 75.9	383.8 ± 96.3
Vitamin A - Total (RE)	904.1 ± 92.3[a]	1021.2 ± 151.5	757.8 ± 6.0
Thiamin (mg)	1.68 ± 0.14	1.53 ± 0.22	1.87 ± 0.15
Riboflavin (mg)	1.79 ± 0.11	1.70 ± 0.16	1.90 ± 0.13
Niacin (mg)	18.6 ± 1.8	19.0 ± 2.6	18.2 ± 2.8
Vitamin B_6 (ug)	1.74 ± 0.26	1.78 ± 0.30	1.68 ± 0.49
Vitamin B_{12} (ug)	2.82 ± 0.52	3.11 ± 0.83	2.45 ± 0.62
Folacin (ug)	296 ± 38	259 ± 48	342 ± 59
Pantothenic Acid (mg)	4.78 ± 0.47	4.52 ± 0.43	5.10 ± 0.97
Vitamin C (mg)	161 ± 28	174 ± 36	144 ± 50
Vitamin E (mg)	11.92 ± 1.53	9.84 ± 1.86	14.53 ± 2.03
Calcium (mg)	897 ± 64	810 ± 100	1004 ± 33
Copper (mg)	1.86 ± 0.21	1.54 ± 0.20	2.27 ± 0.32

Iron (mg)	16.1 ± 1.6	14.2 ± 1.94	18.5 ± 2.5
Magnesium (mg)	348 ± 42	302 ± 44	404 ± 74
Phosphorus (mg)	1326 ± 127	1188 ± 132	1498 ± 223
Sodium (mg)	2240 ± 273	2330 ± 454	2127 ± 312
Potassium (mg)	3050 ± 340	2840 ± 421	3313 ± 598
Selenium (ug)	121.0 ± 13.7	106.3 ± 14.6	139.3 ± 23.9
Zinc (mg)	9.55 ± 0.77	8.83 ± 1.08	10.45 ± 1.00
Protein (%)	14.2 ± 0.7	14.6 ± 1.1	13.8 ± 1.1
Carbohydrates (%)`	55.2 ± 2.2	54.6 ± 3.7	56.0 ± 2.5
Fat (%)	30.8 ± 2.5	30.8 ± 4.2	30.8 ± 4.7
Polyunsaturated: saturated fat	0.73 ± 0.13	0.74 ± 0.12	0.73 ± 0.27
Sodium:Potassium	0.85 ± 0.15	0.92 ± 0.21	0.75 ± 0.21
Calcium:Phosphorus	0.69 ± 0.04	0.66 ± 0.02	0.73 ± 0.09

*Determined by Food Processor II Nutrient Analysis Software (ESHA Research, Salem, OR) prior to the study.
**Mean ± SEM.
[a]Significant difference between 75% and 100% diet group, $p < 0.01$.
Comparison's made by student's unpaired t-test.

3. ANTHROPOMETRY

A. MALES

Anthropometric measures of male subjects pre and post-treatment are shown in Table 7. The total amount of weight lost within the experimental diet period was 1.5 ± 0.3 kg ($p < 0.01$). Thus, the percent weight lost was 1.7 ± 0.3 %. Also, BMI decreased by 0.5 ± 0.1 ($p < 0.01$) as a result of the weight change.

Although skinfold measures at each specific site did not change independently, the sum of the four skinfolds resulted in a 5.2 ± 1.9 mm decrease ($p < 0.05$) which corresponded to a decrease in body fat (-1.4 ± 0.2 kg, $p < 0.01$; -1.3 ± 0.4%, $p < 0.05$).

Table 7: Anthropometric measures of male subjects before and after the dietary and exercise treatment.

Variable	Pre-treatment (n = 8)	Post-treatment (n = 8)	Change
Weight (kg)	84.2 ± 4.9	82.7 ± 4.6	1.5 ± 1.3**,a
Body Mass Index (kg/m^2)	27.1 ± 1.3	26.6 ± 1.3	-0.5 ± 0.1[a]
Triceps Skinfold (mm)	11.2 ± 3.5	10.0 ± 2.2	-1.2 ± 2.3
Subscapular Skinfold (mm)	15.8 ± 3.7	14.6 ± 2.4	-1.2 ± 3.6
Suprailiac Skinfold (mm)	18.1 ± 5.8	21.1 ± 8.3	3.0 ± 4.4
Biceps Skinfold (mm)	7.6 ± 1.8	7.8 ± 2.3	0.2 ± 1.8
Sum of Skinfolds (mm)	59.2 ± 13.3	54.0 ± 12.9	-5.2 ± 1.9[b]
Body Fat (%)	19.9 ± 2.7	18.7 ± 2.9	-1.3 ± 0.4[b]
Total Body fat (kg) (TBF)	17.8 ± 3.7	16.5 ± 3.7	-1.4 ± 0.2[a]
Fat-free mass (kg) (FFM)	66.4 ± 3.9	66.2 ± 3.1	-0.1 ± 0.6

**Mean ± SEM.

[a] Significant difference between pre- and post-treatment, $p < 0.01$.

[b] Significant difference between pre- and post-treatment, $p < 0.05$.

Comparisons made with correlated t-tests.

B. FEMALES

Anthropometric measures of female subjects, pre- and post-diet, are shown in Table 8. The body weight in the subjects of the 100% diet group did not change, while body weight decreased (-1.7 ± 0.3 kg; $p < 0.001$) in the subjects of the 75% diet group. This corresponded to a 2.6 ± 0.4% weight loss in the 75% diet group. Furthermore, BMI decreased by 0.7 ± 0.03 kg/m^2 ($p < 0.01$) in the 75% diet group as a result of the weight change.

There was a decrease in the sum of skinfolds (-15.7 ± 2.4 mm; $p < 0.01$) in the 75% diet group which corresponded to a decrease in body fat (-2.3 ± 0.4 kg, $p < 0.01$; -2.9 ± 0.6 %, $p < 0.05$). These changes were due to decreases in the following skinfold measures ($p < 0.05$): triceps, suprailiac, axilla, and chest. No such differences in body fat, sum of skinfolds, or individual skinfold measures were found for subjects in the 100% diet group.

Table 8: Anthropometric measures of female athletes according to the dietary subgroup before and after the respective diet period.

Variable	100% Diet (n = 7)			75% Diet (n = 7)		
	Pre-diet	Post-diet	Change	Pre-diet	Post-diet	Change
Weight (kg)	58.5 ± 2.4	58.5 ± 2.4	-0.04 ± 0.03	65.1 ± 2.4	63.4 ± 2.2[a]	-1.7 ± 0.3**, [b]
Body Mass Index (kg/m^2)	21.7 ± 0.9	21.7 ± 0.9	-0.04 ± 0.03	22.6 ± 0.8	21.9 ± 0.8[a]	-0.7 ± 0.03[b]
Triceps Skinfold (mm)	11.6 ± 1.7	13.2 ± 1.7	1.6 ± 0.7	16.1 ± 1.5	14.8 ± 0.8	-1.1 ± 0.9[c]
Subscap-ular Skin-fold (mm)	13.7 ± 2.7	13.6 ± 2.6	-0.1 ± 0.6	15.8 ± 2.2	13.4 ± 1.6	-1.6 ± 2.2
Suprailiac Skinfold (mm)	14.2 ± 3.1	14.0 ± 2.5	-0.1 ± 1.0	20.4 ± 2.1	14.6 ± 2.3[a]	-4.4 ± 1.4[c]
Abdominal Skinfold (mm)	15.7 ± 2.0	16.3 ± 2.3	0.6 ± 1.2	20.6 ± 1.5	16.1 ± 0.6	-4.8 ± 2.2[c]
Axilla Skinfold (mm)	12.4 ± 2.8	12.4 ± 2.3	-0.3 ± 1.0	15.0 ± 1.5	12.3 ± 1.2[a]	-2.1 ± 0.7
Chest Skinfold (mm)	4.9 ± 1.1	4.3 ± 0.4	-0.6 ± 1.2	7.8 ± 1.2	4.8 ± 0.5[a]	-3.0 ± 0.9
Thigh Skinfold (mm)	14.2 ± 1.3	14.4 ± 1.0	0.3 ± 1.2	16.8 ± 2.0	16.1 ± 1	-0.8 ± 1.3
Sum of Skinfold (mm)	86.6 ± 12.6	88.2 ± 11.2	1.6 ± 3.9	107.8 ± 6.8	92.1 ± 4.8[d]	-15.7 ± 2.4[e]
Body Density (kg/m^3)	1.060 ± 0.010	1.060 ± 0.004	0.0001 ± 0.001	1.050 ± 0.002	1.060 ± 0.002[d]	0.007 ± 0.001[e]
Body Fat (%)	18.2 ± 2.2	18.1 ± 1.9	-0.1 ± 0.8	21.8 ± 1.2	18.9 ± 0.9[a]	-2.9± 0.6[c]
Total Body Fat (kg)	10.9 ± 1.6	10.7 ± 1.4	-0.2 ± 0.5	14.2 ± 1.1	12.0 ± 0.9[d]	-2.3 ± 0.4[e]
Fat-Free Mass (kg)	47.7 ± 1.7	47.7 ± 1.6	0.05 ± 0.5	50.9 ± 1.8	51.3 ± 1.6	0.5 ± 0.5

**Mean ± SEM.

[a]significant difference within group, pre- versus post-diet, $p<0.05$·

[b]significant difference between the change in the 100% group versus the change in the 75% group, $p < 0.001$.
[c]significant difference between the change in the 100% group versus the change in the 75% group, $p < 0.05$.
[d]significant difference within group, pre- versus post-diet , $p<0.01$.
[e]significant difference between the change in the 100% group versus the change in the 75% group, $p < 0.01$.
Comparisons made by a 2 x 2 RM ANOVA for each variable.

4. EXERCISE DATA:

A. MALES

Subjects exercised (ie jogging) for a 30 minute session each day throughout the study period. The average energy expended per session is shown in Table 9. The highest energy expended occurred on day 1 of the study (314 ± 27 kcal/day) while the lowest occurred on day 8 of the study (253 ± 11 kcal/day). Exercise data for one subject was not obtained as he failed to record his heart rate values.

In order to evaluate whether or not there was a decrease in energy expenditure with the consecutive days of exercise a trend analysis was performed. No linear, quadratic, or cubic trend was significant for this set of data. The energy expended was not significantly different between any of the days exercised.

Table 9: Average energy expended per exercise session for male subjects*.

Day	Energy Expenditure (Kcal/30 min exercise session) (n = 7)
1	314 ± 27**
2	306 ± 20
3	299 ± 21
4	274 ± 29
5	296 ± 23
6	292 ± 18
7	290 ± 29
8	253 ± 11
9	270 ± 16

*Determined from heart rate values, (which was then used to determine energy expenditure through the calculation of oxygen consumption from heart rate; refer to Appendix 17 for equation relating heart rate and oxygen consumption).
**Mean ± SEM.

Heart rate values determined at specific time points during the exercise session are shown in Table 10. The average maximum heart rate for all of the subjects was 186 ± 7 beats/minute. Heart rate reserve follows a trend of increasing throughout the 30 minute exercise session. Heart rate and heart rate reserve increased linearly with time in a significant manner, ($p < 0.0001$, $p < 0.0000$ respectively) determined by trend analysis. The highest heart rate reserve occurred between 15 and 25 minutes of exercising.

Table 10: Average exercise heart rate and reserve heart rate values for male subjects at various time points during the 30 minute exercise session§.

Time (minutes)	**Heart Rate (beats/min) (n = 7)**	**Heart Rate Reserve* (n = 7)**
0	82 ± 3	45 ± 3**
5	146 ± 5	79 ± 3
10	150 ± 5	81 ± 2
15	152 ± 6	82 ± 3
20	152 ± 6	82 ± 3
25	151 ± 6	82 ± 3
30	148 ± 5	80 ± 3

§Average values for a nine day period.

* Percent maximum heart rate.

**Mean ± SEM.

Throughout the study, the average energy expenditure from exercise for each subject was 288 ± 18 kcal/day. This corresponded to an energy deficit of 12 ± 0.1 % when energy expenditure was expressed relative to the average maintenance energy intake (2491 ± 21 kcal; refer to Table 11). This was similar to the desired energy deficit of 11%. The actual dietary energy deficit created was 22 ± 0.5% (548 ± 5 kcal), which (when added to the deficit created by exercise) gave rise to a total deficit of 34 ± 1 % (841 ± 26 kcal).

Table 11: Energy intake and energy expenditure of the male subjects.

Variable	Value (n = 8)
(A) Maintenance energy requirement (kcal)	2491 ± 21**
(B) Energy expenditure from exercise*(kcal/day)	288 ± 18
(C) Energy deficit from diet (kcal/day)	548 ± 5
(D) Total deficit (kcal/day) (B + C)	841 ± 26
(E) Net Caloric Intake (kcal/day) (A - D)	1647 ± 13
(F) % Energy Deficit of Maintenance Energy from Exercise (B/A x 100)	12 ± 1
(G) % Energy deficit of Maintenance energy from diet (C/A x 100)	22 ± 1
(H) % Total Energy deficit of Maintenance Energy (D/A x 100)	34 ± 1
% Energy Requirement Satisfied (E/A x 100)	66 ± 1

**Mean ± SEM.

*Determined from the relationship between heart rate and oxygen consumption.

B. FEMALES

Table 12 presents the activity characteristics of the female athletes. Although the distance run per week (37 ± 4 km) was not greater than or equal to 50 km per week (one of the inclusion criteria; refer to Appendix 22 for the distance run per week by each subject), all of the subjects were involved in at least one other aerobic activity other than running, as was evident by the large amount of time spent exercising each day (see Appendix 21 for activities in which the female subjects were involved and Appendix 23 for the time spent by each subject exercising each day). The activity characteristics did not differ between the two diet groups.

Table 12: Activity characteristics of female athletes according to dietary subgroup during the study.

Characteristic	**All (n = 14)**	**100 % Diet (n = 7)**	**75 % Diet (n = 7)**
Run distance per week (km/week)	37.1 ± 3.7	42.4 ± 4.3	31.9 ± 5.6**
Time spent exercising each day (min/day)*	99.9 ± 6.4	105.2 ± 10.1	94.7 ± 8.0
Energy Expenditure from exercise (kcal/day)§	832 ± 97	829 ± 119	834 ± 142

**Mean ± SEM.
No significant differences.
Comparisons made by student's unpaired t-tests.
*Calculated from daily exercise recording forms.
§Calculated by taking average of days 1 to 9; determined by the Weight Loss Programmer (Version 3.1, Ohio Distinctive Software, Cleveland, Ohio, 1992).

Table 13 displays the energy expended during exercise per day for the female subject group over a nine day period. The 100% and 75% diet groups did not differ with respect to the amount of energy expended per day on any day during the study except on day 6 where the energy expenditure for the 100% diet group (1009 ± 128 kcal/day) was significantly larger than the, 75% diet group (673 ± 66 kcal/day). Appendix 25 contains the results of energy expended per day for the female subjects over 11 days. The energy expended by the female subjects on days 10 and 11 was not included in Table 13 because the sample size was too small to represent values obtained for the entire group.

The average exercise energy expenditure of days one to nine for the entire group of subjects, the 100% diet group, and the 75% diet group were respectively: 833 ± 97 kcal/day, 829 ± 119 kcal/day, and 834 ± 142 kcal/day. The average exercise energy expenditure on days one to nine did not differ between groups.

The highest energy expended per day for the entire group of subjects, and subjects in the 75% diet group occurred on day nine while the highest energy expended for the 100% diet group occurred on day 6. The lowest energy expended per day for the entire group of subjects, the 100% diet group, and the 75 % diet group occurred on days 7, 7, and 6 respectively.

The slopes of the graphs of days exercised versus energy expended for the diet groups were compared to determine: if the energy expenditure progressively decreased with the amount of days exercised, and if this differed between groups. The resulting slope values for the entire group of subjects, the 100% diet group, and the 75% diet group when considering days 1 to 9 were respectively: -7.80 ± 8.36 kcal/days^2, -12.07 ± 10.5 kcal/days^2, and -3.53 ± 13.66 kcal/days^2 (Figure 1). The slope values were not significantly different between the two diet groups and no significant linear trends were found within each diet group with successive days of exercise.

Figure 2: Energy expenditure from exercise throughout the diet period.

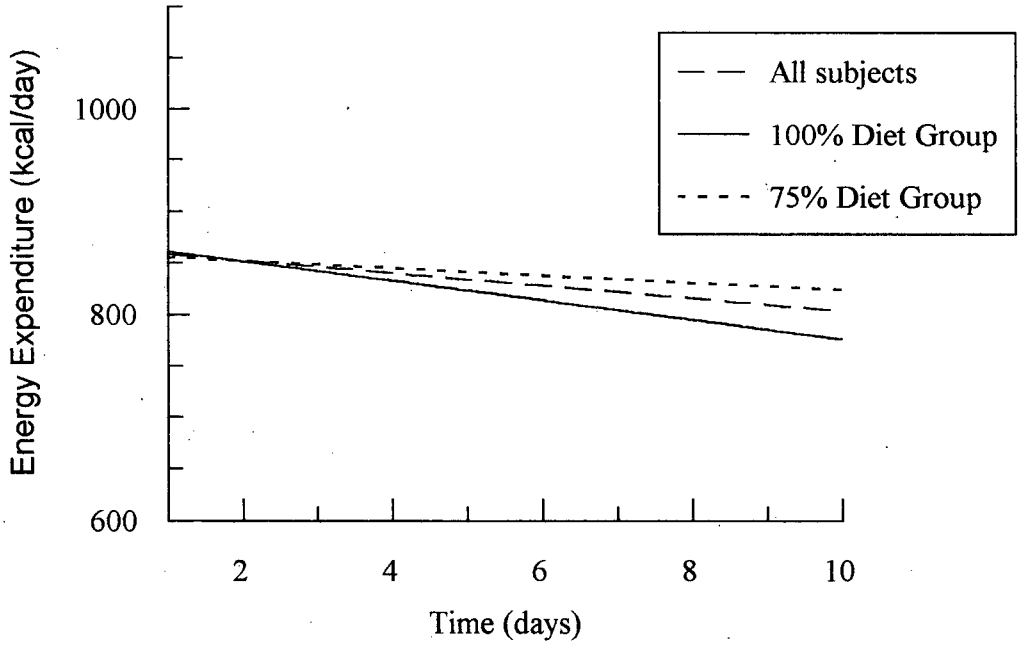

Table 13: Average energy expended from exercise per day for female athletes according to the dietary subgroup.

Day	Sample size (All subjects)	Energy Expenditure (kcal/day)* All	100% Diet group	75% Diet group
1	14	923 ± 97	914 ± 156 (n = 7)	932 ± 129 (n = 7)**
2	14	796 ± 95	751 ± 108 (n = 7)	841 ± 164 (n = 7)
3	13	789 ± 73	784 ± 88 (n = 7)	794 ± 124 (n = 7)
4	13	862 ± 97	943 ± 108(n = 7)	780 ± 164 (n = 7)
5	13	881 ± 110	837 ± 108 (n = 5)	920 ± 121 (n = 7)
6	13	854 ± 87	1009 ± 128 (n = 7)	673 ± 66[a] (n = 6)
7	11	729 ± 90	710 ± 171 (n = 5)	744 ± 102 (n = 6)
8	14	741 ± 76	717 ± 98 (n = 7)	765 ± 125 (n = 7)
9	13	926 ± 150	795 ± 110 (n = 7)	1056 ± 283 (n = 6)

*Determined by the Weight Loss Programmer (Version 3.1, Ohio Distinctive Software, Cleveland, Ohio, 1992).
**Mean ± SEM.
[a]Significant difference between 100% and 75% diet groups, $p < 0.05$.
Comparisons made by student's unpaired t-tests.

5. SUBSTRATE OXIDATION:

A. MALES

Table 14 displays the resting energy expenditure data for the male subjects. Their were no changes in the resting energy expenditure and the resting RQ value as a result of the diet and exercise treatment. However, it should be noted that data for only three subjects were used to make these comparisons.

Table 14: Resting energy expenditure data during diet treatment* for male subjects.

Variable	**Pre-treatment (n = 3)**	**Post-treatment (n = 3)‡**
REE§ (kcal/day)	1860 ± 155	1920 ± 122**
(kcal/kg body weight)	21.6 ± 0.9	22.8 ± 1.0
(kcal/kg fat-free mass)	27.4 ± 0.1	28.4 ± 0.7
RQ (VCO_2/VO_2)	0.84 ± 0.03	0.80 ± 0.09

*Measured at the beginning and end of each diet treatment.
‡Data for 3 subjects was not assessable; 2 subjects could not schedule the tests.
§ Calculated by formula of Weir (1949).
**Mean ± SEM.
No significant differences.
Comparisons made by correlated t-tests.

Post-prandial RQ values of male subjects pre- and post-treatment are presented in Table 15. The post-prandial RQ values significantly decreased ($p < 0.05$) at the following time points: 60 minutes (-0.09 ± 0.01); 120 minutes (-0.04 ± 0.01); 150 minutes (-0.08 ± 0.01). The decrease in the post-prandial RQ at 90 minutes(-0.02 ± 0.01) almost showed a significant difference ($p = 0.07$).

Table 15: Post-prandial RQ* values after consumption of a test breakfast for male subjects.

Time (minutes)	Pre-treatment* (n =3)	Post-treatment (n = 3)
30	0.90 ± 0.02	0.83 ± 0.00**
60	0.89 ± 0.02	0.80 ± 0.02[a]
90	0.86 ± 0.01	0.83 ± 0.02
120	0.86 ± 0.03	0.83 ± 0.03[a]
150	0.88 ± 0.02	0.80 ± 0.01[a]

*Measured at the beginning and end of the diet treatment.
**Mean ± SEM.
[a] Significant difference, $p < 0.05$.
Comparison made by correlated t-tests.

B. FEMALES

The resting energy expenditure data of the female subjects pre- and post-diet is shown in Table 16. The resting energy expenditure did not differ between the diet groups at each time point, or pre- and post-diet within each diet group, even when corrected for body weight and FFM. The resting RQ value of the 75% diet group decreased (-0.06 ± 0.02; $p < 0.05$), while the value for the 100% diet group did not change.

Table 16: Resting energy expenditure data during diet treatment* for female athletes according to the dietary subgroup.

Parameter	100% Diet (n = 7)		75% Diet (n = 7)	
	Pre-diet	Post-diet	Pre-diet	Post-diet
REE§				
(kcal/day)	1344 ± 78	1341 ± 46	1511 ± 98	1464 ± 63**
(kcal/kg body weight)	22.9 ± 0.7	23.2 ± 0.5	23.2 ± 1.0	23.1 ± 0.8
(kcal/kg FFM)	28.1 ± 1.1	28.4 ± 0.8	29.7 ± 1.7	28.6 ± 1.0
RQ (VCO_2/VO_2)	0.87 ± 0.03	0.84 ± 0.02	0.83 ± 0.03	0.77 ± 0.02[a,b]

* Measured at the beginning and end of each diet period
§ Calculated by the formula of Weir (1949)
**Mean ± SEM.
a significant difference within the diet group, pre- versus post-diet ($p<0.05$).
b significant difference between the change in the 100% diet group versus the change in the 75% diet group, ($p<0.05$).
Comparisons made with a 2 x 2 RM ANOVA for each variable.

Table 17 displays the post-prandial RQ values pre- and post-diet in the female athletes according to the dietary subgroup. There were no significant differences in t he post-prandial RQ values between the 100% and 75% diet groups either pre-diet or post-diet.

The post-prandial RQ values for the 75% group decreased from the pre-diet value at the following time points: 30 minutes (-0.07 ± 0.03; $p < 0.05$); 120 minutes (-0.10 ± 0.02; $p < 0.001$); 150 minutes (-0.09 ± 0.03; $p < 0.05$). The changes in the post-prandial RQ values for the 75 % group were significantly larger than changes occurring in the 100% diet group at 120 minutes ($p < 0.001$) and 150 minutes ($p < 0.05$). There was no change in the post-prandial RQ values for the 100% diet group, pre- and post-diet.

Table 17: Post-prandial RQ* values after consumption of a test breakfast of female athletes according to the dietary subgroup.

	TIME (min)									
	30		60		90		120		150	
Diet Type	pre-diet	post-diet	pre-diet	post-diet	pre-diet	post-diet	pre-diet	post-diet	pre-diet	post-diet
100% diet group†	0.90 ± 0.01	0.94 ± 0.02	0.91 ± 0.03	0.90 ± 0.02	0.90 ± 0.03	0.87 ± 0.02	0.89 ± 0.03	0.90 ± 0.02	0.89 ± 0.02	0.88 ± 0.02**
75% diet group†	0.95 ± 0.02	0.88 ± 0.02[a]	0.89 ± 0.03	0.87± 0.01	0.85 ± 0.02	0.82± 0.02	0.87 ± 0.03	0.79 ± 0.02[b,c]	0.88 ± 0.02	0.80 ± 0.01[a,d]

* Measured at the beginning and end of each diet period.
† n = 7.
**Mean ± SEM.
a significant difference within group, pre- versus post-diet ($p<0.05$).
b significant difference within group, pre- versus post-diet ($p<0.001$).
c significant difference in the change in the100% diet group versus the change in the 75% diet group ($p<0.01$).
d significant difference in the change in the 100% diet group versus the change in the 75% diet group ($p<0.05$).
Comparison made with a 2 x 2 RM ANOVA at each time point.

In order to evaluate whether the change in RQ over time was significantly different pre- and post-diet within each diet group, and at each time point between each diet group, the slopes of the lines of time versus RQ were evaluated statistically. No significant differences were found between slope values pre- and post-diet within each diet group, and at each time point between each diet group.

6. MUSCLE FIBER ANALYSES:

A. MALES

There was no significant difference in calpain activity pre- (9.40 ± 2.30 units/g wet weight) and post-treatment (13.63 ± 2.59 units/g wet weight) where the change in activity was 4.23 ± 4.60 units/g wet weight (n = 4) as assessed by correlated t-tests.

B. FEMALES

Table 18 shows the pre- and post-diet myofibrillar calpain activity for the female subjects. There were no differences in myofibrillar calpain activity between the diet groups at either time point. Furthermore, there were no differences in pre- and post-diet myofibrillar calpain activity within each diet group.

Table 18: Myofibrillar calpain activities of female athletes according to the dietary subgroup before and after dietary period.

Diet Type	Calpain Activity (units/g wet weight)		
	Pre-Diet	Post-Diet	Change
100%* (n = 6)	28.82 ± 4.9	34.84 ± 10.57	6.02 ± 7.43**
75%* (n = 6)	20.82 ± 2.49	42.98 ± 12.49	22.17 ± 14.34

**Mean ± SEM.
No significant differences.
Comparisons made with a 2 x 2 RM ANOVA.

7. MUSCLE FUNCTION:

A. MALES

Table 19 shows the torque values for the eccentric contractions of the quadriceps muscles for the male subjects. There were no significant differences between pre- and post-diet values for any of the average or peak torque values for eccentric contractions. Note that the sample size for the average torque parameters was 6 instead of 8 because pre-treatment data was inaccessible for 2 subjects because baseline muscle function measurements were obtained from a previous study which did not look at the average torques.

Table 19: Eccentric contractions of quadriceps muscles of male subjects before and after the diet and exercise period.

Velocity (degrees/sec)	Pre-treatment Torque (Nm)	Post-treatment Torque (Nm)	Change in Torque (Nm)
Peak torque (n = 8)			
30	179 ± 19	177 ± 13	-2 ± 18**
90	177 ± 18	206 ± 7	28 ± 21
120	177 ± 21	195 ± 19	18 ± 27
180	175 ± 25	175 ± 20	0 ± 25
Average torque (n = 6)			
30	173 ± 9	153 ± 11	-20 ± 11
90	158 ± 10	156 ± 14	-2 ± 22
120	140 ± 12	158 ± 16	18 ± 25
180	146 ± 15	137 ± 18	-9 ± 16

**Mean ± SEM.
No significant differences.
Comparisons made with correlated t-tests.

Table 20 shows the torque values for the concentric contractions of the quadriceps muscles for the male subjects. There were no significant differences between pre- and post-diet values for any of the concentric peak torque values. However, the concentric average torque obtained at a movement velocity of 30 deg/sec decreased (-22 ± 6 Nm; $p < 0.05$) from the pre-diet value.

Table 20: Concentric contractions of quadriceps muscles of male subjects before and after diet and exercise treatment.

Velocity (degrees/sec)	Pre-treatment Torque (Nm)	Post-treatment Torque (Nm)	Change in Torque (Nm)
Peak torque (n = 8)			
30	163 ± 24	157 ± 10	-5 ± 16**
90	150 ± 14	159 ± 18	9 ± 18
120	154 ± 20	165 ± 17	11 ± 10
180	144 ± 16	152 ± 16	8 ± 8
Average torque (n = 6)			
30	144 ± 18	123 ± 12	-22 ± 6[a]
90	132 ± 16	133 ± 21	0 ± 11
120	138 ± 21	142 ± 23	4 ± 6
180	125 ± 16	130 ± 20	5 ± 9

**Mean ± SEM.

[a] Significant difference between pre- and post-diet values, $p < 0.05$.
Comparisons made with correlated t-tests.

In order to evaluate if the change in torque with increasing movement velocity was significantly different pre- and post-treatment, the slope of the graph of movement velocity versus torque value was evaluated statistically. There were no significant differences between any of the pre- and post-treatment slope values for all contraction and torque types.

B. FEMALES

The peak and average eccentric torques of the female subjects according to the dietary subgroup pre- and post-diet are displayed in Table 21. The eccentric average torque of the 100% diet group increased at a movement velocity of 90 degrees/second (36 ± 19 Nm; $p < 0.05$), while the eccentric average torque of the 75% diet group at a movement velocity of 180 degrees/second increased (19 ± 13 Nm; $p < 0.05$). There were no differences in the eccentric peak torque within each diet group or between the diet groups.

Table 21: Eccentric contractions of quadriceps muscles of female athletes according to the dietary subgroup before and after the diet period.

Velocity (deg/sec)	**100 % Diet (n = 7) (Nm)**		**75% Diet (n = 7) (Nm)**	
	pre-diet	**post-diet**	**pre-diet**	**post-diet**
Peak torque				
30	111± 17	121 ± 16	121 ± 11	127 ± 13**
90	86 ± 15	125 ± 19	112 ± 9	124 ± 12
120	129 ± 16	131 ± 19	121 ± 10	126 ± 15
180	132 ± 18	138 ± 17	116 ± 13	135 ± 15
Average torque				
30	90 ± 14	100 ± 14	100 ± 9	115 ± 13
90	69 ± 15	98 ±1 5[a]	91 ± 7	101± 10
120	97 ± 13	94 ± 15	98 ± 6	107 ± 11
180	95 ± 14	97 ± 14	85 ± 12	106 ± 9[a]

**Mean ± SEM.
[a] Significant difference from pre-diet value, $p < 0.05$.
Comparisons made with a 2 x 2 RM ANOVA for each condition.

Table 22 shows the peak and average concentric torques of the female subjects according to the dietary subgroup before and after the diet period. The concentric peak torque in the 75% diet group changed from the pre-diet value (-10 ± 6 Nm; $p < 0.05$). Furthermore, this change was significantly larger than that seen in the 100% diet group ($p < 0.05$). The concentric average torque at a movement velocity of 30 degrees/second in the 100% diet group increased (11 ± 5 Nm; $p < 0.05$).

Table 22: Concentric contractions of quadriceps muscles of female athletes according to the dietary subgroup before and after the diet period.

Velocity (deg/sec)	**100 % Diet (n = 7) (Nm)**		**75% Diet (n = 7) (Nm)**	
	pre-diet	**post-diet**	**pre-diet**	**post-diet**
Peak torque				
30	118 ± 11	124 ± 8	116 ± 8	109 ± 9**
90	107 ± 15	116 ± 6	108 ± 10	97 ± 11
120	106 ± 10	114 ± 6	104 ± 8	93 ± 9[a,b]
180	102± 9	106 ± 9	98 ± 8	101 ± 5
Average torque				
30	98 ± 9	110 ± 6[a,b]	96 ± 6	93 ± 7
90	94 ± 13	102 ± 6	96 ± 7	85 ± 10
120	92 ± 10	97 ± 7	92 ± 7	84 ± 8
180	85 ± 13	88 ± 8	86 ± 6	83 ± 6

**Mean ± SEM.

[a] Significant difference from pre-diet value, $p < 0.05$.

[b] Significant difference between the change in the 100% diet group versus the change in the 75% diet group, $p < 0.05$.

Comparisons made with a 2 x 2 RM ANOVA for each condition.

To evaluate whether the change in torque with increasing movement velocity was significantly different pre- and post-diet within each diet group, and at each time point between the 100% and 75% diet groups, the slope of the graph of movement velocity versus torque value was evaluated statistically. There were no significant differences between any of the pre- and post-diet slope values within each diet group for any contraction or torque type. There were also no significant differences between the 100% and 75% diet group slope values at each time point.

8. FACTORS RELATING TO CHANGES IN CALPAIN ACTIVITY

A. MALES

Stepwise multiple regression analysis was not performed on the calpain data with substrate oxidation parameters incorporated as the subject number was too low to provide representative results.

B. FEMALES

Stepwise multiple regression analysis was performed on the calpain data incorporating substrate oxidation variables (REE, post-prandial RQ values at all time points) to determine which substrate oxidation variables were most important in determining calpain activity. The pre-diet substrate oxidation parameters did not predict the pre-diet calpain activity.

The correlations between the post-diet substrate oxidation parameters and the calpain activity in the 100% diet group are shown in Table 23. The post-diet calpain activity was most strongly correlated to the RQ value obtained 120 minutes post-prandially (beta = -3.56; $p < 0.010$), as compared to the correlation obtained 150 minutes post-prandially (beta = 2.55; $p < 0.020$).

Table 23: Post-diet substrate oxidation parameters correlated to the post-diet calpain activity in the 100% diet group (n = 7).

Parameter	R Square	Adjusted R Square	B	Beta (ß)	Probability
RQ, 120 minutes	0.721	0.434	-1874.665	-3.56	0.010
RQ, 150 minutes	0.927	0.790	1639.186	2.66	0.020

Constant = 173.880

9. FACTORS RELATING TOCHANGES IN MUSCLE FUNCTION

A. MALES

Simple linear regression analysis was performed on the calpain data and the muscle function parameters for the purpose of determining the extent to which muscle function (dependent variable) was affected by the myofibrillar calpain activity (independent variable). None of the pre-diet muscle function parameters were significantly linearly related to pre-diet calpain activity.

The significant post-diet correlations between muscle function parameters and calpain activity in the male subjects is displayed in Table 24. The eccentric average torque obtained at a movement velocity of 120 degrees/second and the eccentric peak torque were significantly correlated to the post-diet calpain activity in a positive fashion.

Table 24: Post-diet muscle function parameters correlated to post-diet myofibrillar calpain activity in male subjects (n = 4).

Muscle function parameter	Multiple R	Adjusted R square	B	ß	Probability
eccentric peak, 120 deg/sec	0.992	0.977	8.547	0.99	0.0078
eccentric average, 120 deg/sec	0.984	0.951	6.131	0.98	0.0164

The correlations between the change in muscle function parameters and the change in calpain activity in the male subjects is displayed in Table 25. Both the change in the eccentric average torque at a movement velocity of 120 degrees/second, and the eccentric peak torque at a movement velocity of 120 degree/second were positively related to the change in calpain activity.

Table 25: Correlation coefficients for change in calpain activity to change in muscle function parameters for the male subjects (n = 4).

Muscle function parameter	**Multiple R**	**Adjusted R square**	**B**	**ß**	**Probability**
eccentric peak, 120 deg/sec	0.959	0.879	9.302	0.96	0.0412
eccentric average, 120 deg/sec	0.918	0.859	6.558	0.95	0.0482

B. FEMALES

Simple linear regression analysis was performed on the calpain data and the muscle function parameters for the purpose of determining the extent to which muscle function (dependent variable) was affected by the myofibrillar calpain activity (independent variable). Only the significant correlations between the pre-diet muscle function parameters and calpain activity are shown in Table 26. All pre-diet concentric torques were significantly correlated with pre-diet calpain activity except for the concentric peak torque at a movement velocity of 180 deg/sec and the concentric peak torque at a velocity of 90 deg/sec within the whole group of female subjects.

Table 26: Pre-diet muscle function variables correlated to pre-diet myofibrillar calpain activity in the entire group of athletic females (n = 14).

Muscle function parameter	Multiple R	Adjusted R square	B	ß	Probability
concentric average, 120 deg/sec	-0.697	0.440	-1.477	-0.70	0.0081
concentric average, 180 deg/sec	-0.665	0.391	-1.155	-0.66	0.0131
concentric average, 90 deg/sec	-0.662	0.388	-1.640	-0.66	0.0137
concentric peak, 120 deg/sec	-0.685	0.416	-1.485	-0.68	0.0140
concentric peak, 90 deg/sec	-0.640	0.356	-1.942	-0.64	0.0185
concentric peak, 30 deg/sec	-0.635	0.349	-1.500	-0.63	0.0198
concentric average, 30 deg/sec	-0.620	0.329	-1.100	-0.62	0.0236

Table 27 displays the correlations between muscle function parameter and calpain activity in the 100% diet group. All pre-diet concentric torque values were significantly correlated with pre-diet calpain activity in the 100% diet group, except for the following muscle function parameters where there was an nearly significant linear relationship between each one and pre-diet calpain activity: concentric average torque, 30 deg/sec ($p = 0.0561$); concentric average torque, 180 deg/sec ($p = 0.0822$). The only correlation between muscle function and pre-diet calpain activity within the 75% diet group occurred at a movement velocity of 180 deg/sec where the concentric peak torque was almost significantly correlated to the pre-diet calpain activity ($r = -0.864$; Adjusted $r^2 = 0.661$; $B = -3.235$; Beta = -0.86; $p = 0.0591$).

Table 27: Pre-diet muscle function variables correlated to pre-diet myofibrillar calpain activity in athletic females in the 100% diet group (n = 7).

Muscle function parameter	Multiple R	Adjusted R square	B	ß	Probability
concentric peak, 120 deg/sec	-0.798	0.564	-1.602	-0.80	0.0316
concentric average, 120 deg/sec	-0.779	0.528	-1.599	-0.78	0.0391
concentric average, 90 deg/sec	-0.756	0.486	-1.937	-0.76	0.0493
concentric peak, 30 deg/sec	-0.742	0.461	-1.651	-0.74	0.0561
concentric average, 30 deg/sec	-0.740	0.456	-1.308	-0.74	0.0575
concentric peak, 90 deg/sec	-0.730	0.439	-2.223	-0.73	0.0627
concentric peak, 180 deg/sec	-0.718	0.418	-1.286	-0.72	0.0690
concentric average, 180 deg/sec	-0.696	0.382	-1.185	-0.70	0.0822

All of the significant correlations (or correlations that were almost significant) between post-diet muscle function and post-diet calpain activity occurred within the concentric conditions for both diet groups. The correlations between the post-diet muscle function variables and post-diet calpain activity for the 100% diet group are shown in Table 28. There were significant correlations between the following post-diet muscle function parameters and the post-diet calpain activity for the 100% diet group: concentric peak torque, 90 deg/sec; concentric peak torque, 120 deg/sec; concentric average torque, 120 deg/sec.

Table 28: Post-diet muscle function variables correlated to post-diet myofibrillar calpain activity for athletic females in the 100% diet group.

Muscle function parameter	**Multiple R**	**Adjusted R square**	**B**	**ß**	**Probability**
concentric peak, 90 deg/sec	-0.873	0.714	-0.529	-0.87	0.0104
concentric average, 120 deg/sec	-0.816	0.598	-0.528	-0.82	0.0254
concentric peak, 120 deg/sec	-0.813	0.594	-0.474	-0.81	0.0261
concentric average, 30 deg/sec	-0.704	0.3939	-0.428	-0.70	0.0778
concentric average, 180 deg/sec	-0.699	0.387	-0.519	-0.70	0.0804

The post-diet correlations between muscle function variables and post-diet calpain activity are shown in Table 29. The only significant between a muscle function parameter and post-diet calpain activity occurred with the post-diet concentric peak condition at a movement velocity of 120 deg/sec. Also included in Table 29 were correlations between post-diet muscle function parameters and post-diet calpain activity that were almost significant.

Table 29: Post-diet muscle function variables correlated to post-diet myofibrillar calpain activity for athletic females in the 75% diet group.

Muscle function parameter	**Multiple R**	**Adjusted R square**	**B**	**ß**	**Probability**
concentric peak, 120 deg/sec	-0.882	0.703	0.798	0.88	0.0480
concentric peak, 90 deg/sec	-0.851	0.631	0.869	0.85	0.0678
concentric peak, 180 deg/sec	-0.825	0.573	0.304	0.82	0.0859
concentric average, 120 deg/sec	-0.7300	0.416	0.559	0.73	0.0996
concentric average, 180 deg/sec	-0.711	0.381	0.369	0.71	0.1136

Table 29: Post-diet muscle function variables correlated to post-diet myofibrillar calpain activity for athletic females in the 75% diet group.

Muscle function parameter	Multiple R	Adjusted R square	B	ß	Probability
concentric peak, 120 deg/sec	-0.882	0.703	0.798	0.88	0.0480
concentric peak, 90 deg/sec	-0.851	0.631	0.869	0.85	0.0678
concentric peak, 180 deg/sec	-0.825	0.573	0.304	0.82	0.0859
concentric average, 120 deg/sec	-0.7300	0.416	0.559	0.73	0.0996
concentric average, 180 deg/sec	-0.711	0.381	0.369	0.71	0.1136

Change in muscle function parameters that were significantly correlated to the change in calpain activity for the female athletes occurred only in the 75% diet group and can be seen in Table 30. The only significant correlation occurred between the change in concentric peak torque at a movement velocity of 180 deg/sec and the change in calpain activity. There were almost significant correlations between the change in muscle function and calpain activity within the 75% diet group for the eccentric peak condition at movment velocities of 90 and 180 deg/sec (Table 30).

Table 30: Correlation coefficients for change in calpain activity to change in muscle function variables for the female athletes in the 75% diet group (n = 7).

Muscle function parameter	**Multiple R**	**Adjusted R square**	**B**	**ß**	**Probability**
ecccentric peak, 180 deg/sec	0.936	0.844	-0.956	-0.94	0.0061
concentric peak, 180 deg/sec	0.936	0.834	-0.352	-0.94	0.0194
ecccentric peak, 90 deg/sec	0.756	0.464	-0.768	-0.76	0.0824

10. SUMMARY OF RESULTS WITH REFERENCE TO THE STUDY HYPOTHESES

Hypothesis 1:

Changes in fat oxidation, induced by dietary restriction and aerobic exercise, result in no difference in quadriceps muscle group function.

The null hypothesis was confirmed for both male and female subjects. Although there were selective changes in muscle function at specific movement velocities in both subject groups, there were no significant trends in the change in overall muscle function.

Hypothesis 2:

Changes in fat oxidation, induced by dietary restriction and aerobic exercise, result in no difference in proteolysis of skeletal muscle.

The null hypothesis was generally confirmed as there were no significant changes in myofibrillar calpain activity in both the male and female subject groups. It is important to note however, that the small sample size, and the large inter-subject-variability (especially in the female subject group) renders the detection of small differences unlikely.

It is also interesting to note that calpain activity was negatively correlated to a number of the muscle function parameters in the female subject group, suggesting that an inverse relationship exists between the two variables. A conclusive relationship between muscle function and calpain activity can not be made due to the fact that calpain activity was positively correlated to a number of muscle function parameters in the female subject group.

Chapter V

DISCUSSION

1. INTRODUCTION

A brief review of the subjects involved in the study will introduce the discussion. This will be followed by the major findings pertaining to each of the hypotheses: (i) changes in fat oxidation, induced by dietary restriction and aerobic exercise, as related to protease activity (protein degradation); (ii) changes in fat oxidation, induced by dietary restriction and aerobic exercise, as related to muscle function. Subsequently, the overall relationships established through this study will be reviewed, and lastly conclusions will be presented.

2. SUBJECTS

A) MALES:

In accordance with the subject criteria, the non-smoking male subjects were sedentary, as none of them had been regularly active within the last two years. Their lack of activity and trained status was evident by their low VO_2 max value for their age (which was below the average value of 45 ml/min/kg found for men between the ages of 20 to 29 (Canadian Standardized Test of Fitness Operations Manual, 1987)). It was not indicative of a trained person (indicated by a VO_2 max value of greater than 51 ml/min/kg) (Canadian Standardized Test of Fitness Operations Manual, 1987). The subjects' lack of activity was also indirectly reflected by their high body weight which was 20% higher than their ideal body weight.

The large body weight corresponded to a BMI that was outside the healthy limit of 18-25 kg/m^2 (Hunt and Groff, 1990). This value fell into the range of BMI values used to

describe type I obesity (BMI = 25-29 kg/m^2; Garrow, 1981). Lack of activity has been shown to be a contributing factor for obesity (Robinson et al, 1990).

The average percent body fat of the male subjects was within the desired subject criteria range of 10-20%. Furthermore, this percent body fat indicated that the male subjects were in a range where optimal health was attainable (10-25%; Fox et al, 1993).

1) Dietary Intake:

According to the subjects' 4-day diet records, the proportion of carbohydrate, protein, and fat consumed was approximately within the recommended healthy nutrient intakes (50-55% carbohydrate, 12-15 % protein, and 25-30% fat; Faber and Spinnler Benade, 1991).

Analysis of usual intake (four-day food records) showed that energy intake supplied 175% of the recommended energy intake suggested for a 22 year old sedentary male of height and weight 176 cm and 84 kg respectively (Food Processor II Nutrient Analysis Software, ESHA Research, Salem, OR). The positive energy balance produced by the combination of the higher energy intake and low energy expenditure may explain why the subjects were classified as being type I obese. It is well known that the combination of a high energy intake and lack of activity are contributing factors to obesity (Robinson et al, 1990).

It is also possible that the male subjects overestimated their usual intake. An intake above 75% of their recommended suggests that they would have been actively gaining weight, or exercising. The subjects did comment on the fact that they had gained a small amount of weight in the past year. Therefore, the possibility that they overestimated their energy intake is highly likely.

The high consumption of meat could, in part, explain why the following nutrients exceeded the RNI, because meat (chicken, beef, pork, steak) contains a high proportion of at least 1 of these nutrients: protein, thiamin, niacin, vitamin B12, iron, phosphorus,

selenium. The large amount of potassium and sodium consumed may have been due to the large consumption of salted food (fast foods, potato chips).

B. FEMALES

Fourteen female subjects (7 subjects per group) were recruited for the study. In order to determine if this number was large enough to detect a difference in calpain activity, the average standard deviation associated with calpain activity obtained from the female subjects was inserted into the following sample size equation (Brown and Hollander, 1977):

$$\text{Sample Size (n)} = (2z^2 \times v^2) / (\text{Difference}^2)$$

where n = population size per groups; z = 1.96 so that all tests were done at a significance level of $p < 0.05$; Difference = 0.10; v = variability associated with determining calpain activity.

The "Difference" term represents a value required in order to find a significant difference between each group. For this study, a difference in the amount of calpain activity of 10% was selected as meaningful, based on the study of Belcastro et al (1988). The variability term of 25 units/g wet weight was determined from the female subjects.

$$\text{Sample Size (n)} = [2\ (1.96)^2 \times 25^2] / (10^2)$$

Therefore, n = 48, and the total sample size = 96.

Thus, a larger sample size should have been recruited as the total subject number was to low to find a significant difference in calpain activity.

In accordance with the subject criteria, all of the female subjects were runners who had indicated that they ran at least 50 km/week. Eleven subjects were competitively

involved in long distance running, and all of the subjects participated in at least 2 other types of exercise other than running.

All of the subjects possessed VO_2 max values that were greater than the subject criteria of 42 ml/kg/min signifying that the subjects were well-conditioned females (Canadian Standardized Test of Fitness Operations Manual, 1986). Their commitment to athletics was high as they had been involved in running, and had been exercising regularly and/or competitively for the past 9.4 ± 1.0 years. Their dedication to athletics was also seen by the fact that they exercised approximately 100 minutes/day throughout the duration of the study.

Eight subjects were taking oral contraceptives. This was the only type of medication the subjects were ingesting. Only subjects with regular menstrual cycles were accepted into the study due to the inherent difficulties associated with measurements of female athletes with oligomenorrhea or amenorrhea.

All of the subjects were within the desired subject criteria age range (18-35 years) except for one subject, who was 47 years old. This subject was accepted into the study because of her exceptional athletic ability and fulfillment of all other subject criteria.

The average percent body fat for all of the subjects fell within the desired subject criteria range of 16-26%. This value was in accordance with those previously found for female endurance athletes (Fox et al, 1993).

The percent body fat for the athletes in both diet groups was slightly higher than that found for a group of 70 female distance runners, where the relative body fat averaged 16.8 ± 5.5 (Wilmore, 1977). However, a woman who established the best time in the world for a 50 mile run in 1987 had a percent body fat of 35.8%. Thus, the percent body fat is not the sole determinant of the caliber of the athlete.

Comparisons of the demographic data demonstrated that the 100% and 75% diet groups were well matched with regard to age, height, weight, percent body fat, fat-free mass (FFM), and fitness level. The subjects described themselves as weight stable within

the last 3 months. The subjects were also very well matched in terms of fat distribution in accordance with skinfold values.

i) Dietary Intake:

The energy intake found in this study was 84 ± 6% (2052 ± 153 kcal/day) of the energy requirement in the subject group as a whole, when activity level was considered. Mulligan and Butterfield (1990) examined the energy intake of female runners, aged 19-41, and found that they met 75% (1974 kcal/day) of their energy requirement each day. Other researchers have further confirmed the finding of a low energy intake in runners: Clement and Asmundson (1982) found the mean energy intake in 17 female runners, aged 21-22 years, averaging 44 miles/week to be 2026 kcal/day; Lampe et al (1986) found that 9 female distance runners, aged 27-34, running 45 miles/week had a mean energy intake of 1922 kcal/day which was less than mean estimated energy expenditure per day (based on activity level); Prior et al (1990) found that female marathoners and recreational runners had mean energy intakes that were 1720 kcal/day and 1708 kcal/day respectively. Thus, according to four-day diet records of the subjects, the finding in the present study that energy intake was low in female participants of a sport such as running, where weight and/or body fat is an issue, is consistent with findings from other studies (Beals and Manore, 1994).

An energy intake lower than the maintenance energy requirement may be explained in part by under-reporting of food intake (Livingston et al, 1990). For example, Mertz et al (1992) found that this underreporting represented 18-20% of energy required to maintain weight in a group of males. Edwards et al (1993) also found that women runners underreported energy intake, possibly from 4-58% of total energy requirement. In the group of female subjects, under-reporting of energy intake was highly possible because they had reported weight stability over the past 6 months.

Factors affecting the incidence of under-reporting are not well known, nor have strategies to control these factors been successful (Block, 1982; Gibson, 1987; Mertz et al,

1992; Stunkard, 1981). Mertz et al (1992) suggested that in a group of female runners, under-reporting may have been subconscious and motivated by the belief that low body weight is accomplished through eating less.

Many studies have reported the presence of physiological adaptations in athletes when net energy intake was negative due to the weight stability of the subjects. For example, Mulligan and Butterfield (1990) reported weight stability in very active runners over a two month period where 75% of the energy requirement was met. Prior et al (1990) found that recreational runners and marathoners had almost identical intakes (1708 and 1720 kcal/day respectively), yet the marathoners were running nearly three times the weekly mileage of the recreational runners and still reported stable body weights. It is questionable as to whether or not the athletes did actually ingest less food because the method used to determine energy intake varied between studies (Edwards et al, 1993; Schulz et al, 1992; Wilmore, 1991). However, it has been suggested that suboptimal energy intakes may be the result of physiological adaptations, specifically an increased energy efficiency, which allows some female athletes to function normally and maintain energy balance on fewer calories than would be expected based on body size and activity level (Clark et al, 1988; Thompson and Blanton, 1987).

The percent macronutrients ingested in the subjects' diets were very similar to those recommended for healthy individuals (50-55% carbohydrate, 12-15% protein, and 25-30% fat; Faber and Benade, 1991). This is similar to the percentages previously found for male marathon runners (of which the percentage of calories from carbohydrate, protein and fat approximated the general US population; Rokitzki et al, 1994): 54% carbohydrate, 31% fat, and 12% protein.

All subjects met the RNI for saturated and monounsaturated fat intake (10% of caloric intake; Nutrition Recommendations, Health and Welfare Canada). All subjects ingested slightly less than the RNI for polyunsaturated fats, where polyunsaturated fats

were 7.1 ± 0.01% and 8.5 ± 0.02% of total caloric intake in the 100% and 75% diet groups respectively.

All of the subjects in this study ingested lower than the recommended amounts of copper and pantothenic acid. The decreased intake of these nutrients has not been previously documented in athletes, and may have been due to the food types that the subjects chose to ingest. Thus, these findings may have occurred as a result of limitations of the Canadian Nutrient file for assessing intake of these nutrient accurately (Barr et al, 1994).

The subjects in this study ingested large amounts of vitamin C, potassium and sodium. Potassium and sodium were probably ingested in large amounts to replace the electrolytes lost through exercise (Fox, 1984). The high vitamin C intake has been previously documented in athletes: Rokitzki et al (1994) found vitamin C intake to be 2.23 mg/kg body weight for a group of male marathon runners (compared to 2.61 mg/kg for this group of subjects); Faber and Benade (1991) found that a group of elite field athletes consumed 2.12 mg/kg body weight per day (corresponding to an absolute value of 179 mg/day).

3. ANTHROPOMETRICS

The amount of weight loss in the male subject group (1.5 ± 0.3 kg) over the 9 day study period corresponded to a healthy amount of weight loss in the respective time period (Robinson et al, 1990). The amount of weight lost was larger than expected weight loss of 1.0 kg. The expected weight loss was determined by dividing the energy deficit created in nine days (7623 kcal) by the energy deficit needed to lose 1 kg body weight (7700 kcal/kg; Robinson et al, 1990). A larger amount of weight lost than expected was probably due to the loss of water that often accompanies dieting (Ballor, 1991).

The amount of weight lost in the female subjects who were in the 75% diet over the two week diet period was 1.7± 0.3 kg and corresponded to a healthy amount of weight lost in the respective time period (Robinson et al, 1990).

Studies illustrating the components of weight lost in athletes with an imposed energy restriction have not been documented. However, studies looking at weight loss in obese persons suggest that weight lost over short term could have been due to loss of water, fat, protein, and/or glycogen (Brownell et al, 1987). It has been determined that as the length and severity of the energy restriction increases, the contribution of fat stores to total weight lost increases substantially (Brownell et al, 1987).

This is in contrast with results found from this study, where the loss of fat mass of the male subjects and the female subjects who were in the 75% diet group was a large contributor to the decrease in body weight occurring as a result of the imposed moderate energy restriction of relatively short length. These results are consistent with Ballor (1991) who found that a short term moderate energy restriction did result in a significant loss of fat mass in Sprague-Dawley rats, while a severe energy restriction did not. The food allotment of the moderately restricted (MR) and severely restricted (SR) groups was reduced by 25 and 50% respectively for 12 weeks during which they were fed a low-fat vitamin supplemented rat chow mix. The rats were exercised on a motor-driven treadmill set on a 15% slope at 12 m/min for 30 min/day, 5 days/week. There are however, limitations to the study because the control rats were placed on a high fat diet and the rats were still growing throughout the experiment. Also, application of these results to this study may be limited due the fact that Ballor's study used rats and the duration was short term.

It is difficult to compare the loss of fat mass in the male subjects and the female subjects who were in the 75% diet group to the loss of fat mass found in other studies because of the differences in subject type (most studies look at the effect of an energy restriction on obese subjects) and severity and length of the energy restriction. Nonetheless, the change in fat mass which occurred in the male subjects and the female

subjects in the 75% diet group appeared to contribute significantly to the decrease in body weight. It appeared that more fat mass was lost than body mass lost. This may have been due to errors associated with determining total body fat from skinfold measurements.

Determination of total body fat from skinfold measurements is based on the assumption that a direct relationship exists between total body fat and subcutaneous fat. Errors in determining total body fat from skinfold measurements are associated with the validity of this relationship. For example, individuals distribute fat differently, where some may deposit fat more internally versus externally (subcutaneous fat). Therefore, a direct relationship between total body fat and sucutaneous fat may not be consistent among individuals of different sex, age, and race.

Another factor that may contribute to the error associated with skinfold measurements in determining total body fat includes differences in skin thickness and compressibility among individuals and among different skinfold sites. Compressibility of the subcutaneous fat layer may differ between sites and between individuals because the subcutaneous fat layer is not constant.

The decrease in total body fat measured by skinfolds may have been confounded due to hydration changes. The female subjects in the 75% diet group and the male subjects may have been more dehydrated at the end of the study as it has been suggested that a diet restriction could result in less replete glycogen stores and therefore less conservation of body water (glycogen is stored in conjugation with body water; Ballor, 1991). This lower conservation of body water in the male subjects and the female subjects who were in the 75% diet group, while keeping the other constituents stable, tends to increase the overall density of the body. Increasing the body density makes the body appear to contain less fat, suggesting that the final percent body fat measurement for the male subjects and the female subjects in the 75% diet group was smaller than the actual value. As a result, the post-diet total fat mass may have been underestimated in the male subjects and the female subjects who were in the 75% diet group.

Overall, limitations associated with using skinfolds to assess fat mass, as well as changes in hydration status of male subjects and female subjects in the 75% diet group challenges the validity of the loss of a large proportion of fat mass from total weight lost with an imposed short-term energy restriction.

4. EXERCISE

A) MALES

The average energy expenditure throughout the study duration for 30 minutes of jogging was 288 ± 18 kcal/day. During exercise, the male subjects maintained the recommended aerobic training heart rate at a range of 60-80% of maximum heart rate (Fox et al, 1993), indicated to stimulate improvements in endurance or performance (Fox et al, 1993). The heart rate reserve remained fairly constant (80%) between 5 and 30 minutes of exercise, indicating that the intensity of exercise was constant within this time period.

B) FEMALES

Although the distance run per week was less than the desired subject criterion of 50 km/week,the subjects were involved in various other activities such as biking and swimming. Their participation in other activities was evident from the large amount of time spent exercising and each subject did at least two activities per day.

The average energy expenditure per day (when considering days 1 to 9 of the study) was 832 ± 97 kcal/day, with the average energy expenditures being quite similar between the 75% and 100% diet groups. The variability in energy expenditure each day was high. This was probably due to the difference in type, intensity, duration, and frequency of the exercise performed among subjects.

Variability in reported energy expenditure may have also arisen through the difficulty associated with filling out the daily exercise regimen by the subject. For example, although the subjects specified the intensity of exercise performed daily, it may have been difficult to define intensity of exercise as being in one of three categories (defined on the exercise recording form as low, medium, and high according to the amount of effort exerted). Although the intensity of exercise was defined for the subjects on the

exercise recording form and recorded by the subjects, in calculating the energy expenditures from the diaries, it was difficult to interpret what subjects considered to be low, medium and high intensity.

An attempt to circumvent these problems and define the intensity of exercise involved having the subjects record their maximum heart rate during an exercise bout and comparing this heart rate value to the theoretical maximum heart rate for that subject. However, subjects did not always remember to do this. Also, different people have different maximum heart rate values. Thus, a value for maximum heart rate may not be predicted in an accurate manner from the equation 220-age (as was shown with the male subjects).

The amount of energy expended through exercise was high for the female subjects in both diet groups. However, the accuracy of these values is questionable due to limitations associated with the exercise recording form and its interpretation.

5. SUBSTRATE OXIDATION

There was no significant change in resting energy expenditure (REE) after diet and exercise treatment, even when corrected for body weight and fat-free mass (FFM) in both the male and female subjects. This is likely due to the short time period of the study. Furthermore, the sample size may have been too small to elicit a statistical difference in both male (n = 3) and female subjects (there were 7 subjects in each diet group).

In the male subjects there was no significant change in the resting RQ value as a result of the diet and exercise treatment. This signifies that there was no change in substrate oxidation at rest as a result of this treatment. However, the applicability of the results are questionable due to the small sample size.

The female subjects in the 75% diet group, on the other hand, had a significant decrease in the resting RQ value. This means that there was a change in substrate utilization in the female athletes as a result of the imposed energy restriction such that fat

was utilized to a larger extent under resting conditions compared to the pre-diet value. This is consistent with results from Froidevaux et al (1993) who found that the resting RQ value decreased from 0.86 ± 0.03 to 0.77 ± 0.02 in 10 moderately obese women.

The results obtained from the female subjects in the 75% diet group correspond with those obtained from Nelson et al (1992) who found that there was a significantly larger percent of resting energy expenditure derived from lipid after a 5 month diet restriction in obese females. However, the time period of these two studies is very different. The results obtained from the female subjects, concurrent with results obtained from other studies, demonstrate that an energy restriction created by diet and exercise promotes the utilization of fats as an energy source under resting conditions.

Both the male and female subjects experienced a change in substrate oxidation after the consumption of a standard test breakfast. Specifically, for the male subjects there was a significant decrease in the RQ value at 60, 120, and 150 minutes post-prandially. The applicability of these results may be questionable, however, again due to the small sample size.

The female subjects in the 75% diet group had significantly lower RQ values at 30, 120 and 150 minutes post-prandially as compared to their pre-diet value and compared to the change in the 100% diet group. Although the subject size of the male group limits the applicability of the results, the similar decreases in RQ values observed for males as was seen for the females indicated that fat utilization was elevated post-prandially as a result of the diet and exercise program.

The decrease in the RQ value at these time points may have also been due to the lower energy value of the meal that was fed post-diet to the 75% diet group. However, the significant decrease in the resting RQ value suggests that the decrease in RQ values is an effect of the diet , and not the meal.

These results are consistent with findings of Froidevaux et al (1993) who found that the post-absorptive RQ decreased from 0.81 ± 0.04 to 0.76 ± 0.03 in 10 moderately

obese women who lost weight as a result of a diet and exercise program. They also measured the rate of lipid, carbohydrate and protein oxidation before and during the weight loss period for 24 hours in a respiratory chamber and found that the rate of lipid oxidation was significantly increased as a result of weight loss (from 84 ± 31g/day to 141 ± 33 g/day).

6. MUSCLE PROTEIN DEGRADATION

To date, calpain activity has never been documented in human subjects. It is interesting to note in the present study that the myofibrillar calpain activity of the trained female athletes was about three times the value found for the male subjects, both pre- and post-diet. This elevated activity in the female athletes may have been due to a training effect on calpain activity.

It has previously been demonstrated that with high volume exercise, calpain activity increases with increasing intensity (Belcastro, 1995). Calpain activity in the plantaris muscle (which contains predominantly fast fiber types) of rats doubled when intensity was increased from 55% VO_2 max to 75% VO_2 max (Belcastro, 1995). Thus, in human muscle containing mixed fiber types, such as the *vastus lateralis* (the muscle in which calpain activity was assessed), the training program (involving high volume and high intensity exercise) in the female subjects may have caused the calpain activity to be elevated above that found for the male subjects. Reasons for the increased calpain activity in the trained female subjects may be due to: the regulation of phosphorylation of myosin light chain (through the interaction of myosin light chain kinase and calpain); regulation of glycolytic enzymes; myoblast rearrangement to accommodate fusion (through the action of calpain on glycoproteins; Bond and Butler, 1987); regulation of phospholipid turnover (due to the membrane being a site for calpain activation (Johnson, 1990)); modulation of critical transport processes of the membrane (Johnson, 1990); post-translational modification of proteins/enzymes (Johnson, 1990).

The effect of an energy restriction on myofibrillar calpain activity has also not previously been investigated in human subjects. In this study, an imposed energy restriction in the form of diet and exercise appeared to increase in myofibrillar calpain activity (half fold from the pre-treatment activity in the male subjects, two fold from the pre-diet value in the female subjects who were in the 75% diet group). However, these changes in activity remained statistically insignificant in both the female and male subjects. In both subject groups, this may have been due to the small sample size.

The absence of a significant change in myofibrillar calpain activity in the female group of subjects may have been due to high variability in calpain activity. The large standard deviations associated with the calpain activity of the female subjects may be related to the exercise regimen performed by each subject. Female subjects were instructed to exercise "as usual". Differences in intensity, time, frequency, and type of exercise performed may have been responsible for the large variability in myofibrillar calpain activities. The variability associated with exercise intensity has recently been documented, where calpain activity increased with increasing intensity in the plantaris muscle of rats (Belcastro, 1995). This intensity dependent increase in calpain activity was volume dependent, where the intensity effect was not seen when exercise volume was decreased. The type and frequency of exercise performed may also affect calpain activity, although the effect of these factors has not been previously documented. Thus, by controlling the exercise intensity, time, frequency, and type of exercise a lower variability in myofibrillar calpain activity may have been produced, and a statistical difference may have been possible.

In the male subjects, exercise intensity, duration, frequency, and type was controlled. This was associated with a much lower variability in myofibrillar calpain activity, such that the post-treatment variability was almost five times lower in the male subject group compared to the post-diet variability in the female subject group.

Although the subjects were instructed not to exercise 24 hours before their muscle biopsy, the timing of the muscle biopsy relative to the last bout of exercise may have also contributed to the variability in calpain activity in both the female and male subjects. For example, a higher calpain activity may have been detected if a muscle biopsy was taken 24 hours after exercise compared to the activity obtained with a biopsy that was taken 48 hours after exercise. It is unknown how long calpain activity remains elevated post-exercise. Thus, more controlled and standardized biopsy times may have decreased the variability in calpain activity.

Moreover, the site chosen for the biopsy within the vastus lateralis may have introduced variability into the calpain activity in both groups of subjects. Although the variability in calpain activity at different sites in the vastus lateralis has not been documented, it has previously been suggested that fast muscle fibers may be more predisposed to changes in calpain activity compared to slow muscle fibers (Belcastro, 1995). For example, Belcastro (1995) has previously shown that the exercise intensity and duration effects on calpain activity are only seen in the plantaris muscle in the rat, which consists predominantly of fast fibers, while no effect was seen in the rat soleus muscle (consisting of predominantly slow fibers). Therefore, because fiber types are distributed unevenly in different parts of the vastus lateralis (superficial versus central, for example), the imposed energy restriction may have induced changes at sites that were different from the site chosen to assess activity. Thus, localized changes in myofibrillar calpain activity may or may not reflect changes in calpain activity within the whole muscle.

The absence of a significant increase in myofibrillar calpain activity with an imposed energy restriction in both female and male subjects is in contrast to previous results in animals where an increase in myofibrillar calpain activity was observed in calorically restricted and fasted rats (Murray et al, 1991). Calpain activity was measured in hind leg skeletal muscle of mature Wistar rats, divided into four groups: control rats; 50% diet restriction (DR) over 48 hours; 75% DR over 48 hours; fasting for 48 hours. Calpain

activity, expressed as units per gram of muscle, was significantly increased in the 75% dietary restricted group and the fasted group compared to the controls. The calpain activity increased as the severity of the restriction increased (Murray et al, 1991).

Although this study showed that myofibrillar calpain activity is very sensitive to the dietary regimen, it also showed that a more severe energy restriction may be required to elicit changes in the calpain activity. Murray et al (1991) found that the increase in myofibrillar calpain activity of the 50% DR group was not significantly different from the controls. Thus, the 33% and 25% energy restrictions imposed on the male and female subjects respectively, may not have been severe and/or long enough to create the significant changes in myofibrillar calpain activity as seen in the study by Murray and co-workers (1991).

Results from this study are consistent with Kettelhut et al (1994) who found that activation of the calcium dependent pathway involving calpain was not responsible for the increased muscle proteolysis observed during fasting. They suggested that the increase in muscle proteolysis during fasting was attributable to an enhancement of the energy-requiring process by finding an increase in the ATP-dependent proteolytic pathway 1 day after food restriction in the *extensor digitorum* muscles of rats. Thus, the energy restriction imposed on the male and female subjects in this study may have activated other pathways involved in muscle proteolysis other than calpain.

Attaix et al (1994) and Mitch et al (1994) suggest that the ATP-dependent pathway involving ubiquitin and proteasomes is responsible for the enhanced degradation of muscle proteins as a result of energy restriction. This was concluded by observing that under conditions where intracellular ATP was depleted, the increased proteolysis due to fasting was almost completely suppressed (Medina et al, 1992; Wing and Goldberg, 1993). The rise in ATP-dependent proteolysis observed during starvation was associated with increased mRNA level for ubiquitin and the 14 kDa ubiquitin-conjugating enzyme (which mediates the formation of ubiquitin-protein conjugates), accumulation of ubiquitinated

proteins, and increased expression of subunits of the 20S proteasome (Medina et al, 1992; Wing and Goldberg, 1993). Upon refeeding, total and ATP-dependent proteolysis, levels of ubiquitin-protein conjugates, and expression of ubiquitin (Medina et al 1992), the 14 kDa enzyme (Wing and Banville, 1994), or proteasome subunits (Medina et al, 1992) returned to normal. Adenosine triphosphate-ubiquitin-stimulated proteolysis was also enhanced in the soluble extracts of skeletal muscle from fasted rabbits (Medina et al, 1992), in accordance with an increased mRNA level for the proteasome subunit.

Results from the current study are in contrast to those found by Fagan et al (1992) who suggested that the increase in myofibrillar protein degradation observed during an energy depleted state was associated with an increase in calpain activity. This was determined by observing that trans-epoxysuccinyl-L-leucylamido(4-guanidino)butane (also known as E64; a compound that inhibits the calpains and the lysosomal cysteine proteases) in combination with calcium resulted in a decrease in myofibrillar degradation in energy depleted rats.

Because there was no significant increase in calpain activity post-treatment or post-diet in either group of subjects it is also possible that myofibrillar protein degradation did not change as a result of the imposed energy restriction. The lack of a significant increase in myofibrillar protein degradation with an imposed energy restriction is consistent with prior results where an increase in myofibrillar protein degradation was not observed in rats who engaged in an exercise and a food restriction program or an exercise program (Kasperek et al, 1992). Rats were starved for 24 hours before exercise on a treadmill for 200-215 minutes and then they were re-fed ad libitum the day after exercise. All rats had pair-fed controls. Myofibrillar protein degradation was determined by the measurement of the rates of release of 3-MH from the perfused single Sprague-Dawley rat leg and was measured immediately after and 1 day following the exercise bout.

The findings of Kasperek et al (1992) were indirect, such that re-feeding after 1 day of starvation, resulted in an increased food consumption which obliterated the increased

protein degradation seen in rats who were fed before the exercise bout. Kasperek et al (1992) suggested that exercise prevents an increase in the rate of protein degradation caused by fasting. The mechanism responsible for this exercise-induced muscle protein sparing is unknown, but a conservation mechanism was implicated where muscle proteins are conserved when energy reserves are low (Kasperek et al, 1992).

It is difficult to make conclusions about the effect of an energy restriction on muscle protein degradation because the assessment methods of different studies, measure different aspects of myofibrillar protein degradation. For example, 3-MH reflects actin and myosin degradation, while calpain degradation reflects the degradation of the cytoskeletal and sarcolemmal proteins. Thus, further research is needed to find a method that accurately reflects myofibrillar protein degradation and to elucidate whether calpain degradation rates and 3-MH excretion represent myofibrillar protein degradation rates. A method of assessing muscle protein degradation would allow the effects of an energy restriction on muscle protein degradation to be clarified.

7. MUSCLE FUNCTION

Peak torque is often the parameter used to describe muscle strength. The peak torque values for the active subjects are unique in that they include both eccentric and concentric strength scores, whereas usually only concentric strength scores are presented (Snow and Blacklin, 1992). The value most often reported in the literature is the concentric knee extension torque at a slow movement velocity (usually 30 degrees/second) and a fast movement velocity (usually 180 degrees/second or 230-250 degrees/second).

The pre- (163 ± 24 Nm; mean, SEM) and post-diet (157 ± 10 Nm; mean, SEM) concentric peak torque values for the male subjects at a movement velocity of 180 degrees/second were larger than the value of 117 ± 23 Nm (Kannus and Beynnon, 1993) found for forty-three healthy males (178 ± 7 cm; 76 ± 8 kg; 29 ± 5 years). The pre- and

post-diet concentric peak values at a movement velocity of 30 degrees/second were lower than that found for elite male sprinters (267 ± 42 Nm; Alexander, 1990), as was expected.

The concentric peak torque values at a movement velocity of 30 degrees/second corrected for body weight pre- (1.89 ± 0.22 Nm/kg body weight) and post-diet (1.92 ± 0.11 Nm/kg body weight) at a movement velocity of 180 degrees/second were also lower compared to corrected torque value obtained for elite male sprinters (3.65 ± 0.6 Nm/kg body weight; Alexander, 1990). These values are lower than that found for physical education majors of 3.0 ± 0.7 Nm/kg body weight at 30 degrees/second (Smith, 1987).

The pre- and post-diet corrected and absolute eccentric peak torque values at a movement velocity of 30 degrees/second for the male subjects were lower than those found for elite male sprinters, (279 ± 57 Nm and 3.83 ± 0.8 Nm/kg body weight; Alexander, 1990). It was expected that the male subjects would have lower strength values than their athletes, as they were quite inactive.

The pre- and post-diet concentric peak torque values for the female subjects in the 100% (118 ± 9 Nm and 124 ± 8 Nm respectively, mean ± SEM) and 75% (116 ± 8 Nm and 109 ± 0 Nm respectively, mean ± SEM) diet groups at a movement velocity of 30 degrees/second were lower than those found for elite female sprinters (171± 40 Nm; mean, SEM; Alexander, 1990) and slightly higher than those found for nonathletic females (96 Nm; Snow and Blacklin, 1992).

The pre-diet corrected concentric peak torque at a movement velocity of 30 degrees/second (where torque was divided by body weight) values in the female subjects who were in the 100% (2.01 ± 0.15 Nm/kg body weight) and 75% (1.79 ± 0.12 Nm/kg body weight) diet groups was much lower than that found for elite female sprinters (2.95 Nm/kg body weight) for a knee extension (Alexander, 1990). This was expected as the subjects in this study were endurance athletes, not power athletes. It has previously been reported that endurance athletes had significantly lower concentric peak torque values at movement velocities ranging from 50 degrees/second to 300 degrees/second (Taylor et al,

1990). Thus, overall the female subjects had pre- and post-diet concentric peak torque values that were better than the average female, but were not as high as those reported for elite athletes.

The corrected pre- and post-diet eccentric peak torque values obtained at a movement velocity of 30 degrees/second for the female subjects who were in the 100% diet (pre-diet, 1.90 ± 0.03 Nm/kg body weight; post-diet, 2.07± 0.04 Nm/kg body weight) and the 75% diet groups (pre-diet, 1.72 ± 0.04 Nm/kg body weight; post-diet 2.00 ± 0.03 Nm/kg body weight) were also higher than the value of 1.52 ± 0.03 Nm/kg body weight previously found for 12 healthy females (Snow and Blacklin, 1992). Eccentric peak torque values could not be compared to those found for elite athletes as this parameter has not been frequently reported. The only significant decreases in muscle function post-treatment in the male subject group occurred with the concentric average contractions at a movement velocity of 30 degrees/second.

The only significant decrease post-diet in peak torque for the female subjects occurred with the concentric average contractions in 75% diet group at a movement velocity of 120 degrees/second. The corrected post-diet concentric average torque at a movement velocity of 30 degrees/second was also significantly lower for the 75% diet group compared to the 100% diet group.

These decreases in quadriceps muscle torque, post-diet in the female subjects who were in the 75% diet group, were accompanied by an increase in function at a movement velocity of 180 degrees/second where the post-diet eccentric average torque was significantly larger than the pre-diet value. This change in strength was unexpected.

This increase in post-diet eccentric average torque at a movement velocity of 180 degrees/second for the female subjects in the 75% diet group may have been due to a greater learning effect of this group after the initial testing period. Additionally, the female subjects in the 100% diet group also showed increases in muscle torque at the completion of the diet treatment, where the post-diet eccentric and concentric average torques at

movement velocities of 90 and 30 degrees/second respectively were significantly larger than the pre-diet value. This may have also been due to a learning effect.

In the 75% diet group, this learning effect may have been due to the fact that at a faster movement velocity, one cycle takes much less time (0.73 seconds) than at 30 degrees/second (4.35 seconds), making it more difficult for the subject to learn to integrate sensory feedback with motor output (Snow and Blacklin, 1992). Therefore, subjects may have had greater difficulty producing a smooth, uninterrupted movement at maximal effort at the faster velocity, especially during the eccentric phase of muscle contraction.

This phenomenon has previously been reported (Snow and Blacklin, 1992) where a movement velocity of 180 degrees/second gave the lowest within session ($r = 0.92$) and between session ($r = 0.75$) correlations compared to those found for a movement velocity of 30 degrees/second. In fact, the eccentric torque at a movement velocity of 180 degrees/second had the highest intra-subject coefficient of variation (8.9%) compared to other eccentric and concentric torque values obtained at movement velocities of 30 and 180 degrees/second (Snow and Blacklin, 1992). Therefore, additional practice may have been required for the female subjects in the 75% diet group to learn to perform as well as they did at the slower velocities.

It is also possible that the increases in muscle torque post-diet in both the female and male subject groups represented training effects. It has previously been shown that the combination of resistance strength training and a very low calorie diet results in increases in strength (Pronk et al, 1992).

Overall, the physical performance measures were not drastically impaired in either the male subjects or the female subjects within or between any dietary intervention group after weight loss. There were no significant trends in the absolute or corrected torque values pre- and post-diet for the male subjects and within each diet group for the female subjects. Furthermore, there were no trends in differences between the diet groups for the female subjects at the two time points. Thus, muscle strength and muscle performance was

relatively unimpaired after 10 days of an energy restriction in both male and female subjects

The fact that there was no overall decreases in muscle function in the female and male subject groups with an imposed energy restriction has been previously documented. Scott et al (1992) looked at the effects of a moderate caloric restriction on maximal physical performance in 36, mildly obese (30-40% body fat), premenopausal women (age 29-49 years). They consumed 1000 kcal/day with a diet composition of 20% fat, 60% carbohydrate, and 20% protein for 8 weeks. Muscle strength was assessed using a Cybex 340 isokinetic device during knee flexion and extension at a movement velocity of 60 degrees/second (Scott et al, 1992). Strength was determined by taking the highest torque value from one of three concurrent maximal efforts. Values for the pre- and post-diet measurements for eccentric (pre-diet, 137 ± 18 Nm; post-diet, 125 ± 20 Nm) and concentric (pre-diet, 80 ± 12 Nm; post-diet, 79 ± 14 Nm) peak torques were not significantly different. Scott et al (1992) suggested that in order to induce any negative changes in maximal physical performance measures, either one or more of the following must occur: a greater degree of caloric restriction is required; or a longer dietary period is needed. Thus, the absence of a change in muscle function in the male and female subject groups with an imposed energy restriction in this study may have been due to the fact that: the energy restriction was not severe enough to induce changes in muscle function; or the 10 day imposed energy restriction period was not long enough to induce changes in muscle function.

However, Pronk et al (1992) imposed a severe caloric restriction (intake was 520 kcal/day) with and without an endurance exercise program for a long period of time (90 days) and found no change in strength within and between groups. Strength was evaluated using the one-repetition maximum method with knee extension and knee flexion. The subject population consisted of severely obese females (n = 109). Thus, the applicability of results found by Pronk et al (1992) to the results of this study may be questioned.

Nonetheless, this study did demonstrate that a severe energy restriction imposed over a long period of time did not result in changes in muscle function.

Similarly, Fogelholm et al (1993) found that muscle function did not change in athletes following a 3 week gradual weight reduction program. Physical performance was assessed in 7 male wrestlers and 3 judokas using a 30-m sprint, vertical jumping height, and anaerobic performance. The 3 week diet restriction period involved a 1000 kcal/day decrease in energy intake by the subjects. There was a $2.7 \pm 0.5\%$ decrease in weight at the end of the diet period, which was similar to the weight loss experienced by the 75% diet group ($2.6 \pm 0.4\%$) in this study. The unchanged or even improved jumping height results indicated the preserved capability of the neuromuscular system to produce force. The results even suggested slightly better muscle mechanical function after the gradual weight loss program. Results from this study are not totally comparable to the results obtained from the female athletes because: different performance assessments were used; different athlete types were used (endurance versus more of a strength athlete); different diet protocols were used to induce weight loss, where the percent macronutrients and energy intake were not directly controlled.

Results from the current study are in contrast with those found by Horswill et al (1990) who found that physical performance was impaired as a result of rapid weight loss. Physical performance was evaluated in 12 highly trained collegiate wrestlers both before and after the 4-day period of a hypocaloric low carbohydrate diet (11.4% protein, 46.7% fat, and 41.9% carbohydrate) with eight 15 second intervals of maximum effort sprints. After a 6% weight loss, there was nearly a three fold decrease in sprint work. It was suggested that the impaired performance after rapid weight loss was due to an alteration in blood volume (due to water loss) or an inhibition of glycolysis due to elevated free fatty acid levels in the blood. Results from this study are not completely comparable to the results obtained from the current study due to the fact that weight loss was rapid, and due to the fact that the ingested diet was high in fat.

It is also possible that the imposed energy restriction in the female and male subjects resulted in a decrease in aerobic capacity, without a subsequent change in muscle strength. For example, Bender and Martin (1986) found that there was a decrease in endurance in 12 healthy females consuming an energy intake at 40% of normal daily intake. Endurance was assessed by measuring time to volitional exhaustion while walking on a treadmill that increased grade by 3% every 3 minutes. The time to volitional exhaustion was shorter following caloric restriction than after the normal diet ($p < 0.001$). Thus, the effect of an energy restriction may affect performance by decreasing endurance. This question was not evaluated in this study and deserves further attention.

Overall, the fact that there was no change in muscle function with an imposed energy restriction is consistent with results obtained from many studies. However, comparisons made with other studies are limited by: the subject population; the diet and/or exercise protocol utilized; the severity and duration of energy restriction.

Results obtained from this study are also limited by factors associated with using the Kin/Com isokinetic dynamometer to assess muscle function. For example variability may have been introduced into the measured torque values as a result of body stabilization, axial alignment, subject motivation, and the subject skill level. Although care was taken to align the knee axis with the machine's exercise-arm axis, the exact positioning was not precise, and there was a tendency for the subject's thigh to come forward during a test. Axial alignment, therefore, likely accounts for a significant portion of the variance.

8. POSSIBLE RELATIONSHIPS ELUCIDATED FROM THIS RESEARCH

Two main associations were evaluated in order to test the hypothesis that substrate oxidation changes induce changes in calpain activity, which subsequently causes muscle function to change:

1) the effect of substrate oxidation on calpain activity

2) the effect of calpain activity on muscle function

A) SUBSTRATE OXIDATION AND CALPAIN ACTIVITY

When stepwise regression analyses were performed on calpain activity and substrate oxidation measures (female subject group only). Significant relationships between calpain activity and substrate oxidation measures in the female subject group were not established pre-diet.

However, significant relationships were established between the post-diet calpain activity and the RQ values obtained 120 minutes and 150 minutes post-prandially. Calpain was inversely related to the RQ obtained 120 minutes post-prandially and was proportionally related to the RQ obtained 150 minutes post-prandially. This means that as the post-diet calpain activity increased the RQ obtained 120 minutes post-prandially decreased, while the RQ obtained 150 minutes post-prandially increased. A decrease in RQ at 120 minutes would indicate an increase in fat oxidation, while an increase in RQ at 150 minutes would indicate a decrease in fat oxidation.

The post-diet calpain activity was determined to a larger extent by the RQ obtained 120 minutes post-prandially, compared to the RQ obtained 150 minutes post-prandially, as was evident by the larger standard correlation coefficient obtained at 120 minutes (-3.56; $p = 0.010$) compared to 150 minutes (2.66; $p = 0.020$). However, the coefficients are close in value, which signifies that although RQ 120 is more substantial in determining post-diet calpain activity, RQ 150 is almost equally contributive to determining the value of the post-

diet calpain activity. Thus, there was no consistent increase in fat oxidation which causally influenced increased calpain activity.

Furthermore, the change in RQ obtained 60 minutes post-prandially which was positively correlated with the change in calpain activity within the 75% diet group suggests that as fat oxidation decreased (signified by an increase in the RQ value) the calpain activity increased.

Therefore, it is not known whether an increase in fat oxidation results in an increase in calpain activity due to the conflicting results obtained. However, it seems likely that changes in substrate oxidation do not change calpain activity as the significant increase in fat oxidation associated with an imposed energy restriction in this study was not consistently associated with an increase in calpain activity.

Overall, the relationship of substrate oxidation and calpain activity remains to be elucidated. Further research is needed to investigate changes in substrate oxidation induced by varying the severity/intensity of diet and/or exercise protocols respectively and the subsequent changes in calpain activity.

B) MUSCLE FUNCTION AND CALPAIN ACTIVITY

There was no overall change in muscle function or in calpain activity. In fact, the exact relationship between these two variables is inconclusive due to the differing results obtained with the male and female subjects. For example, significant positive correlations (obtained through simple linear regression between muscle function parameters and calpain) of the post-treatment calpain activity with eccentric average and peak torques at a velocity of 120 degrees/second for the male subject group suggests that muscle function increases with an increase in calpain activity. The changes between these variables for the male subject group were also significant and were directly proportional to one another.

However, significant correlations (obtained through simple linear regression between muscle function parameters and calpain) between calpain activity and muscle function for the female subjects were negative signifying that as calpain activity increased, muscle function decreased, which was the hypothesized relationship. Most of the significant correlations between calpain activity and muscle function parameters occurred pre-diet and with concentric contractions. In fact, all of the pre-diet concentric average and peak torques were significantly correlated with the pre-diet calpain activity in the whole group of subjects, while only the pre-diet eccentric peak torques were significantly correlated to the pre-diet calpain activity. Although the correlations between the pre-diet concentric contractions and calpain were all highly significant, the pre-diet calpain activity was most strongly related to: concentric average torque, 120 degrees/second (beta = -0.70; p = 0.0081); concentric peak torque, 120 degrees/second (beta = -0.68; p = 0.0140); concentric average torque, 90 degrees/second (beta = -0.66; p = 0.0137); concentric average torque, 90 degrees/second (beta = -0.66; p = 0.0131).

The only significant correlations that occurred between post-diet muscle function parameters and post-diet calpain activity for the female subjects occurred with the concentric contractions in the 100% diet group. Similar to pre-diet correlations, a high calpain activity was associated with a low torque value. The post-diet calpain activity in the 100% diet group was most strongly related to: concentric peak torque, 90 degrees/sec (beta = -0.87; p = 0.0140); concentric average torque, 120 degrees/sec (beta = -0.82; p = 0.0254); concentric peak torque, 120 degrees/sec (beta = -0.81; p = 0.0261).

A highly significant negative correlation between the change in concentric peak torque at a movement velocity of 180 degree/second and calpain activity within the 75% diet group revealed that the change in calpain activity and muscle function assessed at a high movement velocity were inversely related.

Because more tension per motor unit is generated with eccentric contractions (Vander et al, 1990), it was thought that the effect of changes in sarcomere structure

induced by calpain may have been more profound in eccentric versus concentric contractions. However, this effect was not seen in the current study as the correlation between calpain activity and eccentric contractions were much less frequent. This may have been due to the fact that results obtained from eccentric contractions were more erratic, as the subjects found that the downward phase of contraction was more difficult to perform maximally. The greater amount of difficulty associated with producing smooth, uninterrupted movement during the lengthening phase of the quadriceps muscle group contractions has previously been documented in a group of 12 healthy women (Snow et al, 1992).

The extent to which whole quadriceps muscle group function is determined by calpain activity evaluated in a small sample of muscle (15-35 mg) is questionable. For example, calpain may have induced localized changes in muscle function within the vastus lateralis for which the vastus lateralis muscle may have been able to compensate. It is unknown: if calpain activity varied within the muscle; if calpain produced localized or whole muscle functional changes; or if the whole muscle was able to compensate for localized changes in calpain activity if they did occur. Although an inverse relationship is suggestive between myofibrillar calpain activity and quadriceps group muscle function in the female subject group, a directly proportional relationship is suggestive in the male subject group. Therefore, the relationship between calpain activity and muscle function is inconclusive.

The lack of a significant decrease in quadriceps muscle group function and change in myofibrillar calpain activity with an imposed energy restriction in the female subject group could have been due to metabolic adaptations in muscle protein structure previously present before the period of diet manipulation. The fact that these women had been competitively involved in endurance sports for the past 9.4 ± 1.0 years may have induced the myofibrillar protein adaptations allowing them to maintain muscle function with a succeeding imposed energy restriction.

The reasons for the discrepancies between the results obtained from the males and the females may be due to the difference in sample size. Furthermore, the difference in the exercise regimen performed by the male and the female subjects may have affected the relationship as the exercise regimen performed by the female subjects was much more intense and of longer duration than that performed by the male subjects. The total energy deficit created in the male subject group may not have been large enough to produce changes in muscle function and/or calpain activity. Additionally, the difference in training status between the males and the females may have influenced the relationship between calpain and muscle function. In summary, more research is required to define the relationship between myofibrillar calpain activity and muscle function under well-controlled conditions.

9. FURTHER RESEARCH:

Future research is required on:

1. substrate oxidation and calpain activity, to establish a more specific relationship between the two factors. This may be accomplished by increasing the severity and length of an imposed energy restriction.

2. exercise and calpain activity, where the effects of endurance exercise frequency, type, intensity and duration need to be established.

3. exercise and calpain activity, where the length of time in which calpain activity is modified post-exercise needs to be established.

4. dietary/exercise factors related to calpain activity such as lean body mass, training status, athlete type (strength or endurance), etc.

5. establishing the reliability and validity of the calpain assay so that the variability in calpain activity can be established.

6. the variability in calpain activity at different sites of a whole muscle and evaluation of calpain activity obtained from a muscle sample and whole muscle calpain activity.

7. the difference in calpain activity between males and females.

8. the relationship between calpain activity and 3-MH release to determine if selective protein degradation rates are proportionally related to contractile protein degradation rates (assessed through 3-MH) for muscle and whole body changes.

9. developing a procedure for the assessment of skeletal muscle protein degradation rates of selective proteins.

10. STUDY LIMITATIONS

1. The lack of control of energy expenditure by the female subjects may have been responsible for producing the large variability in calpain activity. Control of the frequency, time, type, and intensity of exercise of the female subjects may have introduced less variability in calpain activity, and therefore enhanced statistical power.

2. The lack of control of the timing of the muscle biopsy may have also contributed to the variability in calpain activities, as some subjects may have exercised 24 hours before the biopsy, while others may have been resting for more than 24 hours.

3. Lack of conclusive knowledge as to whether myofibrillar calpain activity obtained from a small muscle sample reflects the overall calpain activity of the whole muscle.

4. Lack of validity of the calpain assay used to assess calpain activity in the vastus lateralis in human subjects.

5. Relating structural changes at the level of the myofibril to whole muscle group function.

6. Small sample size.

CONCLUSION

This study initially set out to determine if an imposed energy restriction in the form of diet and exercise resulted in an increase in fat oxidation, which subsequently may have increased calpain activity, which subsequently may have decreased muscle function. Overall, two relationships were established throughout this investigation:

1) an imposed energy restriction resulted in an increase in resting fat oxidation in the female subjects (nonsignificance in the male subjects).

2) high calpain activity was correlated with low muscle function for some parameters (only in female subjects).

The results from this research support the concept that an imposed energy restriction results in an increase in fat oxidation. This was evident by the decreases in the post-prandial RQ values as a result of the imposed energy restriction in both male and female subjects and a decrease in the resting RQ value for the female subjects.

Additionally, it was inconclusive as to whether the changes in substrate oxidation induced by the energy restriction resulted in increases in calpain activity. The relationship of calpain and substrate oxidation as well as other factors that may be related to calpain activity (muscle damage for example) still requires further investigation.

Although there were selective changes in muscle function, there was no change in quadriceps muscle function due to the lack of consistent trends within both sets of data. Increases in muscle torque observed in the female subjects were most likely due to a learning effect.

The fact that myofibrillar calpain activity did not change with an imposed energy restriction indicated that a calpain induced activation of muscle proteolysis did not occur, alluding to the idea that another mechanism may be involved in increasing muscle proteolysis (if increased at all).

However, involvement of calpain cannot be completely ruled out. Perhaps the energy restriction was not severe enough or of long enough duration. Furthermore, characteristics of the female subjects (athletically trained; previously restricting their energy intake) may have induced myofibrillar adaptations that may have resulted in no change in calpain activity when a subsequent energy restriction was imposed. Additionally, the ability to find a statistical difference may have been limited by small sample size in both subjects groups and a high variability in the female subject group (resulting from an uncontrolled exercise regimen).

Whether changes in calpain activity directly affect changes in muscle function was not demonstrated. This also may have required a more severe and lengthy energy restriction.

Both of these associations, energy restriction on substrate oxidation, and calpain activity on muscle function, have meaningful nutritional and functional implications. Energy restriction induces changes in substrate oxidation. Although the results between substrate oxidation and calpain activity are inconclusive, changes in substrate oxidation may induce an increase in myofibrillar calpain activity. Further research is required to establish this in human subjects. Furthermore, the relationship between calpain activity and muscle function is inconclusive and may depend on gender and training status. Definition of this relationship may be important because an increase in calpain activity may result in a decrease in muscle function which can affect performance of individuals in occupations involving physical labor, as well as performance of athletes in both recreational and competitive sports.

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Appendix 1

Three-day rotating menu plan for a caloric intake of 2700 Kcal.

	Breakfast		Lunch		Dinner		Snack	
	Amt	Type	Amt	Type	Amt	Type	Amt	Type
Day 1	238 ml 22 g 88 g 7 g 20 g 25 g 238 ml	orange juice Cheerios Whole Wheat Bread Margarine Peanut Butter Jam Skim milk	222 g 14 g 130 g 10 g 100 g 70 g 250 ml	Spaghetti Margarine Tomato Sauce Parmesan Banana Ice cream Skim milk	60 g 88 g 14 g 20 g 166g 15 g	Cheese Whole wheat bread Margarine Mayonn-aise Apple Salad	89 g 14 g 250 ml	Muffin Margarine Skim milk
Day 2	250 ml 30 g 88g 7 g 20 g 25 g 250 ml	Apple juice Shreddies Whole Wheat Bread Margarine Peanut Butter Jam Skim Milk	145 g 21 g 250 g 28 g 166 g	Mixed vegetables Margarine Rice Shortbread Cookies Apple	60 g 50 g 88 g 14 g 20 g 120 g 200 g	Tomato Cucumber Whole wheat bread Margarine Mayonn-aise Grapes Yogurt	89 g 14 g 250 ml	Muffin Margarine Skim milk
Day 3	250 ml 26 g 88 g 7 g 20 g 25 g 250 ml	Orange Juice Raisin Bran Whole Wheat Bread Margarine Peanut Butter Jam Skim Milk	350 g 80 g 30 g	Meat Lasagna Grapes Chocolate chip cookies	80 g 88 g 14 g 20 g 160 g 55 g 100 g	Tuna Whole wheat bread Margarine Mayonn-aise Yogurt Banana Carrot sticks	89 g 14 g 250 ml	Muffin Margarine Skim milk

Appendix 2

Mean nutrient intake in experimental diets*.

Nutrient	Day 1	Day 2	Day 3	Average
calories (kcal)	2740	2634	2681	2685
Protein (g)	96.7	72.0	126.0	98.2
Carbohydrates (g)	362.0	416.0	368.0	382.0
Fiber (g)	31.0	35.9	31.6	32.8
Total Fat (g)	97.5	76.0	81.0	84.8
Saturated Fat (g)	39.3	23.3	33.2	31.9
Monounsaturated Fat (g)	45.1	34.5	39.0	39.5
Polyunsaturated Fat (g)	38.5	31.2	32.2	34.0
Cholesterol (mg)	166	83.7	156	135
Vitamin A - Carotene (RE)	251.0	688	3017	1319
Vitamin A - Preformed (RE)	1566.0	990	1142	1233
Vitamin A - Total (RE)	1817.0	1678	4159	2551
Thiamin (mg)	1.84	2.04	2.15	2.01
Riboflavin (mg)	3.03	2.34	2.87	2.74
Niacin (mg)	20.7	20.9	31.8	24.5
Vitamin B_6 (mg)	2.61	1.51	2.52	2.22
Vitamin B_{12} (mg)	5.32	2.94	6.46	4.91
Folacin (ug)	315	252	375	314
Pantothenic Acid (mg)	7.12	6.37	6.26	6.58
Vitamin C (mg)	179	52	166	132
Vitamin E (mg)	27.5	16.6	26.2	23.4
Calcium (mg)	2007	1287	1917	1737
Copper (mg)	1.77	1.86	2.30	1.98
Iron (mg)	16.0	17.1	29.5	20.9

Magnesium (mg)	507	465	504	492
Phosphorus (mg)	2151	1765	2252	2056
Sodium (mg)	4357	2792	4254	3801
Potassium (mg)	3763	3140	4004	3636
Selenium (mg)	178	182	224	195
Zinc (mg)	12	11.1	14.2	12.4
Protein (%)	15	11	19	15
Carbohydrates (%)`	53	62	54	56
Fat (%)	32	26	27	28
Polyunsaturated: saturated fat	1.0 : 1.0	1.3 : 1.0	1.0 : 1.0	1.1 : 1.0
Sodium:Potassium	1.2 : 1.0	0.9 : 1.0	1.1 : 1.0	1.0 : 1.0
Calcium:Phosphorus	0.9 : 1.0	0.7 : 1.0	0.9 : 1.0	0.8 : 1.0

*Determined by Food Processor II Nutrient Analysis Software (ESHA Research, Salem OR).
prior to the study.
No significant differences.
Comparison's made by student's unpaired t-test.

Appendix 3

DAILY PHYSICAL ACTIVITY RECORD

A. Running:

1. Description of run:

2. Time length of run:

3. Approximate mileage covered in the run:

4. Frequency (# of occasions during the day):

5. Intensity:

a) Check one of the following off using the criteria listed in the instructions:

Light:_____ Medium:_____ Heavy:_____

Light: slight change from normal state

Medium: some perspiration, faster than normal breathing

Heavy: heavy perspiration, heavy breathing

b) Heart rate values at:

Beginning:_____ Middle:_____ End:_____

of exercise

6. Does this reflect a normal, average run for you?

Yes_____ No_____

Appendix 3 (continued)

For activities other than running please fill out the following questionnaire daily.

Indicate the physical activities in which you have participated in on this day:

	Number of occasions during the day	Average number of minutes spent on each activity				Slight change from normal state	Some perspiration, faster than normal breathing	Heavy perspiration, heavy breathing
	FREQUENCY	DURATION				INTENSITY		
		1-15	15-30	31-60	60+	LIGHT	MEDIUM	HEAVY
Walking for Leisure								
Walking for Exercise								
Exercise Classes								
Home Exercises								
Popular Dance								
Gardening								
Bicycling								
Swimming								
Tennis								
Baseball/Softball								
Racquetball								
Volleyball								
Basketball								
Golf								
Bowling								
Ice Hockey								
Cross Country Skiing								
Ice Skating								
Others - Please specify:								
_____								
_____								
_____								

Appendix 4:

MALE VOLUNTEERS REQUIRED
FOR
DIET COMPOSTION AND MUSCLE FUNCTION
STUDY

Male subjects are required to participate in a test period of 10-12 days where diet and exercise regimens will be imposed for the purpose of looking at the effects of weight loss. Diets will will be provided at approximately 75% of usual caloric intake, and subjects will exercise approximately 30 minutes per day. Subjects are required to eat most meals for 10-12 days at the School of Family and Nutritional Sciences.

The following test will be completed at the beginning and end of the diet and exercise period:

1. Metabolic Rate
2. Body Composition
3. Muscle Function

Subjects should be:

- healthy males between 18-35 years
- non-smokers
- average body weight
- sedentary
- free from chronic illness

What subjects get:

- free meals for 10 days

For information call: 822-6869

Investigators:

Dr. Linda McCargar, School of Family and Nutritional Sciences
Dr. Angelo Belcastro, School of Human Kinetics

Please take one:

822-6869 822-6869 822-6869 822-6869 822-6869 822-6869 822-6869 822-6869 822-6869 822-6869 822-6869 822-6869 822-6869 822-6869 822-6869 822-6869

Appendix 5:

FEMALE RUNNERS REQUIRED FOR DIET COMPOSITION AND MUSCLE FUNCTION STUDY

Female runners are required to participate in a 14 day period where a dietary regimen will be imposed for the purpose of looking at the effects of dieting in athletes. Diets will be provided at approximately 75% or 100% of usual caloric intake, and subjects will be expected to exercise according to their regular schedule. Subjects are required to eat breakfast and lunch at the School of Family and Nutritional Sciences, as ALL MEALS WILL BE PROVDIED FOR 14 DAYS by the School of Family and Nutritional Sciences.

The following tests will be completed in this time period:

1. Metabolic Rate
2. Body Composition
3. Muscle Function (through a muscle biopsy and a Kin-com test)

Subjects should be:

- Healthy females between 18-35 years old
- Running at least 50 km/week
- Average body weight
- Free from chronic illness
- Taking oral contraceptives

What subjects get:

- Body composition analysis
- Diet analysis
- Muscle function and muscle protein analysis
- Free meals for 14 days at a 100% or a 75 % maintenance diet

For information call: 822-6869

Investigators:

Dr. Linda McCargar, School of Family and Nutritional Sciences.
Dr. Angelo Belcastro, School of Human Kinetics.
Sabina Parkes, School of Family and Nutritional Sciences.

Appendix 6

CONSENT FORM

The University of British Columbia

Investigators: S.C. Parkes BSc., Dr. L. McCargar., Dr. A. Belcastro, Dr S. Barr.

Title of the Project: Human Muscle Function and Structure Following a Combined Energy Reduced Diet and Increased Energy Expenditure

Many people in Canada are involved in weight reduction programs, which involve exercising and calorie reduction. The purpose of this project is to determine if the energy deficit created by exercise and a low calorie intake comprimises muscle function and muscle structure.

I understand that as a participant in this study, I will be required to:

1. Keep one 4-day food record, answer questions regarding my diet and lifestyle.

2. Have body weight, height, and body composition (using calipers) measured at the beginning and end of the study.

3. Be willing to follow a diet for 10 days which encourages a decreased intake of calories. This diet will include regular foods that will be provided by the Nutritional Sciences department at UBC.

4. Be willing to eat breakfast and lunch at UBC, and to eat dinner and a snack as prepared by a UBC student wherever you feel comfortable.

5. Be willing to participate in an exercise program consisting of 30 minutes/day for a 10 day period. Each 30 minute session will be lead by a knowledgable supervisor.

6. Be willing to come to UBC and have my metabolic rate measured at the beginning and end of the study.

7. Be willing to do a 24 hour urine collection.

8. Be willing to undergo a muscle biopsy by a trained doctor, as well as undergo a leg muscle function test

on a Cybex machine at the Sports medicine clinic at UBC.

It is unlikely that any side effects will occur as a result of participating in this study. My participation in this study is voluntary. I am aware that I may refuse to participate or that I may withdraw from the study at any time.

If I have further questions concerning this study, I can contact Sabina Prkes at 224-9175 or Dr. Linda McCargar at 822-6869, or Dr. Angelo Belocastro at 822-3685.

Thus, I _____ hereby volumtarily consent to participate in this study and acknowledge that I ahve received a copy of this consent form. I have read and understood the contents of this form.

Date: _____

Signature of Participant: _____

Signature of Witness: _____

THE UNIVERSITY OF BRITISH COLUMBIA

Appendix 7

School of Family and Nutritional Sciences
Division of Human Nutrition
2205 East Mall
Vancouver, B.C. Canada V6T 1Z4

CONSENT FORM

The University of British Columbia

Investigators: S.C. Parkes BSc., Dr. L. McCargar, Dr. A. Belcastro, Dr. S. Barr.

Title of the Project: Human Muscle Function and Structure Occurring with a High Level of Energy Expenditure and a Low Energy Intake

Many female athletes training at a high level of intensity in Canada have irregular eating patterns, of which a low energy intake is one example. The purpose of this project is to determine if the energy deficit created by a high level of activity and a low calorie intake compromises muscle function and structure, and therefore athletic performance.

I understand that as a participant in this study, I will be required to:

1. Keep a 4-day food record.
2. Have my body weight, height, and body composition (with calipers) measured.
3. Keep a record of the exercise performed for a 14 day period.
5. Be willing to come to UBC and have my metabolic rate measured twice.
6. Be willing to do two three-day urine collections.
7. Be willing to undergo a muscle biopsy by a trained doctor, as well as undergo a leg muscle function test on a Kin-com machine at the Rehabilation Centre at UBC.
8. Be willing to undergo a VO_2 max test at the Sports Medicine Clinic at UBC.

9. Be willing to follow a diet for 10 days which encourages a decreased intake/normal intake of calories. This diet will include regular foods that will be provided by the Nutritional Sciences Department at UBC.

10. Be willing to eat breakfast and lunch at UBC, and to eat dinner and a snack as prepared by a UBC student wherever you feel comfortable.

Overall, the study requires about 2.5 hours/day. This includes time required for ingestion of all meals for the day, recording exercise done that day, and measurements made at the beginning and end of the study.

It is unlikely that any side effects will occur as a result of participating in this study as an experienced physician will perform the biopsy. However, four moderate hematomas in 1000 biopsies have been reported. The muscle biopsy may cause some early discomfort, such as mild local tenderness, and may cause mild to moderate limitations in daily activities.

My participation in this study is voluntary. I am aware that I may refuse to participate or that I may withdraw from the study at any time.

The principal investigators, the 3 co-investigators and their assistants, and I will be the only people who will have access to the data until it is in published form. As a subject, I will have a code number by which all my data and information will be identified. My code number will be kept confidential by the investigators.

If I have further questions concerning this study, I can contact Sabina Parkes at 224-9175 or Dr. Linda McCargar at 822-6869, or Dr. Angelo Belcastro at 822-3685.

Thus, I _____ hereby voluntarily consent to participate in this study and acknowledge that I have received a copy of this consent form. I have read and understood the contents of this form.

Date: _____

Signature of Participant: _____

Signature of Witness: _____

Appendix 8

GUIDELINES FOR KEEPING A FOOD RECORD

A FOOD RECORD IS A DETAILED DESCRIPTION OF EACH FOOD OR BEVERAGE ITEM TAKEN OVER 24 HRS OF A DAY. AN ACCURATELY COMPLETED FOOD RECORD CAN PROVIDE VALUABLE INFORMATION ABOUT THE NUTRITIONAL CONTENT OF AN INDIVIDUAL'S USUAL DIET.

TO ASSESS YOUR DIET RECORD CORRECTLY, WE MUST BE ABLE TO CLEARLY PICTURE THE FOODS AND BEVERAGES THAT YOU HAVE RECORDED. THE GUIDELINES BELOW DESCRIBE THE INFORMATION THAT IS IMPORTANT FOR YOU TO RECORD. PLEASE READ THESE GUIDELINES BEFORE YOU START.

PLEASE KEEP A RECORD OF EVERYTHING THAT YOU EAT OR DRINK ON THE ATTACHED FORMS FOR 4 DAYS IN A ROW. INCLUDE 1 WEEKEND DAY IN THE RECORDING PERIOD.

1. THE PORTION SIZE (QUANTITY) NEEDS TO BE ACCURATELY RECORDED
   -Please don't guess if you can measure!

It may be helpful to measure how much your regular glasses, cups and bowls contain before you start. You can describe portion sizes in as many ways as you like. The attached food pictures are provided to help you with portion sizes.

For example, you might record:

Volume

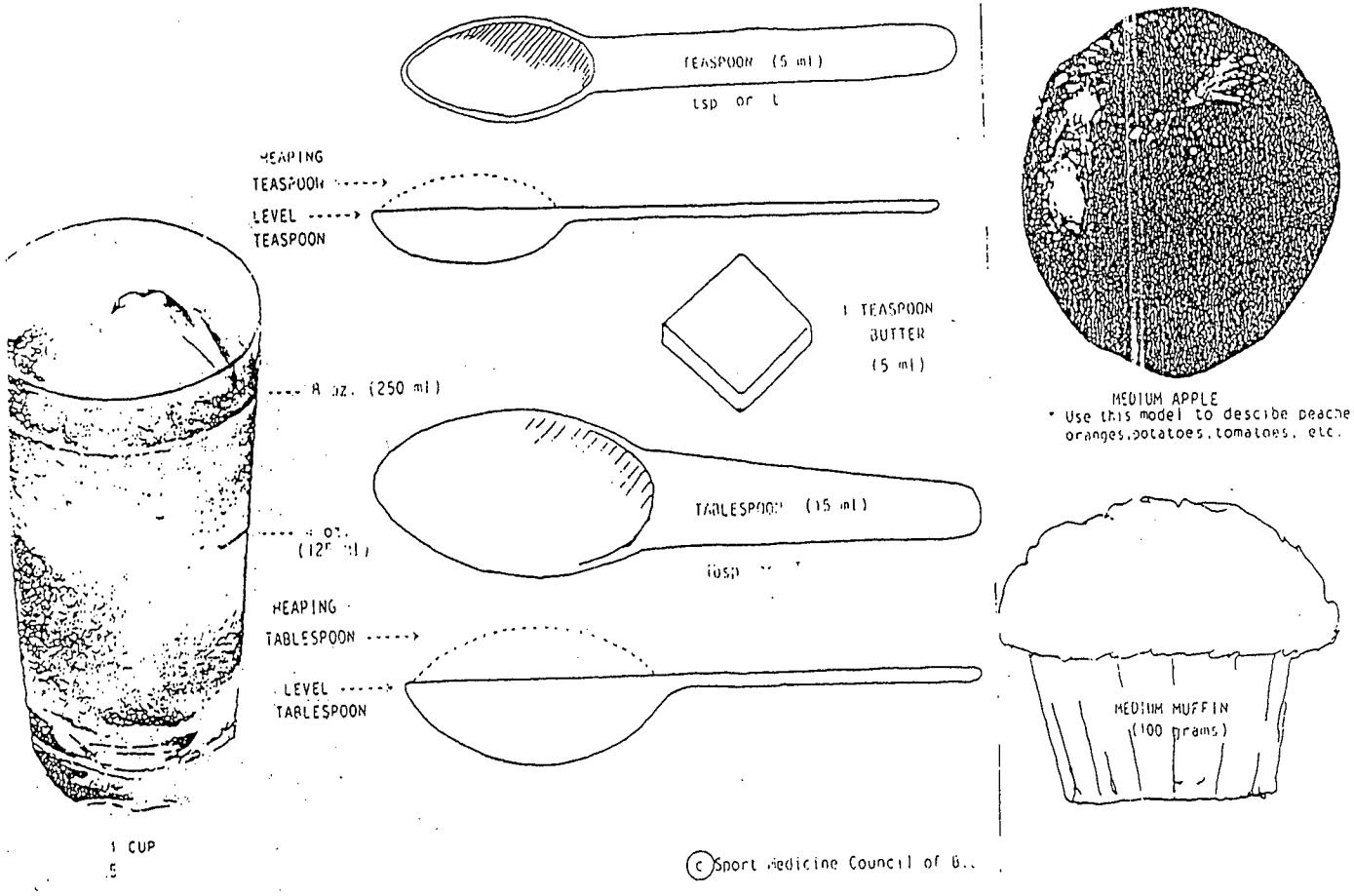
1 cup or 8 oz or 250 mL of 2% milk
1 tablespoon or 15 mL or peanut butter or cream cheese
1 teaspoon or 5 mL of sugar or honey

Size

1 "2 inch by 3/4 inch by 3/4 inch" piece of cheddar cheese
1 medium egg, poached
1 small apple
1 2" diameter digestive biscuit
1 medium bran muffin

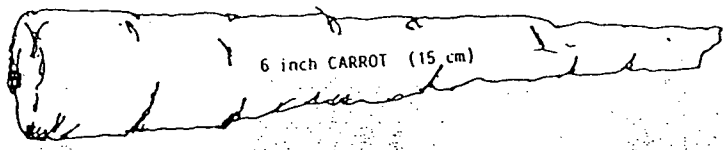
Weight

2 ounces or 60 grams of lean hamburger meat or chicken or fish (use labels on packages to help you)

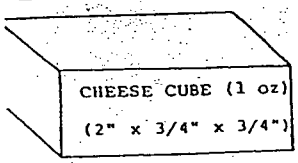


SCALE (cm) - TO HELP IN DETERMINING DIMENSIONS

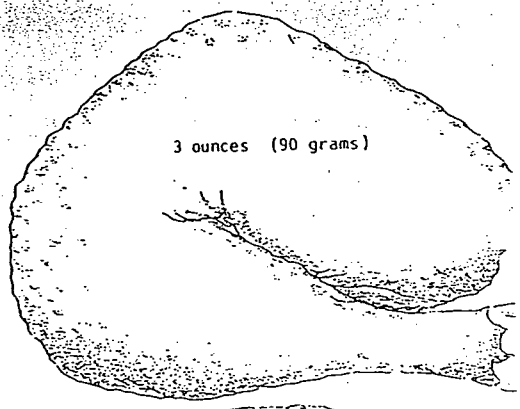
. SIZE



III. WEIGHTS



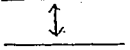
ounces of raw meat = 3 ounces of cooked meat



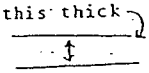
If chicken breast and wing or one leg and thigh cooked equal to about 3 ounces meat.

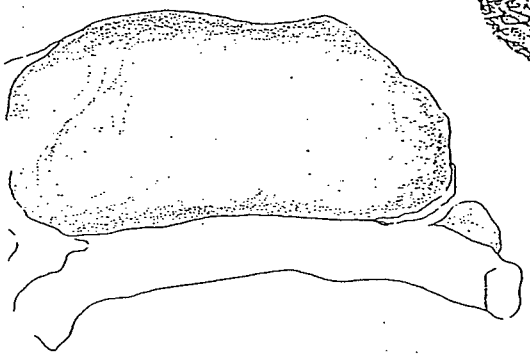
1 ounce= 30 grams

One hamburger pattie this thick is equal to 3 ounces.

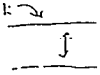


One hamburger pattie this thick is equal to 2 ounces.



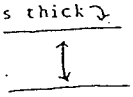


A pork chop this thick is equal to 3 ounces.

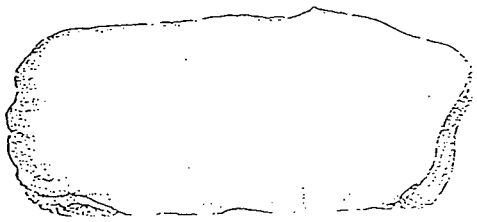


A pork chop this thick is equal to 2 ounces.

One slice of meat this thick equal to 3 ounces.



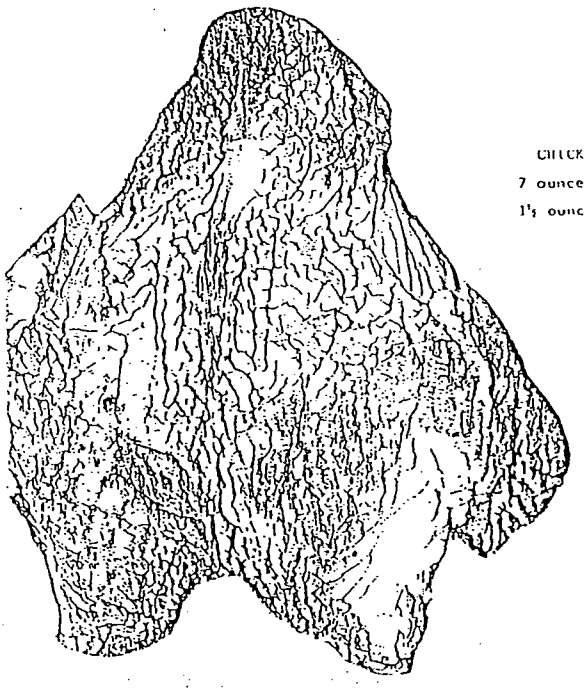
Two slices of meat this thick are equal to 3 ounces.



WEIGHTS CONT'D

STEWING MEAT (cooked)

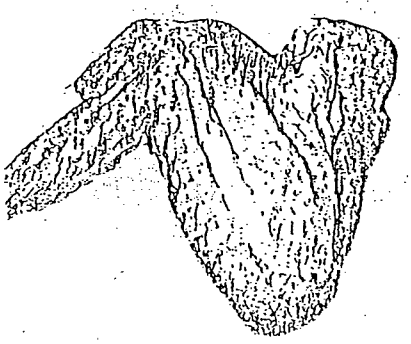
One piece of cooked stewing meat this size is equal to ½ ounce.



CHICKEN BREAST (cooked)

7 ounces lean flesh

1½ ounces skin plus fat



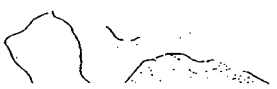
CHICKEN WING (cooked)

½ ounce lean flesh

½ ounce skin plus fat

Two slices are equal t

One slic is equal



Appendix 9

TIME & PLACE			FOOD & BEVERAGE ITEMS	QUANTITY	
Hour & Min. AM/PM	HOME	AWAY	Use a separate line for each items. Describe carefully as if writing a recipe.	Specify each measure: g. oz. tsp. cup	Fo Us C

Coder _____ ID _____ Number _____ Ente

Appendix 10

1983 Metropolitan Life Insurance Values for Desirable Weights for Height for Adults

Weights are for the lowest mortality in ages 25-59 years. Individuals were weighed wearing indoor clothing weighing 1.4 kg (3 lb) and shoes with 2.5 cm (1 in) heels.

Males				Females			
7A. Desirable Weight (kg)							
Height cms	Small Frame	Medium Frame	Large Frame	Height cms	Small Frame	Medium Frame	Large FrameAge
158	58.3-61.0	59.6-64.2	62.8-68.3	148	46.4-50.6	49.6-55.1	53.7-59.8
159	58.6-61.3	59.9-64.5	63.1-68.8	149	46.6-51.0	50.0-55.5	54.1-60.3
160	59.0-61.7	60.3-64.9	63.5-69.4	150	46.7-51.3	50.3-55.9	54.4-60.9
161	59.3-62.0	60.6-65.2	63.8-69.9	151	46.9-51.7	50.7-56.4	54.8-61.4
162	59.7-62.4	61.0-65.6	64.2-70.5	152	47.1-52.1	51.1-57.0	55.2-61.9
163	60.0-62.7	61.3-66.0	64.5-71.1	153	47.4-52.5	51.5-57.5	55.6-62.4
164	60.4-63.1	61.7-66.5	64.9-71.8	154	47.8-53.0	51.9-58.0	56.2-63.0
165	60.8-63.5	62.1-67.0	65.3-72.5	155	48.1-53.6	52.2-58.6	56.8-63.6
166	61.1-63.8	62.4-67.5	65.6-73.2	156	48.5-54.1	52.7-59.1	57.3-64.1
167	61.5-64.2	62.8-68.2	66.0-74.0	157	48.8-54.6	53.2-59.6	57.8-64.6
168	61.8-64.6	63.2-68.7	66.4-74.7	158	49.3-55.2	53.8-60.2	58.4-65.3
169	62.2-65.2	63.8-69.3	67.0-75.4	159	49.8-55.7	54.3-60.7	58.9-66.0
170	62.5-65.7	64.3-69.8	67.5-76.1	160	50.3-55.2	54.9-61.2	59.4-66.7
171	62.9-66.2	64.9-70.3	68.0-76.2	161	50.8-56.7	55.4-61.7	59.9-67.4
172	63.2-66.7	65.4-70.8	68.9-77.5	162	51.4-57.3	55.9-62.3	60.5-68.1
173	63.6-67.3	65.9-71.4	69.1-78.2	163	51.9-57.8	56.4-63.3	61.0-68.8
174	63.9-67.8	66.4-71.9	69.5-78.9	164	52.5-58.4	57.0-63.4	61.5-69.5
175	64.3-68.3	66.9-72.4	70.1-79.5	165	53.0-58.9	57.5-63.9	62.0-70.2
176	64.7-68.9	67.5-73.0	70.7-80.3	166	53.6-59.5	58.1-64.5	62.6-70.9
177	65.0-69.5	68.1-73.5	71.3-81.0	167	54.1-60.0	58.7-65.0	63.2-71.7
178	65.4-70.0	69.6-74.0	71.9-81.2	168	54.6-60.5	59.2-65.5	63.7-72.4
179	65.7-70.5	69.2-74.6	72.3-82.5	169	55.2-61.1	59.7-65.1	64.3-73.1
180	66.1-71.1	69.7-75.1	72.8-83.3	170	55.7-61.6	60.2-66.6	64.8-73.8
181	66.6-71.6	70.2-75.6	73.4-84.0	171	56.2-62.1	60.7-67.1	65.3-74.5
182	67.1-72.1	70.7-76.5	73.9-84.7	172	56.8-62.6	61.0-67.6	65.8-75.2
183	67.7-72.7	71.3-77.2	74.5-85.4	173	57.3-63.2	61.8-68.2	66.4-75.9
184	68.2-73.4	71.8-77.9	75.2-86.1	174	57.8-63.7	62.3-68.7	66.9-76.4
185	68.7-74.1	72.4-78.6	75.9-86.3	175	58.3-64.2	62.8-69.2	67.4-76.9
186	69.2-74.8	73.0-79.3	76.6-87.6	176	58.9-64.8	63.4-69.8	68.0-77.5
187	69.8-75.5	73.7-80.0	77.3-88.5	177	59.5-65.4	64.0-70.4	68.5-78.1
188	70.3-76.2	74.4-80.7	78.0-89.4	178	60.0-65.9	64.5-70.9	69.0-78.6
189	70.9-76.9	74.9-81.5	78.7-90.3	179	60.5-66.4	65.1-71.4	69.5-79.1
190	71.4-77.6	75.4-82.2	79.4-91.2	180	61.0-66.9	65.6-71.9	70.1-79.5
191	72.1-78.4	76.1-83.0	80.3-92.1	181	61.6-67.5	66.1-72.5	70.7-80.2
192	72.8-79.1	76.8-83.9	81.2-93.0	182	62.1-68.0	66.6-73.0	71.2-80.7
193	73.5-79.8	77.6-84.8	82.1-93.9				

7B. Desirable Weight (lbs)

Height ft inch	Small Frame	Medium Frame	Large Frame	Height ft inch	Small Frame	Medium Frame	Large Frame
5 2	128-134	131-141	138-150	4 10	102-111	109-121	118-131
5 3	130-136	133-143	140-153	4 11	103-113	110-123	120-134
5 4	132-138	135-145	142-156	5 0	104-115	113-126	122-137
5 5	134-140	137-148	144-160	5 1	106-118	115-129	125-140
5 6	136-142	139-151	146-164	5 2	108-121	118-132	126-143
5 7	138-145	142-154	149-168	5 3	111-124	121-135	131-147
5 8	140-148	145-157	152-172	5 4	114-127	124-138	134-151
5 9	142-151	148-160	155-176	5 5	117-130	127-141	137-155
5 10	144-154	151-163	158-180	5 6	120-133	130-144	140-159
5 11	146-157	154-166	161-184	5 7	123-136	133-147	143-163
6 0	149-160	157-170	164-188	5 8	126-139	136-150	146-167
6 1	152-164	160-174	168-192	5 9	129-142	139-153	149-170
6 2	155-168	164-178	172-197	5 10	132-145	142-156	152-173
6 3	158-172	167-182	176-202	5 11	135-148	145-159	155-176
6 4	162-176	171-187	181-207	6 0	136-151	148-162	158-179

Source. Ref. 2, p.63; values are based on data collected in the 1979 build study of the Society of Actuaries and the Association of Life Insurance Medical Directors of America

Appendix 11

Anthropometric Measurement Standards

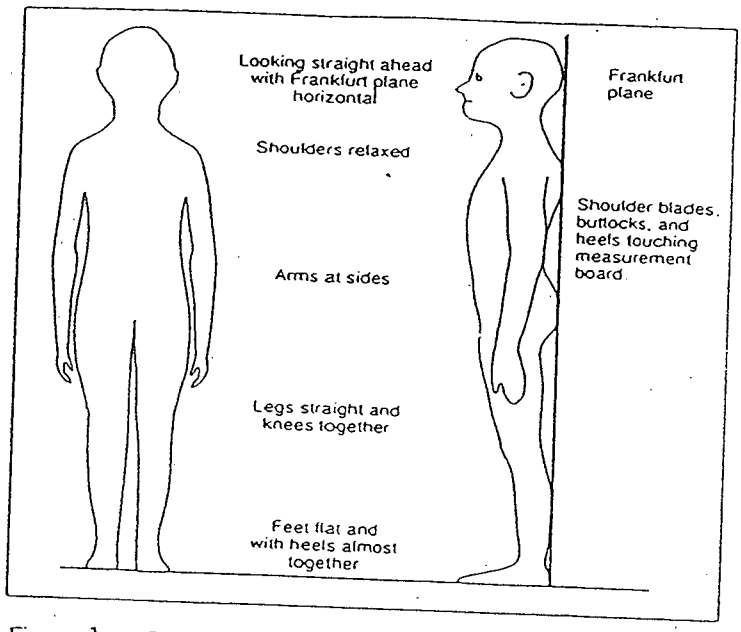


Figure 1: Positioning of subject for height measurement. Horizontal line is the Frankfurt plane, which should be in a horizontal position when height is measured. Reproduced from Robbins GE, Trowbridge FL. In: Nutrition Assessment: A Comprehensive Guide for Planning Intervention by M.D. Simko, C. Cowell, and J.A. Gilbride, p.77, with permission of Aspen Publishers, Inc., © 1984.

Subscapular Skinfold

Recommended Technique

The subscapular skinfold is picked up on a diagonal, inclined infero-laterally approximately 45° to the horizontal plane in the natural cleavage lines of the skin. The site is just inferior to the inferior angle of the scapula (see Figure 3). The subject stands comfortably erect, with the upper extremities relaxed at the sides of the body. To locate the site, the measurer palpates the scapula, running the fingers inferiorly and laterally, along its vertebral border until the inferior angle is identified. For some subjects, especially the obese, gentle placement of the subject's arm behind the back aids in identifying the site. The caliper jaws are applied 1 cm infero-lateral to the thumb and finger raising the fold, and the thickness is recorded to the nearest 0.1 cm (see Figure 4).

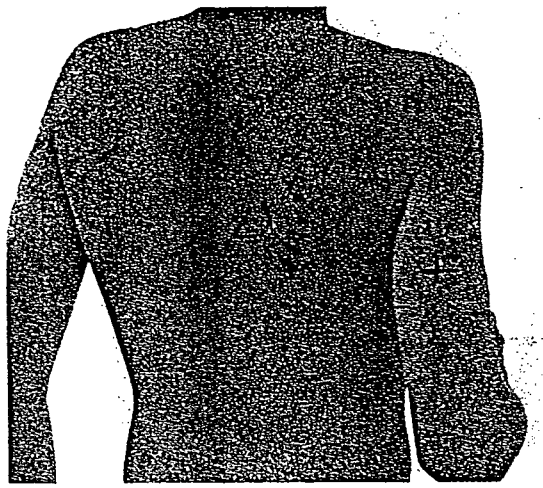


Figure 3 Landmarks for subscapular and triceps skinfolds.

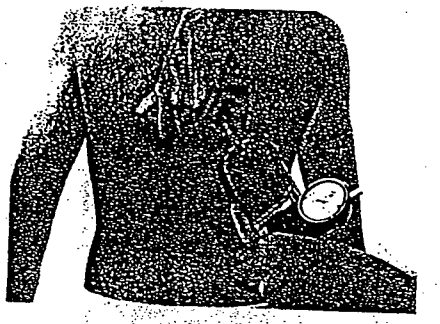


Figure 4 Measurement of subscapular skinfold.

Purpose

Subscapular skinfold thickness is a measure of subcutaneous adipose tissue and skin thickness on the posterior aspect of the torso. It is an important measure of nutritional status and, in combination with other skinfold measurements, is a useful predictor of total body fat, blood pressure, and blood lipids.

Literature

The International Biological Programme recommended the subscapular skinfold thickness as one of 21 basic measurements to be included in survey studies of growth and physique (Weiner & Lourie, 1981). Cameron (1978), citing the work of Durnin and associates (Durnin & Rahaman, 1967; Durnin & Womersley, 1974) recommended the subscapular skinfold thickness, in combination with the triceps, biceps, and suprailiac skinfold thicknesses, as the smallest number of skinfolds representative of body fat. Together with the triceps skinfold thickness, this site is used in health-related fitness tests for children.

There has been general agreement on the location of the subscapular skinfold site, although some authors recommend measuring a verticular skinfold (Cameron, 1978). A diagonal fold, in the natural cleavage of the skin, at the inferior angle of the scapula, is recommended because this makes it easier to raise a fold.

Reliability

The reproducibility of the subscapular skinfold measurement is good. Intrameasurer errors range from 0.88 (Lohman, 1981) to 1.16 mm (Wilmore & Behnke, 1969). Intermeasurer errors range from 0.88 (Sloan & Shapiro, 1972) to 1.53 mm (Johnston et al., 1972).

Sources of Reference Data

Children

Johnston et al., 1972, 1974

Adults

Durnin & Womersley, 1974
Stoudt et al., 1970

Midaxillary Skinfold

Recommended Technique

Midaxillary skinfold thickness is measured at the level of the xiphi-sternal junction, in the midaxillary line, with the skinfold horizontal (see Figure 5). The subject stands erect, except that young infants sit on the lap of the mother or caretaker. Care is taken to ensure that the subject does not flex the trunk towards the side being measured. The left arm is slightly abducted and flexed at the shoulder joint (see Figure 6). A bra can be worn while the measurement is made, but the strap may have to be undone. The measurer stands facing the side of the subject to be measured, elevates a horizontal skinfold with the left hand, and measures its thickness to the nearest 0.1 cm (see Figure 7).

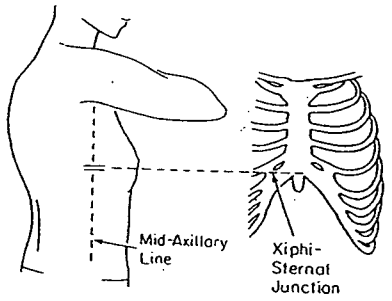


Figure 5 Illustration of the level of the xiphi-sternal junction at which the midaxillary skinfold is measured.

Purpose

Midaxillary skinfold thickness is a guide to the total amount and the distribution of trunk subcutaneous adipose tissue. It is less highly associated than

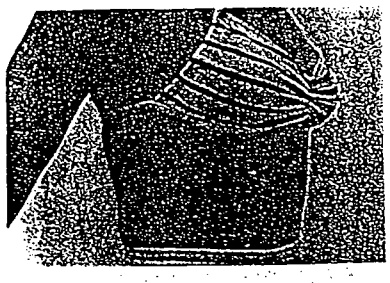


Figure 6 Subject position for midaxillary skinfold measurement.

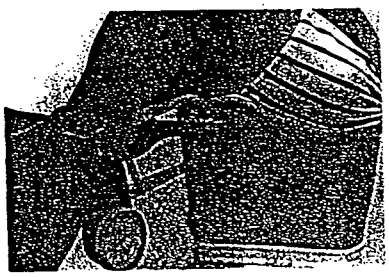


Figure 7 Measurement of midaxillary skinfold.

the subscapular skinfold thickness with the total trunk adipose tissue. It is easier to measure the midaxillary skinfold than the subscapular skinfold in bedfast individuals, and the former skinfold is less likely to be affected by edema. Also, the midaxillary skinfold is easier to measure than most other trunk skinfolds in the obese because it tends to be thinner (Johnston et al., 1974).

Literature

The literature includes few descriptions of the positioning of a subject for the measurement of the midaxillary skinfold, but the positioning in the recommended technique is in agreement with the usual practice.

Most have related the level of measurement to a bony landmark, most often the xiphoid process (Oberman et al., 1965; Pascale et al., 1956; Young, 1964). Less commonly, the measurement has been made at the level of the fifth rib (Slaughter et al., 1978; Wilmore & Behnke, 1969), or the ninth or tenth rib (Lohman et al., 1975). It has been measured midway between the nipples and the umbilicus (Johnston et al., 1974), and at the level of the nipples (Johnston et al., 1972). The latter level usually corresponds to the fifth rib in the midaxillary line except in women.

The midaxillary skinfold, as the name implies, is measured in the midaxillary line (Johnston et al., 1974; Pascale et al., 1956; Young, 1964), but Pařižková (1961) measured in the anterior axillary line.

The fold should be parallel to the cleavage lines of the skin at the site. These lines are nearly horizontal in the midaxillary line at the level of the xiphoid process. Nevertheless, this measurement was made across a vertical fold by Wilmore and Behnke (1969). Pascale et al. (1956) measured a vertical fold unless "the lines of Langer resulted in tension of the skinfold. Then the skinfold was taken along these lines." A fold at 45° to the horizontal was measured by Slaughter et al. (1978). Differences in thickness between horizontal and vertical folds are minimal (Chumlea & Roche, 1986).

Reliability

Using the value *SD* of difference/$\sqrt{2}$, the interobserver reliability was 1.47 mm in children aged 6 to 11 years in a National Center for Health Statistics Survey (Johnston et al., 1972) and was 0.36 mm for children and 0.64 mm for adults in the Fels Longitudinal Study (Chumlea & Roche, 1979). The intraobserver reliability has been reported also as the SE from a regression/$\sqrt{2}$ with values of about 1.0 mm (Wilmore & Behnke, 1969). Zavaleta and Malina (1982) reported a technical error of 0.95 mm for Mexican-American boys and of 2.08 mm for Health Examination Survey data. Lohman (1981) estimated the intrameasurer error as 1.22 mm.

Sources of Reference Data

Children

Johnston et al., 1972, 1974

Adults

Oberman et al., 1965
Young, 1964

Pectoral (Chest) Skinfold

Recommended Technique

It is recommended that the same pectoral (chest) skinfold site be used for both males and females (see Figure 8). Pectoral skinfold thickness is measured using a skinfold with its long axis directed

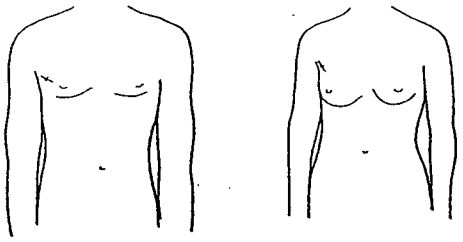


Figure 8 Illustration of location of pectoral skinfold in males and females.

to the nipple. The skinfold is picked up on the anterior axillary fold as high as possible; the thickness is measured 1 cm inferior to this (see Figure 9). The measurement is made to the nearest 0.1 cm while the subject stands with the arms hanging relaxed at the sides (see Figure 10).

For a patient confined to bed, the measurement is made while the patient is supine, with arms relaxed at the sides. For a patient confined to a wheelchair, the measurement can be made with the subject in a wheelchair with the arms relaxed at the sides.

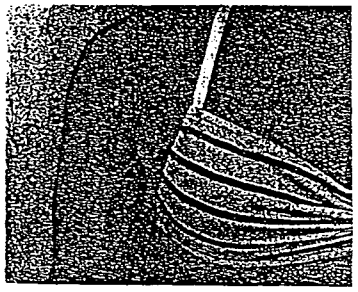


Figure 9 Location of pectoral skinfold on the anterior axillary fold.

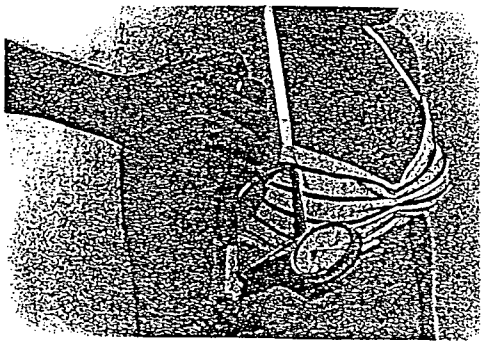


Figure 10 Measurement of the pectoral skinfold.

Purpose

Pectoral skinfold thicknesses have high correlations with body density determined by hydrostatic weighing (Pollock et al., 1975, 1976). This measure has been selected by regression analysis for inclusion in equations to predict body density from anthropometric values (Pascale et al., 1956).

Literature

Pectoral skinfold thicknesses are not measured commonly. Its exclusion from many studies may result from the vague descriptions in the literature and the complexity of the measurement. Complications include the need for the removal of a T-shirt or undergarment and the need to measure away from the mammary gland in women. In most studies, a distinction is not made between the sexes in the methods for the measurement of pectoral skinfold thicknesses.

Three sites for the measurement of pectoral skinfold thicknesses are described: (a) the midpoint between the anterior axillary fold and the nipple (Pascale et al., 1956; Pollock et al., 1980); (b) juxtanipple (Pascale et al., 1956; Forsyth & Sinning, 1973); and (c) medial to the anterior axillary fold (Katch & Michael, 1968). Skĕrlj et al. (1953) appear to describe a pectoral skinfold site located between those described in methods (b) and (c) above. Hertzberg et al. (1963) describe the pectoral skinfold site as juxtanipple, but their illustration shows the site as described under (a) above. The literature does not indicate the direction from the nipple of the juxtanipple skinfold site.

Skĕrlj et al. (1953) describe the site as being at the axillary border of the pectoralis major muscle and state that the location is somewhat more proximal for women than for men. Pollock et al. (1984) describe the pectoral skinfold site as (a) above for males and used one third of the distance between the anterior axillary fold and the nipple for women. Depending on the size of of the mammary gland, this description for women would lead to variable site location. The main intent of describing a separate site for females was to keep the measurement away from the glandular tissue of the mammary gland.

The literature is vague, but the general impression is that most investigators measured the thickness of an oblique fold along the line of the anterior axillary fold. Hertzberg et al. (1963) measured the thickness of a vertical fold.

The same site for both males and females is desirable. Although the midpoint between the anterior axillary fold and the nipple is used most commonly for males, it is not appropriate for females. Because of the variability in the size of the mammary gland, it is difficult to use the nipple as a reference point to locate the site. Also, in most cases it would be difficult to exclude mammary tissue from measurements at the site described under (a). The recommended site allows the measurement to be made while a woman wears a two-piece bathing suit or bra.

Reliability

Intrameasurer reliability coefficients are very high, ranging from .91 to .97 (Pollock et al., 1975, 1976). The standard error of measurement (*SEM*) generally averages 1 to 2 mm. Data from 68 adults showed a correlation of .96 between trials measured on separate days with a *SEM* of 1.45 mm (Pollock, unpublished data, 1985).

Intermeasurer correlations are generally above .9, but the *SEM* may vary as much as 3 to 5 mm with inexperienced measurers, or when the site is not standardized (Lohman et al., 1984). Jackson et al. (1978) reported a correlation among measurers of .98 with a *SEM* of 2.1 mm. An intermeasurer correlation of .93 with a *SEM* of 1.7 mm has been recorded (Pollock, unpublished data, 1985).

Sources of Reference Data

Children

none reported

Adults

none reported

Abdominal Skinfold

Recommended Technique

For the measurement of abdominal skinfold thickness, the subject relaxes the abdominal wall musculature as much as possible during the procedure and breathes normally. The subject may be asked to hold his or her breath near the end of expiration if there is bothersome movement of the abdominal wall with normal respiration. The subject stands erect with body weight evenly distributed on both feet. Children stand on a platform to allow the measurer appropriate access to the skinfold site.

Select a site 3 cm lateral to the midpoint of the umbilicus and 1 cm inferior to it (see Figure 11). The decision whether to measure to the left or right of the umbilicus should be consistent within a study. Raise a horizontal skinfold with the left hand and measure its thickness to the nearest 0.1 cm (see Figure 12).

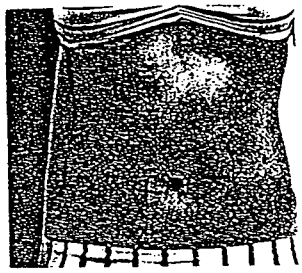


Figure 11 Location of abdominal skinfold site.

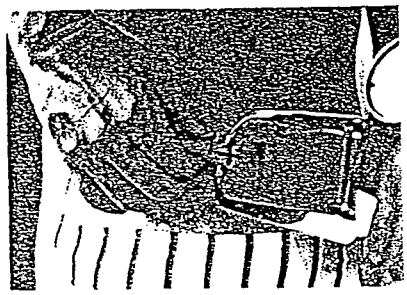


Figure 12 Measurement of abdominal skinfold.

Purpose

The abdominal skinfold is measured commonly and has been included in many studies of body fatness and in many regression equations (Lohman, 1981). Abdominal skinfold thickness changes markedly with weight reduction (Després et al., 1985). It is relatively easy to access, is relatively large, differs considerably among subjects, and is reasonably reproducible with the recommended technique.

Literature

Several locations have been used for measurement of the abdominal skinfold. These include adjacent to the umbilicus; level of the umbilicus but 5 cm to the left of it; slightly inferior to the umbilicus and 1 cm to the right of it, and a quarter of the distance between the umbilicus and the anterior superior iliac spine (Edwards, 1950; Lohman, 1981; Pařízková & Zdenek, 1972; Skĕrlj et al., 1953; Weiner & Lourie, 1981). Most have measured horizontal fold (Behnke & Wilmore, 1974), but others have measured a vertical fold (Sinning et al., 1985; Steinkamp et al., 1965). Some subjects have a "crease" in the region of the umbilicus that precludes selection of a single site for all, and in the obese it is difficult to raise a discrete skinfold.

Reliability

Wilmore and Behnke (1969) reported a test-retest correlation of .979 for measurements made 1 day apart in young men. An intrameasurer technical error of 0.89 mm was reported by Zavaleta and Malina (1982).

Sources of Reference Data

Children

None reported

Adults

None reported

Suprailiac Skinfold

Recommended Technique

The suprailiac skinfold is measured in the midaxillary line immediately superior to the iliac crest (see Figure 13). The subject stands with feet together and in an erect position. The arms hang by the sides or, if necessary, they can be abducted slightly to improve access to the site (see Figure 14). In those unable to stand, the measurement can be made with the subject supine. An oblique skinfold is grasped just posterior to the midaxillary line following the natural cleavage lines of the skin. It is aligned inferomedially at 45° to the horizontal (see Figure 14). The caliper jaws are applied about 1 cm from the fingers holding the skinfold, and the thickness is recorded to the nearest 0.1 cm (see Figure 15).

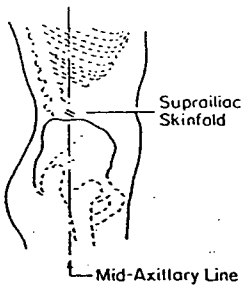


Figure 13 Diagram to illustrate the location of the suprailiac skinfold in the midaxillary line superior to the iliac crest.

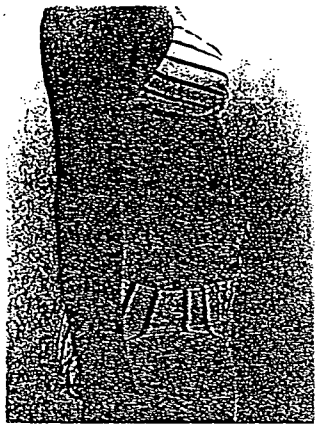


Figure 14 Subject position for measurement of the suprailiac skinfold.

Purpose

Suprailiac skinfold thicknesses are commonly used as indices of body fatness together with other skinfold thicknesses (Durnin & Womersley, 1974). Suprailiac skinfold thicknesses are useful in the study of subcutaneous adipose tissue distribution, which is important in regard to risk of disease (Lapidus et al., 1984; Larsson et al., 1984).

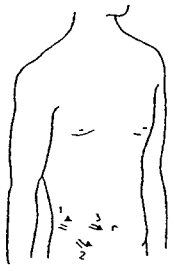
Literature

In most studies, the subjects stood for the measurement of suprailiac skinfold thicknesses. Considerable variation regarding the location and direction of the suprailiac skinfold occurs in the

Figure 15 Measurement of the suprailiac skinfold.

literature. Thicknesses at the various locations appear highly correlated with each other and with body density (Sinning & Wilson, 1984), so that no one position appears to offer unique information. Relatively large systematic differences in thicknesses among locations emphasize the need to standardize the technique for the measurement of the suprailiac skinfold.

The selection of a site on the midaxillary line superior to the iliac creast has the advantage of being easily located in reference to anatomical landmarks. The direction of the fold parallel to the cleavage lines of the skin matches the general approach to skinfold measurement of this manual. The use of a vertical fold (Behnke & Wilmore, 1974), horizontal fold (Johnston et al., 1974), or oblique folds at more anterior locations (Pollock et al., 1975; Ross and Marfell-Jones, 1983) is common (see Figure 16).



1. Standardized Suprailiac Site
2. After Pollock et al
3. After Ross and Marfell-Jones

Figure 16 Location of recommended suprailiac site in reference to other frequently measured suprailiac sites.

The recommended site of measurement is very similar to the site sometimes described for the waist skinfold (Behnke & Wilmore, 1974; Brown & Jones, 1977; Skĕrlj et al., 1953). Because of this similarity, the waist skinfold procedure is not described separately.

Reliability

Wilmore and Behnke (1969) reported a test-retest correlation of .970 for values recorded 1 day apart in young men. Technical errors of 1.53 mm in children and youth (Johnston et al., 1974) and of 1.7 mm in adults (Haas & Flegal, 1981) have been reported. In each study, the errors for suprailiac skinfold thicknesses were larger than those for other skinfold sites. Intrameasurer technical errors of 0.3 to 1.0 mm have been reported by others (Buschang, 1980; Meleski, 1980; Zavaleta & Malina, 1982).

Sources of Reference Data

Children

Baker et al., 1958
Ferris et al., 1979
Johnston et al., 1974 (horizontal fold)
Montoye, 1978
Schutte, 1979
Zavaleta, 1976

Adults

Katch & Michael, 1968

Thigh Skinfold

Recommended Technique

The thigh skinfold site is located in the midline of the anterior aspect of the thigh, midway between the inguinal crease and the proximal border of the patella (see Figure 17). The subject flexes the hip to assist location of the inguinal crease. The proximal reference point is on the inguinal crease at the midpoint of the long axis of the thigh. The distal reference point (proximal border of the patella) is located while the knee of the subject is extended.

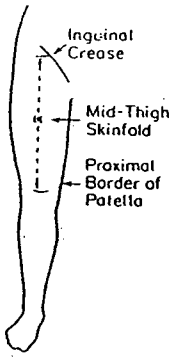


Figure 17 Location of the midthigh skinfold site.

The thickness of a vertical fold is measured while the subject stands. The body weight is shifted to the other foot while the leg on the side of the measurement is relaxed with the knee slightly flexed and the foot flat on the floor (see Figure 18). If the maintenance of balance is a problem, the subject holds the top of the measurer's shoulder, a counter top, or high-backed chair. For patients confined to a bed or wheelchair, the thigh skinfold is measured while the patient is supine. The caliper jaws are applied about 1 cm distal to the fingers holding the fold; the thickness of the fold is recorded to the nearest 0.1 cm.

Purpose

Thigh skinfold thicknesses have moderate to high correlations with body density determined by hydrostatic weighing (Wilmore & Behnke, 1969, 1970). Thigh skinfold thickness has been selected by regression analysis as one of the skinfold measures included in equations to predict body density from anthropometric values.

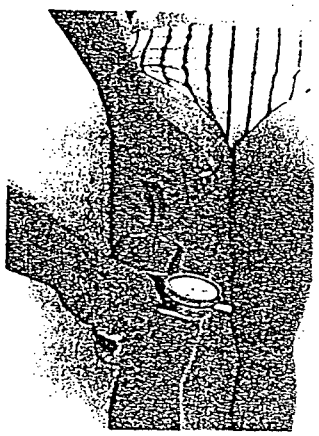


Figure 18 Measurement of midthigh skinfold.

Literature

A few early studies refer to both anterior and posterior thigh skinfold sites, but most refer only to the anterior site: thus, further discussion will relate to this site only.

Although description of the thigh skinfold site appears to be standardized among many studies, considerable variation can be found (Lohman et al., 1984). The most common description of the thigh skinfold site is on the anterior aspect of the thigh, midway between the hip and knee (Wilmore & Behnke, 1969; Zuti & Golding, 1973). Sloan et al. (1962) used the midpoint from the inguinal crease to the proximal margin of the patella. Others give a more general description, such as halfway down the rectus femoris muscle (Young et al., 1962).

The investigators mentioned previously described their measurements as being made with the subject in the standing position, leg relaxed. Some measure with the leg flexed 90° at the knee by placing the foot on a box. This technique is recommended by Ross and Marfell-Jones (1984). All investigators measure thigh skinfold thicknesses with a vertical fold aligned in the long axis of the thigh.

Reliability

Intrameasurer reliability coefficients are very high, ranging from .91 to .98 (Pollock et al., 1976; Wilmore & Behnke, 1969; Zuti & Golding, 1973), although the standard error of measurement (*SEM*) generally averages between 1 to 2 mm. Recent data on 68 adults showed a correlation of .985 between trials taken on separate days with a *SEM* of 1.4 mm (Pollock et al., unpublished data, 1985). Others have reported intrameasurer technical errors of 0.5 to 0.7 mm (Meleski, 1980; Zavaleta, 1976).

Intermeasurer correlations are generally above .9, but the *SEM* may be as much as 3 to 4 mm with inexperienced measurers or when the sites are not standardized (Lohman et al., 1984). Jackson et al. (1978) reported a correlation among measurers of .97 and a *SEM* of 2.4 mm for measurers of varying experience who had trained together. In an unpublished study, Pollock (1986) showed an intermeasurer correlation of .975, with a *SEM* of 2.1 mm.

Triceps Skinfold

Recommended Technique

The triceps skinfold is measured in the midline of the posterior aspect of the arm, over the triceps muscle, at a point midway between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna. The level of measurement is determined by measuring the distance between the lateral projection of the acromial process and the inferior border of the olecranon process of the ulna, using a tape measure, with the elbow flexed to 90° (see Figure 24). The tape is placed with its zero mark on the acromion and stretched along the upper arm, extending below the elbow. The midpoint

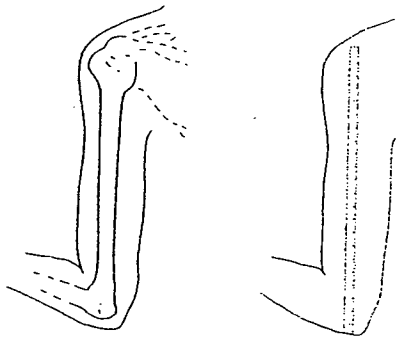


Figure 24 Location of mid-arm level for triceps skinfold

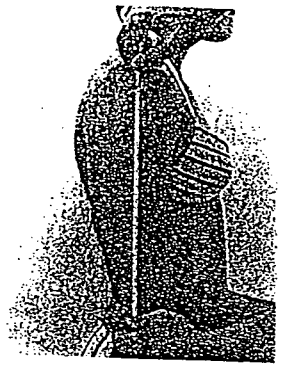


Figure 25 Marked midpoint for triceps skinfold site

is marked on the lateral side of the arm (see Figure 25).

The subject is measured standing, except for infants and the handicapped. The skinfold is measured with the arm hanging loosely and comfortably at the subject's side (Figure 26). The caliper is held in the right hand. The measurer stands behind the subject and places the palm of his or her left hand on the subject's arm proximal to the marked level, with the thumb and index finger directed anteriorly. The triceps skinfold is picked up with the left thumb and index finger approximately 1 cm proximal to the marked level, and the tips of the calipers are applied to the skinfold at the marked level (see Figure 26). The site of measurement must be in the midline posteriorly.

Figure 26 Measurement of triceps skinfold

when the palm is directed anteriorly. Particular problems will be faced when measuring the obese and muscular subjects with little fat at this site. If necessary in the case of obese subjects, an assistant may pick up the fold with two hands, but this gives larger readings than if one hand is used (Damon, 1965).

Purpose

The triceps skinfold is measured more commonly than any other, partly because it is so accessible. It is closely correlated with percentage of body fat and with total body fat but is less well correlated with blood pressure than are trunk skinfolds. It is often included in studies of fat patterning.

Literature

The level of the site is marked with the arm flexed at a right angle at the elbow, and the skinfold is measured with the arm hanging loosely at the side. Positioning is not crucial, except that the subject should be relaxed and the palm directed anteriorly so that the posterior midline can be determined. Most measure subjects in a standing position, though nonambulatory patients may be measured when supine. Infants may be measured lying down, or being held on someone's lap. When supine or sitting positions are used, the recommended technique can still be applied with little modification.

Reliability

In general, measurement error increases with the age of the subject and with increasing levels of fatness. Intermeasurer technical errors vary from 0.8 to 1.89 mm (Johnston et al., 1974; Johnston & Mack, 1985). Intrameasurer technical errors vary from 0.4 to 0.8 mm (Johnston et al., 1974, 1975; Malina & Buschang, 1984; Martorell et al., 1975).

Sources of Reference Data

Children

Frisancho, 1981
Johnston et al., 1981

Adults

Frisancho, 1981
Johnston et al., 1981

Biceps Skinfold

Recommended Technique

Biceps skinfold thickness is measured as the thickness of a vertical fold raised on the anterior aspect of the arm, over the belly of the biceps muscle (see Figure 27). The skinfold is raised 1 cm superior to the line marked for the measurement of triceps

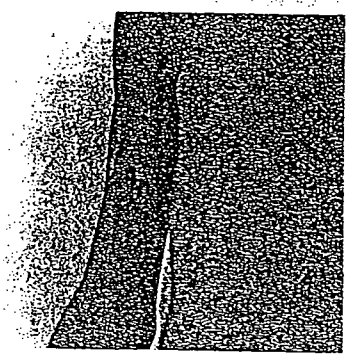


Figure 27. Location of biceps skinfold site.

skinfold thickness and arm circumference, on a vertical line joining the anterior border of the acromion and the center of the antecubital fossa (see Figure 28). The subject stands, facing the measurer, with the upper extremity relaxed at the side, and the palm directed anteriorly. The caliper jaws are applied at the marked level (see Figure 28). The thickness of the skinfold is recorded to the nearest 0.1 cm.

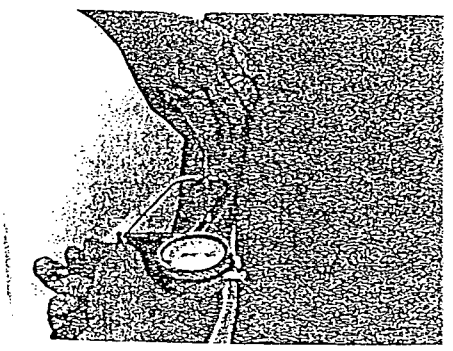


Figure 28. Measurement of biceps skinfold.

Purpose

The biceps skinfold is a measurer of subcutaneous adipose tissue and skin thickness on the anterior aspect of the arm. In combination with other skinfold measurements, it is a useful predictor of total body fat (Durnin & Womersley, 1974). Together with triceps skinfold thickness it may assist the calculation of the "muscle plus bone" cross-sectional area at this level. It can be useful in the obese, in whom many other skinfolds cannot be measured.

Literature

Biceps skinfold thickness is not measured commonly. Consequently, the site has been poorly described, and the reproducibility of measurement is not well established. The International Biological Programme included the biceps skinfold thickness as one of 10 possible skinfold thickness measurements for use in studies of growth and physique, nutritional status, and work capacity (Weiner & Lourie, 1981). More recently, Cameron (1978), based presumably on the work of Durnin and associates (Durnin & Rahaman, 1967; Durnin & Womersley, 1974), recommended biceps skinfold thickness in combination with the triceps, subscapular, and suprailiac skinfold thicknesses, as the fewest skinfold thicknesses representative of body fat; this recommendation is unlikely to be valid in both sexes and in different age groups. The biceps skinfold thickness is used mainly by workers who employ the Durnin equations to estimate percent body fat or study the obese.

Previous descriptions of the biceps skinfold have placed the site generally at the position recommended here. Subject positioning has varied between studies from sitting with the upper extremity resting supinated on the subject's thigh (Durnin & Rahaman, 1967), to standing with the upper extremity held relaxed at the side, palm facing forwards (Cameron, 1978), as is recommended. This brings the site into the anterior midline of the arm, and muscle contraction and skin tension are low.

Reliability

The standard deviation of differences for repeated measurements of biceps skinfold thicknesses by one investigator was 1.9 mm, and the standard deviation of the differences between three measurers was 1.9 mm (Edwards et al., 1955). Technical errors for intrameasurer differences are about 0.2 to 0.6 mm (Meleski, 1980; Zavaleta, 1976).

Sources of Reference Data

Children

Harsha et al., 1978
McGowan et al., 1975

Adults

Durnin & Rahaman, 1967
Durnin & Womersley, 1974
Edwards et al., 1955

Appendix 12

The equivalent fat content, as a percentage of body-weight, for a range of values for the sum of four skinfolds (biceps, triceps, subscapular and supra-iliac) of males:

Skinfolds (mm)	Males (age in years)				Females (age in years)			
	17–29	30–39	40–49	50+	16–29	30–39	40–49	50+
15	4·8	—	—	—	10·5	—	—	—
20	8·1	12·2	12·2	12·6	14·1	17·0	19·8	21·4
25	10·5	14·2	15·0	15·6	16·8	19·4	22·2	24·0
30	12·9	16·2	17·7	18·6	19·5	21·8	24·5	26·6
35	14·7	17·7	19·6	20·8	21·5	23·7	26·4	28·5
40	16·4	19·2	21·4	22·9	23·4	25·5	28·2	30·3
45	17·7	20·4	23·0	24·7	25·0	26·9	29·6	31·9
50	19·0	21·5	24·6	26·5	26·5	28·2	31·0	33·4
55	20·1	22·5	25·9	27·9	27·8	29·4	32·1	34·6
60	21·2	23·5	27·1	29·2	29·1	30·6	33·2	35·7
65	22·2	24·3	28·2	30·4	30·2	31·6	34·1	36·7
70	23·1	25·1	29·3	31·6	31·2	32·5	35·0	37·7
75	24·0	25·9	30·3	32·7	32·2	33·4	35·9	38·7
80	24·8	26·6	31·2	33·8	33·1	34·3	36·7	39·6
85	25·5	27·2	32·1	34·8	34·0	35·1	37·5	40·4
90	26·2	27·8	33·0	35·8	34·8	35·8	38·3	41·2
95	26·9	28·4	33·7	36·6	35·6	36·5	39·0	41·9
100	27·6	29·0	34·4	37·4	36·4	37·2	39·7	42·6
105	28·2	29·6	35·1	38·2	37·1	37·9	40·4	43·3
110	28·8	30·1	35·8	39·0	37·8	38·6	41·0	43·9
115	29·4	30·6	36·4	39·7	38·4	39·1	41·5	44·5
120	30·0	31·1	37·0	40·4	39·0	39·6	42·0	45·1
125	30·5	31·5	37·6	41·1	39·6	40·1	42·5	45·7
130	31·0	31·9	38·2	41·8	40·2	40·6	43·0	46·2
135	31·5	32·3	38·7	42·4	40·8	41·1	43·5	46·7
140	32·0	32·7	39·2	43·0	41·3	41·6	44·0	47·2
145	32·5	33·1	39·7	43·6	41·8	42·1	44·5	47·7
150	32·9	33·5	40·2	44·1	42·3	42·6	45·0	48·2
155	33·3	33·9	40·7	44·6	42·8	43·1	45·4	48·7
160	33·7	34·3	41·2	45·1	43·3	43·6	45·8	49·2
165	34·1	34·6	41·6	45·6	43·7	44·0	46·2	49·6
170	34·5	34·8	42·0	46·1	44·1	44·4	46·6	50·0
175	34·9	—	—	—	—	44·8	47·0	50·4
180	35·3	—	—	—	—	45·2	47·4	50·8
185	35·6	—	—	—	—	45·6	47·8	51·2
190	35·9	—	—	—	—	45·9	48·2	51·6
195	—	—	—	—	—	46·2	48·5	52·0
200	—	—	—	—	—	46·5	48·8	52·4
205	—	—	—	—	—	—	49·1	52·7
210	—	—	—	—	—	—	49·4	53·0

In two-thirds of the instances the error was within ± 3·5% of the body-weight as fat for the women and ± 5% for the men.

*From Durnin and Womersley, 1974.

Appendix 13: Anthropometric recording form for male subjects

Name:_____ Age:_____

Date:_____

WEIGHT:

Trials:_____kg _____kg _____kg

HEIGHT:_____cm

BMI = _____kg/m^2

SKINFOLDS:	1	2	3	average
Tricep:	_____	_____	_____	_____
Bicep:	_____	_____	_____	_____
Sub-scapular:	_____	_____	_____	_____
Suprailiac:	_____	_____	_____	_____

Sum of Skinfolds:_____

% Body Fat:_____

Appendix 14: Anthropometric recording form for female subjects

Name:_____ Age:_____

Date:_____

WEIGHT

Trials: _____kg _____kg _____kg

HEIGHT:_____cm

BMI = _____kg/m^2

SKINFOLDS:

	1	2	3	AVERAGE
Tricep				
Subscapular				
Suprailiac				
Abdominal				
Chest				
Axilla				
Front Thigh				

Sum of Skinfolds:_____mm

% Body fat:_____

Appendix 15:

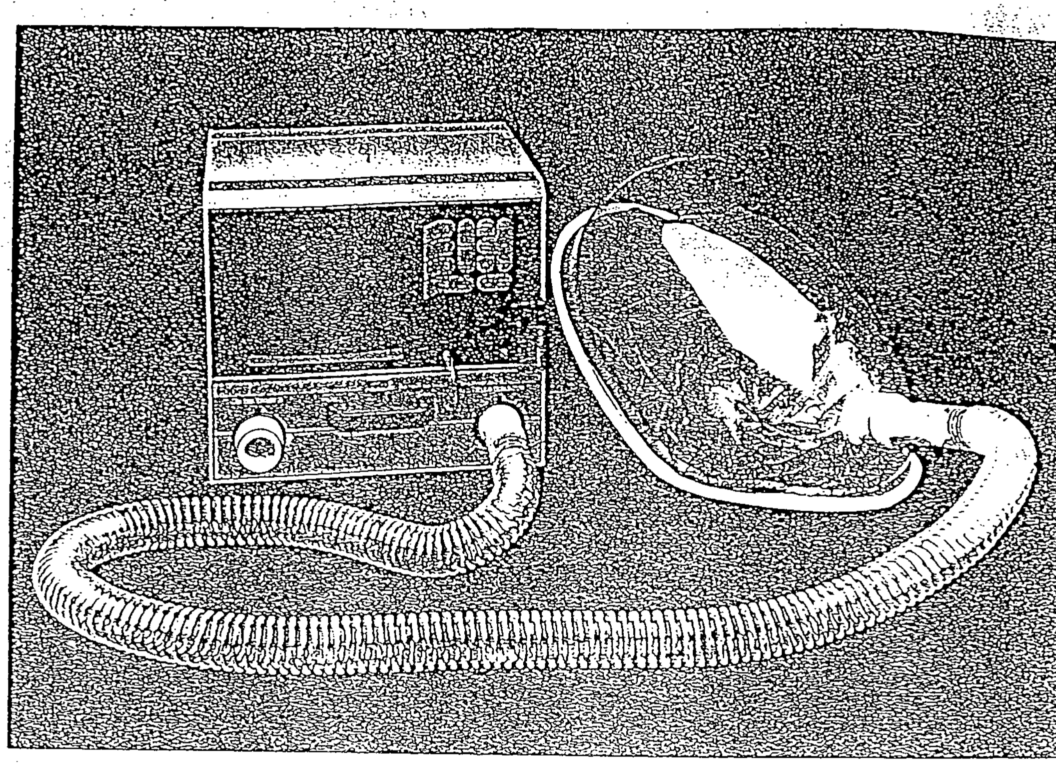
Maximum heart rate, heart rate training range and regression equation (used to determine oxygen inspired/min) for each male subject:

Subject Code	Maximum Heart Rate (beats/min)	Training Heart Rate Range (beats/min)	Regression equation
1	170	102-136	y = 45.9x - 4382.9
2	156	94-125	y = 31.0x - 1822.3
3	183	110-146	y = 28.4x - 1602.5
4	187	112-150	y = 28.2x - 2515.6
5	198	119-158	Not applicable§
6	205	123-164	y = 38.8x - 3940.4
7	189	113-151	y = 24.6x - 1673.3
8	210	126-168	y = 28.8x - 2757.7

*y = Volume of oxygen inspired, ml/min.; x = average heart rate value (beats/min).
§Heart rate values were not available for this subject.

Appendix 16

SensorMedics DeltaTrac™
Metabolic Monitor



Description

The SensorMedics DeltaTrac Metabolic Monitor is a new generation [illegible] indirect calorimeter. DeltaTrac employs revolutionary [illegible] for the measurement of oxygen uptake (VO2), carbon dioxide production (VCO2) and minute [illegible] (VE). It sets new standards [illegible] [illegible]

Features

- Built in [illegible]
- VE without a [illegible]
- True diluti[illegible]
- Low drift [illegible]
- Very easy [illegible]

Unaffected by

- High Peak [illegible]
- PEEP
- Pressure [illegible]
- Elevated [illegible]
- High VE [illegible]
- Water va[illegible]

Recommended Applications

- [illegible] metabolic rate
- [illegible] service
- [illegible]
- [illegible] patients
- [illegible] patients
- [illegible]
- [illegible] Weight [illegible]

SensorMedics

Appendix 17: Kin-com data collection sheet for male and female subjects

Name:_____ Date:_____

Age:_____ Body weight:_____

Leg tested:_____

EXERCISE STIMULUS

Lever arm:_____ Reps:_____

Position:_____ ROM:_____

Exercise tests: 30/90/120/180 deg/sec, concentric and eccentric knee extension, 4 reps

Date	File Name	Velocity	Torque	
			Eccentric	Concentric
		30		
		90		
		120		
		180		

Appendix 18

The University of British Columbia
Office of Research Services
Clinical Screening Committee for Research Involving Human Subjects

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	NUMBER
McCargar, L.J.	Family & Nutr Sci	C94-0025

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
UBC Campus

CO-INVESTIGATORS:
Barr, S.I., Family & Nutr Sci; Belcastro, A.N., Human Kinetics; Parkes, S.C., Family & Nutr Sci

SPONSORING AGENCIES

TITLE:
Human muscle function and structure following a low energy intake plus a high activity level

APPROVAL DATE	TERM (YEARS)	AMENDED:	MODIFICATION OF:
MAY 4 1994	3		

CERTIFICATION:

The protocol and consent form for the above-named project have been reviewed by the Committee and the experimental procedures were found to be acceptable on ethical grounds for research involving human subjects.

B McGillivray
Dr. B. McGillivray, Chair *or*
Dr. A. Hannam, Associate Chair

Shirley A. Thompson
Dr. R. D. Spratley
Director, Research Services

This Certificate of Approval is valid for the above term provided there is no change in the experimental procedures

Appendix 19

Information regarding male subjects re: ethnic group, employment, and previous activities.

ID#	Ethnic Group	Occupation	Previous Activities
1	Caucasian	Student/Salesperson	Soccer, hockey, weights
2	Caucasian	Student	Running, skiing
3	Caucasian	Student	Hockey
4	Caucasian	Student	Hockey
5	Caucasian	Student	Hockey,weights
6	Caucasian	Student	Hockey, weights
7	Caucasian	Student	None
8	Caucasian	Student	Hockey, skiing, weights

Appendix 20

EQUATIONS FOR BODY WEIGHT CALCULATIONS:

1. Percentage ideal body wight = $\frac{\text{actual weight}}{\text{usual weight}} \times 100$

2. Percentage weight loss =

$$\frac{(\text{usual weight} - \text{actual weight})}{\text{usual weight}} \times 100$$

Appendix 21

Information regarding female subjects re: ethnic group, employment, type of exercise, etc..

ID #	Ethnic Group	Occupation	Type of Exercise	Years involved in activities
1	Caucasian	Student	Run, Bike, Swim, Weights (Wts)	16
2	Caucasian	Student	Run, Bike, Wts	15
3	Caucasian	Student	Run, Bike, Walk, Field Hockey	9
4	Caucasian	Student	Run, Bike, Swim, Wts, Aerob.,skiing	14
5	Caucasian	Business Women	Run, Bike, Swim	6
6	Caucasian	Student	Run, Bike, Swim	5
7	Caucasian	Student	Run, Bike, Wts, Karate	16
8	Caucasian	Student	Run, Walk, LaCrosse, Canoeing	9
9	Caucasian	Fitness Instructor	Run, Bike, Swim	10
10	Caucasian	Student	Run, Bike, Row, Aerob, Swim, Wts	9
11	Caucasian	Student	Run, Bike, Swim, Row, Walk	12
12	Caucasian	Student	Run, Bike, Walk, Aerob, Stairmaster	7
13	Caucasian	Student	Run, Bike, Swim	5
14	Caucasian	Unemployed	Run, Bike, Swim, Walk	10

Appendix 22

Table of the distance run per week (km/week) by the female subjects throughout the study*.

Subject Code	Distance run per week (km/week)
1	43
2	55
3	39
4	42
5	45
6	53
7	20
8	40
9	38
10	10
11	21
12	49
13	45
14	20

Appendix 23:

Time spent exercising during each day of the study for female subjects**.

Subject Code	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Average*
1	75	90	80	110	60	100	D/O*	60	90			83
2	148	107	62	78	160	126	90	118	70	65	120	104
3	57	88	60	138	120	97	98	123	83	105		97
4	97	82	150	188	150	210	125	130	120	105		136
5	100	60	90	100	D/O	90	105	70	105			90
6	85	50	85	70	85	120	D/O	50	85			79
7	155	160	150	120	150	180	135	135				148
8	100	60	40	120	275	50	120	120	80	60	31	96
9	90	80	90	80	30	50	D/O	90	80			74
10	145	105	136	255	90	124	84	105	D/O			131
11	110	60	105	130	85	175	105	120	45	195		114
12	88	43	50	75	105	103	130	112	70	140	43	87
13	85	45	85	70	90	D/O	80	70	50			72
14	150	70	105	60	105	45	120	90	60			89

**Determined from exercise diaries.
*D/O refers to a day off of exercising.

Appendix 24:

Table of the energy expenditure (kcal/day) from exercise of each female subject during each day of the study period.

Subject Code	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Average[§]
1	524	525	559	764	420	669	D/O	419	525			493
2	1441	951	682	783	1196	1130	269	1173	778	718	1077*	927
3	790	821	1199	832	929	943	1139	497	739			876
4	747	547	800	1254	800	1587	683	800	800	950		897
5	635	762	572	1016	D/O	572	1048	445	1048			678
6	701	413	701	578	701	991	413	701	330			614
7	1563	1237	976	1376	977	1171	D/O	879	1075			1027
8	985	845	563	422	1338	845	845	1126	422	422		781
9	1310	694	1301	694	260	434	D/O	1301	694			666
10	767	900	607	1700	600	693	360	750	D/O			797
11	1435	1387	1017	804	1482	775	997	455	1907			1140
12	465	305	355	461	532	518	603	688	993	851	305	552
13	701	371	701	578	743	D/O	660	578	413			527
14	864	1387	1017	804	1482	775	997	455	1907			748

**Determined by the Weight Loss Programmer (Version 3.1, Ohio Distinctive Software, Cleveland, Ohio, 1992).
[§]Includes rest days.
*D/O refers to a day off of exercising.