THE EFFECTS OF ACUTE AND CHRONIC PHYSICAL ACTIVITY, NET ENERGY BALANCE ON SERUM LEPTIN LEVELS IN YOUNG ADULTS

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ABSTRACT

The Effect of Acute and Chronic Physical Activity and Net Energy Balance on Plasma Leptin Concentration in Young Adults

Leptin, a 16 amino acid product of ob gene, is thought to play an essential role in the regulation of adiposity. Physical activity forms an important tool in the management of weight regulation. The exact influence of intense physical activity on plasma leptin concentration is unknown. It is also unclear if the influence of an acute bout of physical activity on serum leptin levels is the same in active and sedentary individuals. This study therefore investigated the effects of exercise on plasma leptin concentration in active and non-active young adults.

This study investigated the effects of exercise on plasma leptin concentration in active and non-active young adults. Active subjects (n =10) consisted of the national junior football players and non-active subjects consisted of college students who did not take part in regular physical activity. The subjects were matched closely for age, race, body mass index (BMI) and % body fat. Overnight fasting blood samples were collected before and immediately after a 45-minute bout of exercise. Lipid profiles and plasma leptin levels were estimated in all blood samples. In addition, skinfold thickness measurements and dietary records were obtained from all the subjects to estimate the % body fat and energy balance respectively.

All data analysed using SPSS and are presented as mean ± SEM. No statistically significant differences were found in BMI, % body fat, total energy balance, lipid profiles or plasma leptin concentrations between the two groups. Plasma leptin concentration did not change significantly immediately following exercise although mean leptin concentrations appear to increase slightly post-exercise.

In conclusion, an acute bout of exercise does not decrease plasma leptin concentration if anything there appears a trend towards a slight rise in plasma leptin. This may be a consequent of its decreased clearance during exercise. Similarly, energy intake or balance did not significantly alter plasma leptin levels in both the active and non-active young individuals. However, further studies are required to confirm these findings.

INTRODUCTION

Leptin is thought to play an essential role in the regulation of adiposity. It was first identified in the obese mice in 1994 and a year later its presence was confirmed in humans (Considine, 1995). It is a 167 amino acid protein product of the *ob* gene. Northern blot or reverse transcription-PCR analysis of the messenger Ribonucleic Acid (mRNA) for the *ob* gene has shown that it is expressed only in adipose tissue (Zhang Y et al., 1994; Ogawa Y et al., 1995). The name leptin is derived from the Greek word '*Leptos*' which means 'thin'.

Human leptin gene is on chromosome 7q 31. Its DNA has more than 15000 base pairs and there are three axons, the major coding sites driving protein synthesis (Auwerx & Staels, 1998). Leptin gene expression is regulated in an opposite fashion by Peroxisome Proliferator-activated receptor γ (PPAR) and CCAAT/enhancer binding protein α (C/EBP α), two transcription factors which play important roles in initiating transcription of adipocyte genes during differentiation (Caro et al., 1996). Leptin gene expression is induced by C/ EBPα, an effect mediated by a C/EBP- binding site in the proximal leptin gene promotor (Miller SG et al., 1996, He Y et al., 1995, Hollenberg et al., 1997).

A locus on chromosome 2p21 (d2SI 788) also seems to be a major determining factor for circulating leptin (Comuzzie AG et al., 1997). Although the gene responsible has not yet been identified, the proposed candidate is the pro-opimelano cortin (POMC) gene (Comuzie et al., 1997). POMC is a precursor for adrenocorticotropic hormone that regulates the

production of glucocorticoid hormones. Glucocorticoids are known to regulate leptin gene expression (De Vos P et al., 1995).

Leptin is produced exclusively in fat cells across a wide range of animal species, including humans (Zhang Y et al., 1994; Considine RV et al., 1996). Plasma leptin concentration increases with increasing adiposity (Considine et al., 1996), primarily due to increased adipose tissue leptin production rather than decreased clearance of leptin in the body. It is mainly produced in white adipose tissue and very small amounts are found in brown adipose tissue (Auwerx & Staels, 1998).

Besides the regulation associated with adipose tissue differentiation, leptin gene expression seems to be tightly controlled by environmental and hormonal factors (Tabel 1). Leptin expression is influenced by food intake. In animal studies especially rodents, leptin levels have been shown to decrease after fasting and increase by re-feeding (Frederich RC et al., 1995, Saladin R et al., 1995, Mac Dougold OA et al., 1995; Moinat M et al., 1995). These changes in leptin expression with feeding in rodents are accounted for by changes in insulin levels. Leptin gene expression has been shown to be positively regulated by insulin (Saladin et al., 1995, Mac Dougold O et al., 1995, Leroy P et al., 1996). The level of expression of the leptin gene varies from one fat depot to another. Circulating leptin levels increase with percent body fat but not with visceral fat (Bray & York, 1997). Plasma leptin levels are higher in women when compared to men (Halaas JL et al., 1995; Collins et al., 1996, Bray GA, 1991; Rosenbaum M et al., 1996; Saad MF et al., 1997) and show a diurnal rhythm in both sexes (Sinha MK et al., 1996-A). Adrenal steroids modulate leptin gene transcription

(De Vos P et al., 1995; Murakami T et al., 1995). In addition, glucocorticoids have been shown to enhance leptin gene transcription and leptin levels in both *in vivo* and *in vitro* studies in rodents and man. The generally higher levels of leptin in females' compared to males, suggests that sex steroids might also affect leptin production (Bray & York, 1997; Rosenbaum et al., 1996).

Table 1: Inducers and suppressors of leptin expression

Inducer	Effect*	Species
Feeding	+	Rodent + man
Fasting	-	Rodent + man
Glucocorticoids	+	Rodent + man
Insulin	+ c or +	Rodent Man
Pertusis toxin	-	Rodent
cAMP	-	Rodent
β-receptor agonists	-	Rodent
Thiazolidinediones	-	Rodent
Ctytokines	+	Rodent
Obesity	+	Rodent + man

Adapted from Auwerx et al. (1998)

* + = Induction, - = suppression, c = no change

Leptin is an important regulator of energy balance, providing a negative feedback to the brain to inhibit body fat accumulation (Gardner J et al., 1998). Administration of leptin or Ob protein to ob/ob mice has been shown to reduce food intake and body fat (Schwartz MW & Seeley RJ, 1997). It appears to play an important role in how the body manages its supply of fat. Recently, leptin has also been shown to increase the activity of the sympathetic nervous system (Collins et al., 1996). In addition, leptin deficient mice have been shown to have an increased activity of the parasympathetic system and low levels of sympathetic activity to brown adipose tissue, a thermogenic tissue (Bray & York, 1997).

The increased energy expenditure occurs in part through increased physical activity (Pelleymounter et al., 1995).

Leptin secreted from adipocytes may be bound to a number of different proteins in the circulation (Sinha MK et al., 1996-A). Approximately 50% of total leptin is bound to proteins in lean individuals and the level of free leptin is higher in obese individuals than in their lean counterparts (Sinha MK et al., 1996-B).

Kidney is an important site of leptin clearance in humans (Jensen et al., 1999) and the leptin receptor messenger RNA (mRNA) is highly expressed in the kidney (Tartaglia et al., 1995; Serradeil Le Gal et al., 1997), suggesting that receptor-mediated transport may be necessary for tissue uptake and catabolism

Human leptin levels have been correlated with basal insulin levels (Rosenbaum et al., 1996; Saad MF ., 1997; Widjaja A et al., 1997). Insulin stimulation of leptin production has been demonstrated in rodents (Zheng D et al., 1996-A; Saladin R et al., 1995). Acute changes in insulin levels (Maffei M et al., 1995, Vidal H et al., 1996) however have little effect on leptin expression as insulin level must have to be raised for a longer time to induce leptin expression (Wabiitsch M et al., 1996; Malmstrome R et al., 1996; Widjaja A et al., 1997; Dallongeville J et al., 1997).

Plasma leptin levels respond slowly to fasting (Frederich RC et al., 1995; Trayhurn P et al., 1995) and do not begin to decrease in humans for 12-14 h after the start of the fast. In

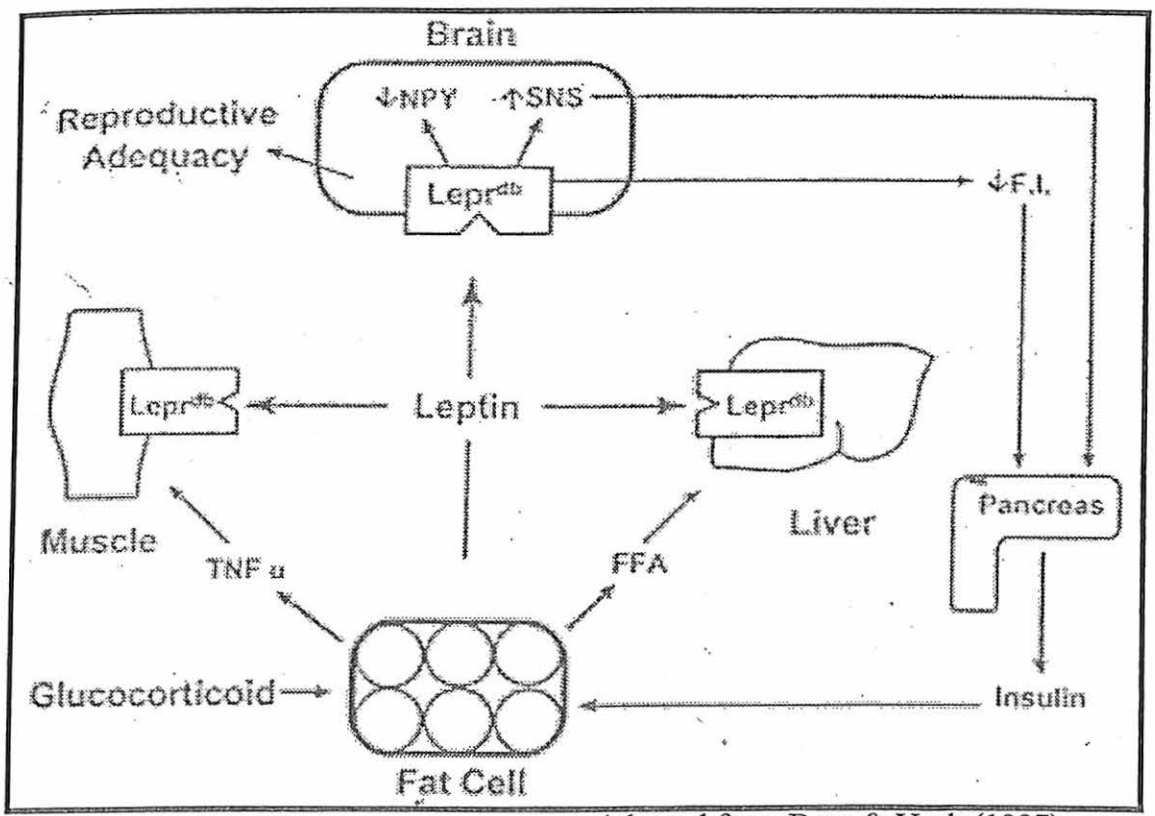
humans, fasting decreases leptin levels (Kolaczynski JW et al., 1996, Boden G et al., 1996), whereas overfeeding seems to increase leptin levels (Kolaczynski JW et al., 1996). Short-term food restriction, resulting in a decrease in caloric intake, however does not seem to effect leptin expression (Maffei M et al., 1995, Vidal H et al., 1996). Leptin gene transcription and plasma leptin are severely reduced by longer starvation (Pratley R et al., 1996; Maffei et al., 1995). Studies on regulatory mechanisms underlying leptin expression after changes in food intake are less clear in human beings than in rodents.

Leptin was seen as an adipocyte derived signaling molecule, which limits food intake and increases energy expenditure (Zhang Y et al., 1994) (Figure 1). Plasma leptin levels are closely correlated with body fat in both humans and rodents, although there is a wide range of individual leptin values at a specific level of body fat (Bray & York, 1997). The percentage of free leptin is higher in obese than in lean individuals (Sinha et al., 1996-B).

The more adipose tissue present, the more leptin is produced. The increase in leptin levels with increasing body fat and obesity suggests that obesity may be associated with "leptin resistance". Adipose tissue leptin mRNA and plasma leptin levels have been found to be closely correlated with the size of the adipose tissue depot (Considine RV et al., 1995; Considine RV et al., 1996; Hamilton BS et al., 1995), suggesting perhaps that obesity is not caused by an absolute deficiency in leptin levels per se (Auwerx & Staels, 1998) but probably by a deficiency in its mechanism of action. These findings gave rise to the "leptin resistance" hypothesis (Caro J et al., 1996; Campfield LA et al., 1996) which argues that obesity is the result of inadequate leptin signaling for a given leptin concentration (Figure 2).

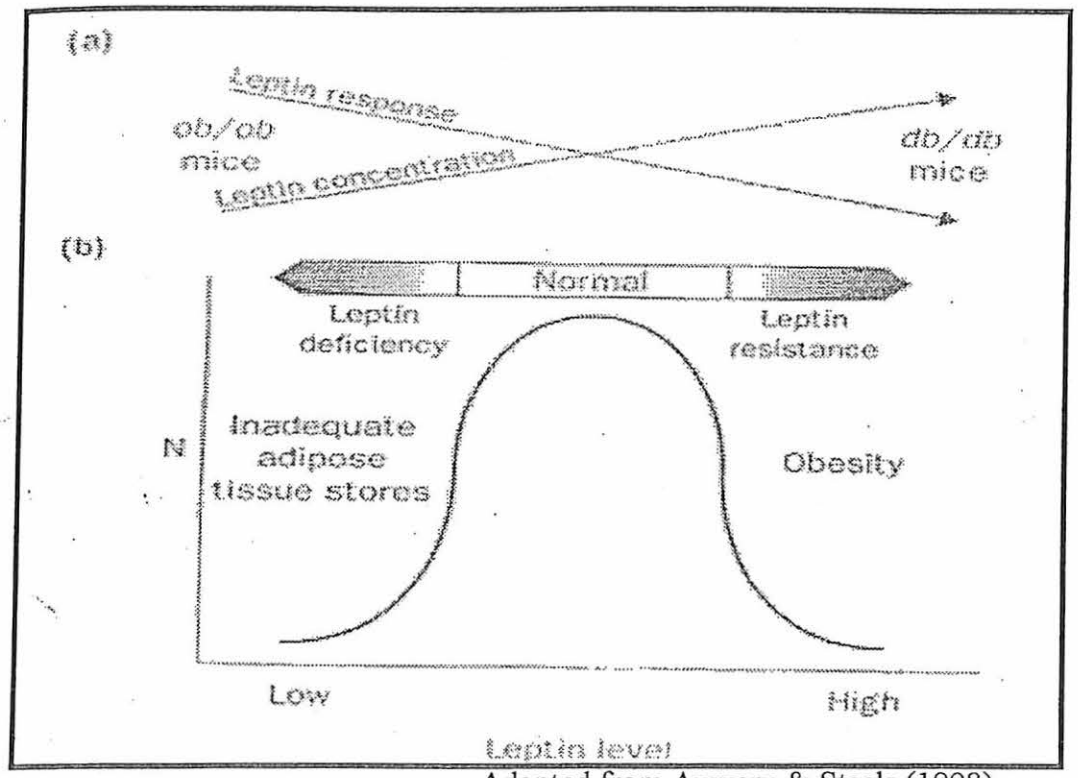
One of the possible mechanism involved in the peripheral effects of leptin resistance is an impaired transport of leptin into the cerebrospinal fluid (Caro JF et al., 1996; Schwartz M et al., 1996).

Figure 1: The Release and Action of Leptin



Adapted from Bray & York (1997)

Figure 2: Leptin resistance in mice (a) and man (b)



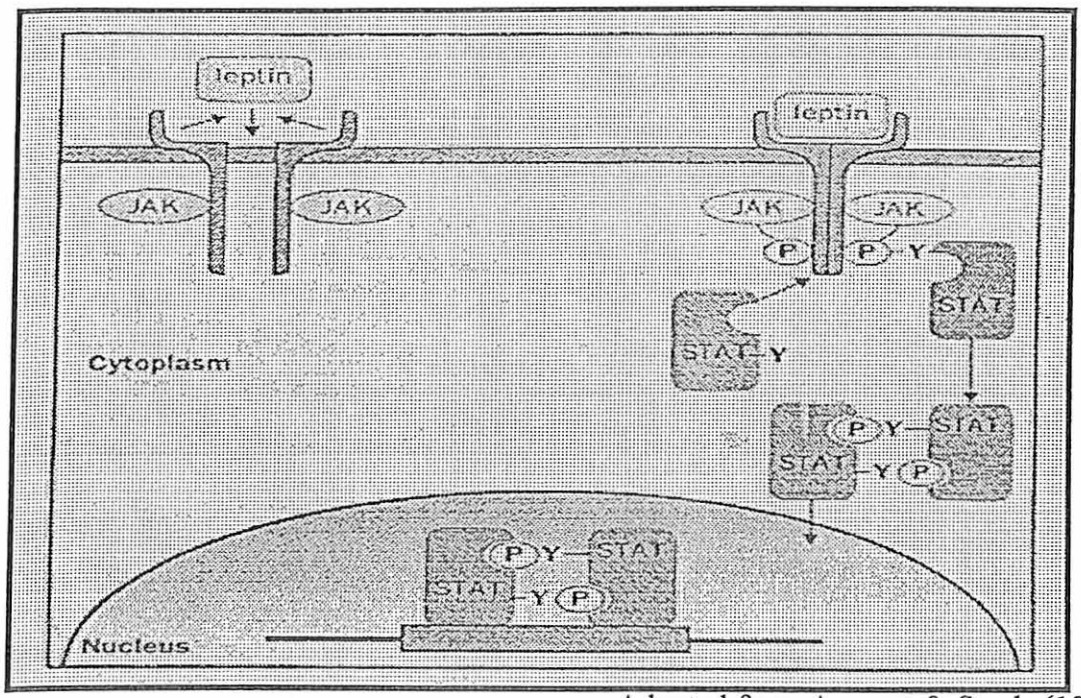
Adapted from Auwerx & Staels (1998)

Leptin achieves most of its metabolic effects by interacting with specific receptors located in the central nervous system and in peripheral tissues (Figure 3; Tartaglia LA et al., 1995, Lee GH et al., 1996, Chen H et al., 1996). The receptor for leptin is a member of the cytokine receptor family (Ghilardi et al., 1996; Madej T et al., 1995., Baumann H et al., 1996). This leptin receptor is a class I cytokine receptor, a family that also includes interleukin-2 receptor, the interferon receptor and the growth hormone receptor (Ihle J, 1996). The receptor transmits the leptin signal via *janus* protein-tyrosine kinase (JAK) 2 (Ghilardi N et al., 1997) to three signal transducers and activators of transcription (STAT 3,5 & 6) (Baumann H et al., 1996; Ghilardi et al., 1996, Vaisse C et al., 1996) a STAT subset known as the "fat-STAT" (Darnell JE, 1996).

JAK and STAT binding are key steps for cytokine class I receptor signaling (Kishimoto et al., 1994). The biological effects of leptin are thought to result from the activation of a Jak-Stat signaling pathway, with specific involvement of Stat-3 protein (Ghilardi n et al., 1996, Madej T et al., 1995, Baumann H et al., 1996, Cusin I et al., 1996, Vaisse C et al., 1996). However, it is not clear whether this is the only signaling pathway that is activated by leptin (Bray & York, 1997)

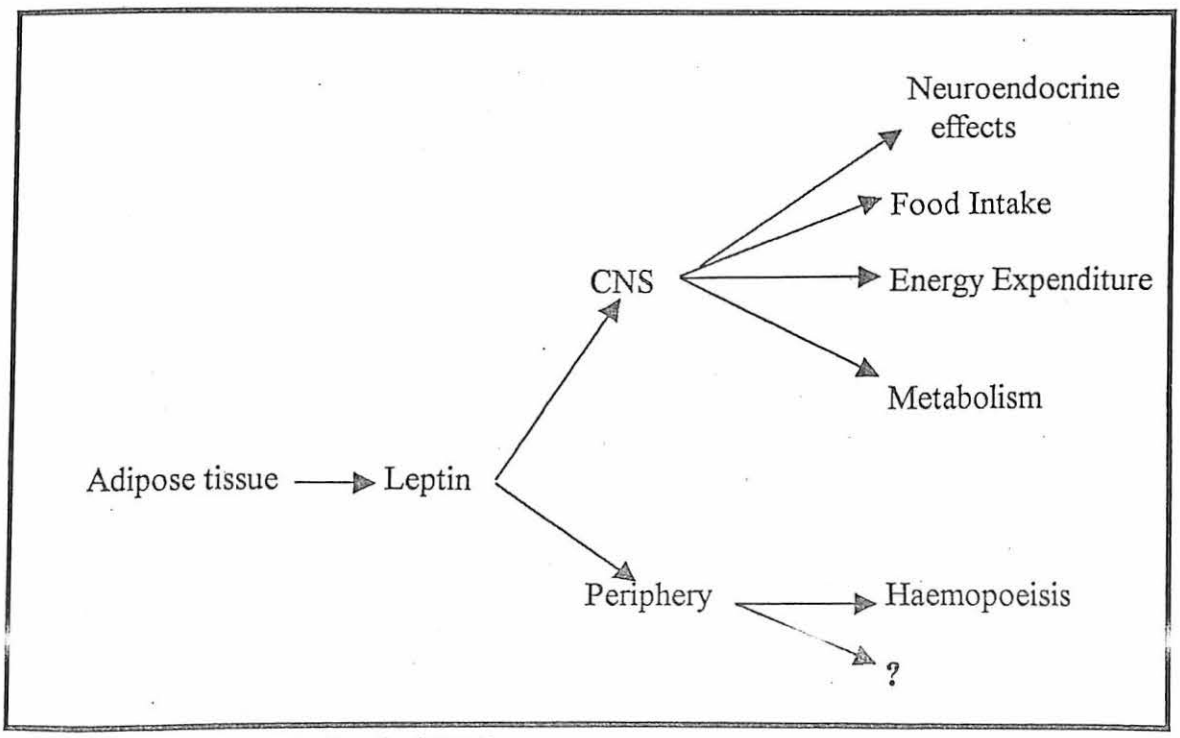
It has been understood that secreted leptin may act as a signal to the central nervous system to indicate the level of body fat and induce the appropriate responses of food intake and energy expenditure (Figure 4) (Bray & York, 1997). However, Ahima RS et al., (1996) suggested that leptin may act as a starvation signal, such that low levels trigger the hypothalamic-pituitary axis to respond to under nutrition.

Figure 3: Leptin Receptor



Adapted from Auwerx & Staels (1998)

Figure 4: Schematic Model of Signaling Pathways of Leptin



Adapted from Auwerx & Staels (1998)

ENERGY BALANCE AND PHYSICAL ACTIVITY

The energy requirement of an individual has been defined by the international technical working group as:

> " *that level of energy intake from food which will balance energy expenditure when the individual has a body size and composition and level of physical activity, consistent with long-term good health; and which will allow for the maintenance of economically necessary and socially desirable physical activity*" (WHO, 1985).

Energy is expended by the human body in the form of resting energy expenditure (REE) or basal metabolic rate (BMR), voluntary activity and the thermic effect of food (TEF). For most people, BMR is the largest component of Total Energy Expenditure (TEE). The second largest component of total energy expenditure is the energy expended in physical activity.

Energy used by the body at rest is defined in terms of either the basal energy expenditure (BEE) or the REE. These are measured as basal metabolic rate (BMR) or resting metabolic rate (RMR). The terms are often used interchangeably. The measurement is made with the body at complete physical and mental rest, relaxed but not asleep, several hours after any strenuous exercise or activity and in a comfortable temperature and environment. In the study of human energy expenditure, the measurement of BMR is an essential element to derive energy requirement estimates for any given population.

A number of factors cause the metabolic rate to vary among individuals. These factors include age, sex, body size and composition. BMR is closely correlated with measures of lean body mass (WHO, 1985). It has been used as the basis for calculating the BMR with the assumption that, because of the need to maintain body temperature, the metabolic rate is affected significantly by the amount of heat lost to the atmosphere by evaporation from the skin. An effect determined to a larger degree by the extent of body surface area. Estimates based on body weight produce results acceptably close to those obtained with body surface area (Mahan & Escott-Stump, 1996).

The proportions of lean body mass to adipose tissue are a function of both sex and age as well as the muscle development. Athletes with greater muscular development show approximately a 5 % increase in basal metabolism over non-athletic individuals. Men have less fat in proportion to muscle than women and the metabolic rates are higher by 5 to 10 % than women of the same weight and height. Nevertheless, based on lean body mass, the BMR for both males and females are similar (Cunningham, 1982).

Exercise does not cause significantly prolonged stimulation of metabolic rate per unit of active tissue, but does cause an increase of 8 % to 14 % higher metabolic rate in men who are moderately and highly active (Horton & Gesissler, 1994). Differences that appear to be related to the individuals and not merely to the extent of the activity itself. Basal metabolic rate is very much influenced by body weight and lean body mass (Poh BK et al., 1999).

In practice, BMR is not commonly measured, instead prediction equations based on age, sex and weight are used (Dubois & Dubois, 1916; Harris & Benedict, 1919). FAO/WHO/UNU (1985) technical report has stated a general predictive equation for the general population. However, there are BMR equations based on the Malaysian population. The equations are as below:

BMR (MJ/day) = 2.84 + 0.064 W

(FAO/WHO/UNU, 1985) (normal population)

BMR (MJ/day) = 0.047 (W) – 0.035 (age) + 3.083

(Ismail et al., 1998) – Adult (normal population)

BMR (MJ/day) = 2.717 + 0.056 W

(Ismail et al., 1994) – Athlete

Energy Balance and the Regulation of Body Weight

The simplest analysis shows that obesity will only develop when energy intake exceeds energy expenditure, but this may neglect the environmental factors which contribute an indirect influence to the energy balance homeostasis. These environmental factors may include genetic susceptibility, metabolic factors and behavioral (Jebb S, 1997). In addition, behavioral, lifestyle, physical activity, eating habits and food composition of the specific population which have remarkable impact upon both energy intake and energy expenditure must be taken into account or observed carefully (Prentice AM, 1997).

Physical activity may affect energy balance both by increasing energy expenditure and by improving the sensitivity of the body's innate appetite control system (Jebb SA, 1997). This was first illustrated by Mayer et al. (1953) in animal studies, who demonstrated that over an intermediate range of physical activity rats were able to match energy intake to energy expenditure but at extremes of activity the balance was disrupted. At high levels of physical activity studies have shown that animals were unable to consume sufficient food and failed to meet their energy needs. Eventually the rat lost weight but at low levels of activity they over ate and gained weight. This shows an inability to down-regulate energy intake to match the low energy needs of the animals (Mayer et al., 1953). In conclusion, this demonstrates there is a vital relationship between physical activity and dietary factors.

Fundamental principles of energy balance

Changes in energy stores = Energy Intake – Energy Expenditures

Positive energy balance occurs when energy intake is greater than energy expenditure. It promotes an increase in energy stores and body weight. Conversely, a negative energy balance occurs when intake is less than expenditure whereby promoting a decrease in energy stores and body weight.

Energy Intake

Total energy refers to all energy consumed as food and drink that can be metabolized inside the body. Table 2 shows the constituent macronutrients present in food and drink that provide energy.

Table 2: Energy Content of Food

Food	Energy contribution	
	(kcal/g)	kJ/g
Fat	9	37
Protein	4	17
Carbohydrate	4	16
Alcohol	7	29

Energy Expenditure

The second element of the energy balance equation is total energy expenditure that has three main components namely:

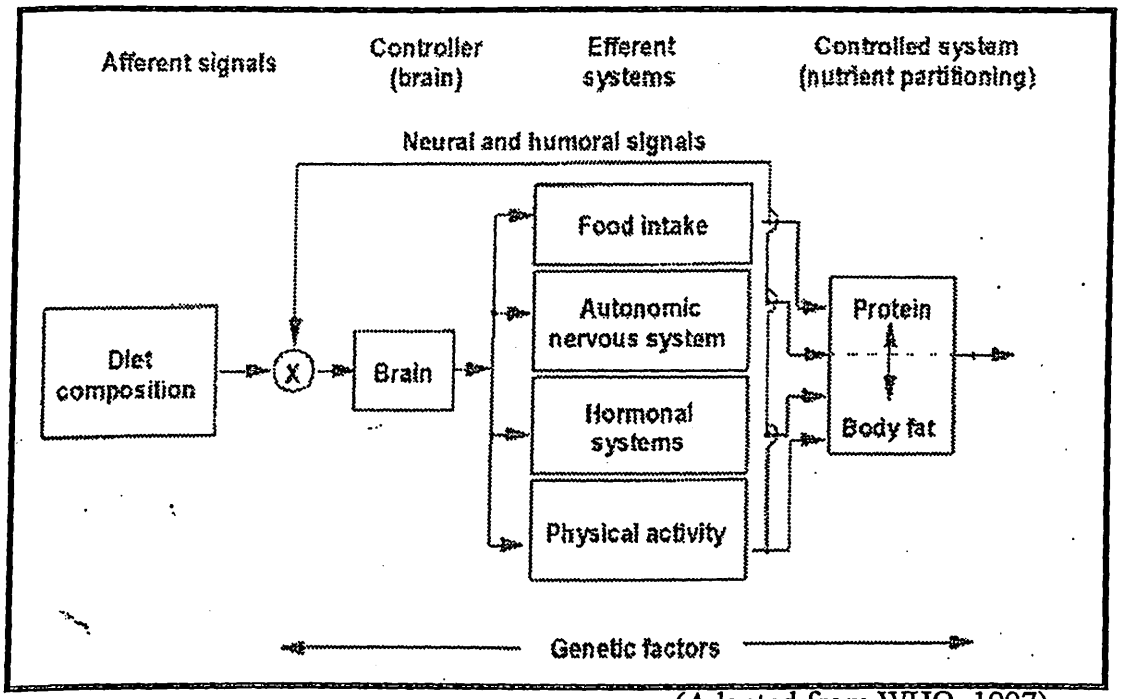
1. Basal Metabolic Rate (BMR)
2. Dietary Thermogenesis
3. Physical Activity

The proportion that each component contributes to total energy expenditure varies according to the regularity and intensity of physical activity. Although the BMR may vary intrinsically between individuals of similar weight by ± 25 %, but within each individual it is tightly controlled (Dallosso HM & James WPT, 1984). The level of physical activity influences energy output of any individual.

The physiological mechanisms responsible for body weight regulation are incompletely understood. Nevertheless, there are many other factors, which may affect directly or indirectly body weight regulation. Some of these may include inflow of dietary nutrients, their distribution or storage and adipose tissue. All the factors or mechanisms that have been mentioned earlier may have been coordinated within the brain resulting to behavioral changes in eating, physical activity and also in body metabolism too. The recent discovery of leptin, which is secreted by adipocytes in proportion to their triglyceride stores and binds to receptors in the hypothalamus, provide interesting insights into possible regulatory signal systems which act to maintain energy balance (Campfield et al., 1995; Bray & York, 1997). The mechanisms are illustrated in Figure 5 that are adapted from the WHO Technical Report on Obesity (1997).

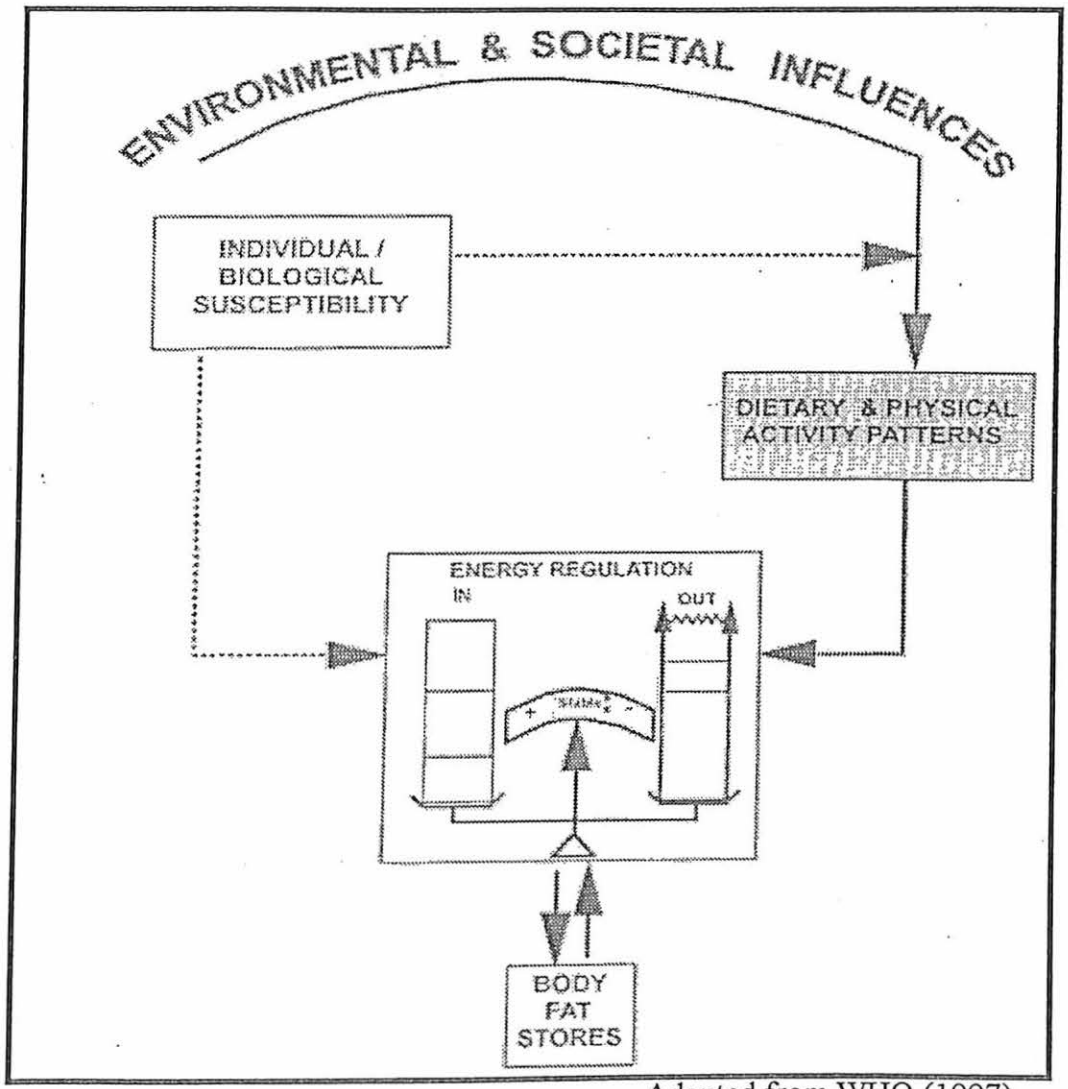
Dietary factors and physical activity patterns have a strong influence on energy balance. As illustrated in Figure 6, dietary factors and physical activity patterns have a direct effect on the outcome of physiological regulation of body weight.

Figure 5: Physiological Processes Involved in Body Weight Regulation



(Adapted from WHO, 1997)

Figure 6: Influences on Energy Balance and Weight Gain (Dietary and Physical Activity Patterns)



Adapted from WHO (1997)

Dietary factors

The macronutrient composition of the diet also influences the extent to which excess energy is stored. According to the Report of WHO Consultation on Obesity (1997), macronutrients with a low storage capacity within the body are preferentially oxidized when intakes exceed requirements: -

1. Protein:

   The protein requirement of an individual is defined as the lowest level of dietary protein that will balance the nitrogen from the body in persons maintaining energy balance at modest levels of physical activity (WHO, 1985).

2. Carbohydrate:

   Small capacity for storage as glycogen. The intake and oxidation of carbohydrate (CHO) are very tightly "auto-regulated", with rapid and substantial changes in CHO oxidation in response to alterations in CHO intake. Excess CHO can also be converted to fat, but human subjects do not use this metabolic pathway to any appreciable extent unless a low fat, high CHO diet is consumed. When CHO is oxidized, however, less fatty acid oxidation is required so dietary fat is stored and endogenous fat retained.

3. Fat:

   The capacity for fat storage in the body is virtually unlimited and excess dietary fat does not acutely increase fat oxidation. Excess dietary fat is readily stored in adipose tissue depots with a very high efficiency (about 96%). Dietary fat has a higher energy density than the other macronutrients.

4. Alcohol:

   No storage capacity within the body and so all ingested alcohol is oxidized immediately. This response dominates oxidative pathways and suppresses the rates at which other fuels are oxidized.

Physical Activity

It is well understood that physical activity plays a vital role in regulation of body weight and fat stores in humans and animals. Inactive lifestyles are heavily implicated in the etiology of obesity. Cross-sectional studies often show an inverse relationship between BMI and physical activity (Rising R et al., 1994; Schulz LO & Schoeller DA, 1994; Davies PS et al., 1995; Westerterp KR et al., 1997) indicating that obese and overweight subjects are less active than their counterparts. However, this correlation normally does not give full explanation about the cause and effect relationship. Nevertheless, low and decreasing levels of physical activity are primary causes of obesity. Obesity is absent among elite athletes while those athletes who give up sports frequently experience an increase in body weight and fatness (Williamson DF, 1996).

Physical activity patterns have an important influence on the physiological regulation of body weight especially when they have a direct effect on total energy expenditure (TEE), fat balance and food intakes (WHO, 1997). Increased energy expenditure is an intrinsic feature of physical activity and exercise. Energy expenditure increases from the basal levels immediately after the initiation of physical activity or exercise and the increase persists for the duration of the activity and sometimes a few hours afterwards.

One of the most important adaptations that takes place following regular physical exercise is the increased capacity to utilize fat relative to carbohydrate during moderate levels of exercise or physical activity. Physically trained individuals metabolize more fat at equivalent

levels of energy expenditure than the untrained individuals. Studies have shown that the rates of fat oxidation or utilization in unfit individuals are increased by approximately 20 % after a 12-week fitness-training program (Hurley et al., 1986).

Physical Activity and Food intake

A delicate balance between energy intake and energy expenditure often is not maintained in sedentary people. This lack of regulating food intake at the lower end of the physical activity spectrum may account for the "creeping obesity" observed in highly mechanized and technically advanced societies. On the other hand, in individuals who exercise on a regular basis, appetite control eventually falls within a "reactive zone" in which food intake is more readily matched to daily expenditure (Mc Ardle et al., 1996).

In considering the effects of exercise on appetite and food intake, focus should mainly be towards the type and duration of exercise. The relationship between physical activity and food intake is very much important as both these factors are the behavioral determinants of body weight (King et al., 1997). The decline in physical activity has been highlighted as a major problem of obesity (Gregory et al., 1990). However, the actual relationship between physical activity and energy intake has yet to be established. Both physical activity and energy intake independently affect energy balance.

A study has shown that acute effect of exercise intensity have some impact on the appetite of young lean men (Thompson et al., 1988). The result shows that there is a brief

suppression of subjective feeling of hunger post exercise but no overall reduction in energy intake. In another study however, Verger et al. (1992), observed that after an hour of swimming exercise there is increased hunger and food intake when compared with rest. There are three possibilities that may happen to energy intake when physical activity is increased:

- ❖ A compensatory effect such that as energy expenditure is increased there is a corresponding rise in energy intake.
- ❖ An exercise-induced suppression of energy intake takes place in which the metabolic or psychological effects of physical activity suppresses energy intake.
- ❖ An exercise-induced alteration in food choice or nutrient selection occurs.

(King et al., 1997)

The mechanism of exercise-induced suppression of energy intake is still not fully understood. Researchers are getting more interested in exercise and energy intake especially with regard to the regulation of energy balance due to increased physical activity. Exercise or increased physical activities have many effects on the body. According to King and his colleagues (1997), there are three main areas that would influence energy intake as a result of exercise. These include:

I. exercise parameters such as mode, duration or intensity of the exercise session

II. subject's characteristic such as age, gender, body composition and training status

III. macronutrient composition (Cotton JR, et al., 1994; Green SM et al., 1994) and the range of available foods (Pliner P et al., 1980; Rolls BJ et al., 1981)

Numerous cross sectional studies have shown significant relationship between physical activity and energy intake in-groups of individuals. Highly active individuals have greater energy intakes than their sedentary counterparts (Blair SN et al., 1981; Maughan et al., 1989; Montoye et al., 1976; Reggiani et al., 1984; Smith et al., 1982; Tremblay et al., 1983). Hardman (1991) stated that, in highly active individuals energy intake and energy expenditure must be monitored for a longer period to gather actual energy intake and energy expenditure of an individual or group (King et al., 1997).

Interestingly, it was found that there is a linear relationship between activity level and energy intake in males (Broeder et al., 1992). In females, however this relationship is not clearly demonstrated (Tremblay et al., 1983; Staten, 1991). Therefore, all these studies suggest that there may be some relationship between exercise and energy intake. However, further studies are required to clearly ascertain the influence of physical activity on food intake at all levels.

Physical Activity and Plasma Leptin Levels

Exercise training has been shown to decrease serum leptin and ob gene mRNA expression in obese and lean rats (Zheng et al., 1996-B; Friedman et al., 1997; Zachwieje et al., 1997; Pasquali et al., 1999). Human studies however have revealed conflicting results. Serum leptin has been shown to decrease (Duclos et al., 1999) or remain unchanged (Hickey et al., 1996; Perusse et al., 1997; Racette et al., 1997; Torjman et al., 1999) immediately following

exercise. Whether leptin production in the body during exercise varies from one site to another also remains unclear. A difference in the ob gene expression in subcutaneous adipose tissue compared to intraperitoneal tissue has been observed (Mazuki et al., 1995). Hamilton et al (1995) on the other hand, found that there was no difference in the ob gene expression in the various adipose tissues during exercise.

Koistinen et al. (1998) examined the effects of exercise with fasting and exercise with feeding on circulating leptin concentrations in healthy man and in type I diabetic patients with normal body weight and well controlled diabetes. They found that there was a slight decrease in serum leptin concentration in healthy fasting male individuals following exercise. But when exercise was performed with feeding, leptin concentration remained unchanged in healthy or NIDDM patients (Koistinen et al., 1998). Therefore, they suggested that although exercise may have reduced circulating leptin levels the effect however is small and can be counterbalanced by feeding or a rise in serum cortisol concentration.

The potential for leptin as a therapeutic agent in the management of obesity is increasingly recognised. The influence of regular exercise on body weight is well accepted. It is possible that the weight reduction that follows exercise is primarily due to increased energy consumption. However, moderate increases in physical activity do not result in similar increases in appetite or food intake. It is believed that this may be due to a change in the level of leptin in the body or perhaps due to its increased sensitivity, whereby it suppresses energy intake. However evidence available in the literature on the effect of exercise on

leptin levels is equivocal and much still needs to be done to clearly establish the influence of physical activity on serum leptin levels and energy balance.

RESEARCH OBEJCTIVE

MAIN OBJECTIVE

The main objective of this study therefore is to determine the effects of acute and chronic physical activity on plasma leptin amongst the Malaysian young adults.

SPECIFIC OBJECTIVE

The more specific objectives of the study however are to:

1. To determine plasma leptin concentration in the both study groups.
2. To determine the lipid profiles including Total Cholesterol (TC), Triglyceride (TG), High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL).
3. To determine subjects' Body Mass Index (BMI) and Waist– Hip Ratio (WHR).
4. To determine the subjects' body composition which include percentage body fat.
5. To determine the total energy balance of the study group subjects'.
6. To compare the difference between:-
    - Mean plasma leptin in the active and control (non-active) groups.
    - Mean lipid profiles (TC, TG, HDL and LDL) level in both groups.
    - Mean BMI and WHR in both active and control groups.
7. To determine the correlation / association between leptin level with :-
    a) Biological parameters (TG, TC, HDL, LDL, BMI, WHR and percentage of body fat).

HYPOTHESIS

Null Hypothesis

There is no difference in: -

1) Mean plasma leptin levels between active and non-active groups.
2) Mean serum lipids (TC, TG, HDL & LDL) between both groups.
3) Mean BMI, WHR, and percentage body fat between active and control groups.
4) Mean energy balance between both groups.

There is no association between plasma leptin level and: -

1) Lipid profiles
2) Body composition (BMI, WHR, % body fat)
3) Total energy balance

METHODOLOGY

Study Design

This study consisted of two groups, namely active group and non-active group. The subjects in both the groups were matched for age, race and selected anthropometric measurements. The active group consisted of under-nineteen (19) national footballers undergoing centralized training at the time of study. The control group consisted of students selected from a community college at Kajang. Subjects for both active and control groups were recruited on a voluntary basis, after having obtained an informed–consent from them. All subjects were informed of the nature and purpose of the study during the first meeting. Confidentiality was assured. Further information about the study was enclosed with the consent form (Appendix 1). This study was approved by the Post-graduate and Human Ethics Committee of USM and the Medical Ethics Committee of FAM. The subjects were instructed to follow normal lifestyles and maintain their daily habits before and during the study so that there won't be any habitual change that could effect the results of the study.

Inclusion Criteria for Active and Non – Active (Control) Subjects

I. Male

II. Healthy (Mentally, emotionally and physically fit)

III. Aged 18 – 25 years old

IV. Absence of any chronic disease

V. Malay

The Selection of Study Variables

Dependent Variable

The dependent variable was plasma leptin, of the subjects.

Independent Variables

The independent variables were exercise, lipid profiles, as described earlier in the objectives, Body Mass Index (BMI), Waist Hip Ratio (WHR), percentage body fat and energy balance.

All measurements were recorded over two sessions and all subjects had to be involved in both sessions.

Session 1: Chronic Exercise

The active group was represented by fit and highly trained individuals (football players). In view of the fact that these footballers had been playing and training for numerous years at this level, they were therefore considered as representing a chronic exercise group. The non-active group consisted of subjects who had sedentary lifestyles. Height, body weight, waist-hip circumference and skin-fold thickness were measured to calculate Body Mass Index (BMI), Waist Hip Ratio (WHR) and body fatness respectively. However, all the anthropometric measurements were measured earlier before the blood collection. Blood samples were only collected on the day of acute exercise (session 2), just before and then after exercise. In addition, the subjects were also instructed to record their dietary intake and

physical activity for a period of 7 days (period between the two sessions) to ascertain their energy balance.

Session 2: The Acute Exercise Session

In the second session, the acute effects of exercise on plasma leptin were investigated among both groups. In this experiment, the subjects from both groups had to exercise for about 45 minutes. Approximately 10 ml of venous blood was collected each time, before the commencement of exercise and immediately after the subjects had completed the 45-min of exercise for analysis of leptin and lipid profiles for both groups, respectively. The flow charts of the study protocol are shown in Appendix 2.

Instruments of Study

1) Questionnaire

A questionnaire (Appendix 3) was used to collect the general data of the subjects. The questionnaire consisted of questions pertaining to their personal data such as name, race, and date of birth together with a 7-day dietary and physical activity record sheets. Subjects were instructed to record their dietary intake and physical activity for a period of 7 days to obtain an estimate of their energy balance.

2) *Anthropometric Measurements*

(a) Height and weight

Height and body weight of each subject was measured to determine the current nutritional status of subjects. The subjects were asked to stand barefooted against the wall while lightly attired. Body weight was measured, using a digital-weighing machine (Tanita), barefooted with light indoor clothing. The height and weight of subjects were measured to the nearest 0.5-cm and 0.1 kg respectively. All measurements were repeated three times and the average was recorded.

(b) Waist and Hip Circumference

Waist Hip Ratios (WHR) were measured to determine the fat distribution in the body. The waist hip circumference ratio is a simple method for describing the distribution of both subcutaneous and intra-abdominal adipose tissue (Larsson et al., 1984; Jones et al., 1986). The WHR is calculated as the ratio of the minimal waist circumference to the circumference at the maximal gluteal protuberance. Subjects were to wear little clothing to ensure that the measuring tape is correctly positioned. Subjects stood erect with the abdomen relaxed, arms at the sides, feet together and with their weight equally divided over both legs during the measurements. Subjects were told to breathe normally and to breathe out gently at the time of the measurement was taken to prevent them from contracting their muscles or holding their breath. The readings were recorded to the nearest millimeter (mm).

3) *Body composition*

Skin-Fold Thickness

Assessment of body fatness by standard techniques provides an estimate of nutritional status of an individual. Presently, there are several method available for assessing body composition. Skin-fold thickness measurement is one of the oldest, inexpensive and most commonly used methods to measure body composition. Skin-fold thickness measurements are said to provide an estimate of the size of the subcutaneous fat depot, which in turn provides an estimate of the total body fat (Durnin & Rahaman, 1967).

The measurement of skin-fold thickness is made by grasping the skin and adjacent subcutaneous tissue between the thumb and forefinger, shaking it gently to exclude underlying muscle and pulling it away from the body just far enough to allow the jaws of the caliper to impinge on the skin (Lukaski, 1987). Duplicate readings are made at each site to improve the accuracy and the reproducibility of the measurements. All skin-fold thickness measurements were taken using Holtain caliper. Total body fat was estimated from the sum of four skin-fold values taken at biceps, triceps, subscapula and suprailiac as recommended by Durnin and Rahaman (1967) and calculated using the Durnin and Womersley equations (1974).

Body density was calculated from the following equations:

BD = c – (m x log sum skin-fold)

Whereby, c = 1.1620 and m = 0.0630

(Durnin & Womersley, 1974)

Later, the percentage of body fat was derived according to the formula from Siri (1961).

$$\% \text{ Body Fat} = (4.95 / BD - 4.50) \times 100\%$$

Intravenous Blood Collection

Ten milliliters (10-ml) of blood was obtained by venepuncture after an overnight fast from all the subjects before the start of exercise. The fresh whole blood was collected and separated into two different tubes. Approximately 4-ml of blood was placed in EDTA vacuum container and the remaining 6-ml was put into a non-EDTA vacuum container and centrifuged at 3500 rpm for 15 minutes at 4°C (Eppendorf Centrifuge 5403). All blood samples were labeled to avoid confusion during the centrifugation sessions. Plasma and serum were isolated from the red cell mass and stored in Eppendorf tubes at -20°C before analysis. Blood was also collected into micro-capillary tubes in duplicate and then centrifuged in the micro-centrifuge for the determination of hematocrit.

Biochemical Analysis

Serum lipids including Total Cholesterol (TC), Triglyceride (TG) and High Density Lipoprotein (HDL) were measured enzymatically using an automated analyzer (Hitachi 911, Japan) at the Chemical Pathology Lab of the School of Medical Sciences, Kubang Kerian, Kelantan.

Estimation of LDL Concentration

Low Density Lipoprotein (LDL) cholesterol concentration was estimated using the following formula (Friedewald et al., 1972):

$$LDL = (\text{Total Cholesterol} - HDL - \text{Triglyceride} / 2.2)\ \text{mmol/L}$$

Note: To convert mg/dL Cholesterol (including HDL and LDL) to mmol/L, multiply mg/dL by 0.026. To convert mg/dL triglyceride to mmol/L, multiply mg/dL by 0.0113.

Leptin Analysis

Leptin concentration in plasma was determined using Sensitive Human Leptin commercially available Radioimmunoassay (RIA) (Linco) Kit. The Linco's Sensitive Human Leptin Radioimmunoassay (RIA) Kit utilizes an antibody made especially against human leptin. Sensitivity of 0.05 ng/ml can be achieved when using a 100 µl sample of serum, plasma or tissue culture media.

Reagents supplied with the kit:

1. Assay Buffer (0.05 M Phosphosaline, pH 7.4, containing 0.025 M EDTA, 0.1% Sodium Azide, 0.05% Triton X-100 and 1% RIA grade BSA), 40 ml.
2. Sensitive Human Leptin Antibody, produced in guinea pig, 26 ml.

3. ^{125}I-Human Leptin Label (<3μCi, <111 kBq). Lyophilled for stability. Hydrate using entire contents (27 ml) of label hydrating buffer. Allow to sit at room temperature for 30 minutes with occasional, gentle mixing.
4. Label Hydrating Buffer, containing normal guinea pig IgG as a carrier. ^{125}I-Human Leptin Label must be hydrated with the entire contents of the label hydrating buffer.
5. Sensitive Human Leptin Standards, 1ml each:

a) 0.05 ng/ml	b) 0.1 ng/ml
c) 0.2 ng/ml	d) 0.5 ng/ml
e) 1.0 ng/ml	f) 2.0 ng/ml
g) 5.0 ng/ml	h) 10.0 ng/ml

6. Quality Controls 1 (Low) and 2 (High), 2 ml each.
7. Precipitating reagent, 260 ml.

All reagents were kept refrigerated between 2°C and 8°C upon arrival before analysis.

Calibration of Pipettes

All pipettes used in the assay preparation were calibrated using a sensitive electronic weighing machine (Analytical Balance, Denver 0066917) and deionised water.

Sample Collection and Storage

a. 100 μml per assay of plasma was used for leptin analysis.
b. Samples had been stored at 4°C a day before the test was conducted.

Protocol Procedure

- Preparation of **assay buffer**
  - 300 µl to the non-specific binding (NSB) tubes (3-4)
  - 200 µl to the reference (Bo) tubes (5-6) and
  - 100 µl to the tubes 7 through the end of the assay
- Pipette 100 µl of Standards and Quality Controls in duplicate in tubes 7 – 22 and 23-26 test tubes respectively. (Flow Chart of Assay Procedure for Leptin Analysis are given Appendix 3)
- Pipette 100 µl of each **Sample** or **Unknown** in duplicate.
- Pipette 100 µl of **Sensitive Human Leptin Antibody** to all tubes **except** total count tubes (1-2) and NSB tubes (3-4).
- Vortex all the tubes, cover them and **incubate** all of them overnight for about 20-24 hours at **room temperature**.

Next Day

- Pipette 100 µl of **^{125}I-Human Leptin Label** to all the test tubes.
- Again vortex, cover and **incubate** all of them for a night (20-24 hours) **at room temperature**.

Precaution:

- Have to be **careful** when dealing with **radioactive substance** and have all the protection when pipetting the ^{125}I-Human Leptin Label by using dispenser.

Second Day

- Add 1.0 ml of cold (4°C) **Precipitating Reagent** to all tubes **except** total count tubes (1-2).
- Once again vortex and incubate for about 20 minutes at 4°C.
- Centrifuge at 4°C all the tubes except the total count tubes (1-2) for 20 minutes at 2500 rpm.
- Immediately **decant** the supernatant from all the tubes **except** the total count tubes (1-2), drain the tubes for 15-60 seconds.
- Had to be very consistent between racks and have to be alert when invert the tubes the pellet may slipped away as they are fragile.
- Counted all tubes in a gamma counter for 3 minutes.
- The calculations for human leptin have been calculated automatically by the gamma counter software that was connected to a computer plus printer.

Data Analyses

Anthropometric Indicators

The criteria for the classification of nutritional status based on anthropometric measurements was according to the recommendations of WHO (1995). Body Mass Index (BMI) is a useful indicator used in the determination of general obesity in subjects. Body Mass Index (BMI) was calculated for all the subjects. BMI is a method of determining overweight and obesity by dividing body weight and height squared. The BMI or Quetelet Index (QI) of each subject were calculated and categorized according to WHO (1995) reference. The formula for BMI or QI is as below:

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

Based on WHO (1995)

The categories of BMI are:

Under-weight	-	< 18.50
Normal	-	18.50 – 24.99
Obesity 1	-	25.00 – 29.99
Obesity 2	-	30.00 – 39.99
Obesity 3	-	40.00 >

The BMI does not calculate body composition (fat versus lean tissue) or considers the degree of fat accumulated within central body cavity. Nevertheless, BMI still has been used widely in determining obesity of populations (Payne & Hahn, 1989).

Waist Hip Ratio (WHR) is an indicator for abdominal or android obesity. Waist circumference is measured midway between the lower rib margin and the iliac crest while hip circumference is measured over the widest circumference of the buttocks. WHR was calculated using the formula below:

WHR = Waist Circumference (cm) / Hip Circumference (cm)

The ratio of WHR should be below 0.95 are classified as normal category for males (Lohman, 1992).

Interpretations for serum lipid parameters are as follows:

Border-high blood cholesterol	5.2 - 6.2 mmol/L
Hypercholesterolemia	> 6.2 mmol/L
Hypertriglyceridemia	> 2.1 mmol/L
Hyperlipoproteinemia	
LDL	>4.9 mmol/L
HDL	> 0.9 mmol/L

According to NCEP (1993)

Statistical Analysis

All statistical analysis was performed using SPSS software Version 9.0 for windows licensed under USM.

- ✓ Descriptive statistics for all variables studied such as socio-economic characteristics, anthropometry measures and biochemical parameters of the subjects.
- ✓ Paired sample t-test was carried out to compare means between active and non active groups for continuos variables such as serum lipid levels, BMI, WHR, % Body Fat and plasma Leptin of the subject in this study.
- ✓ Pearson's Correlation was used to test the association between variables in this study namely, plasma leptin, serum lipids, BMI, WHR and percentage body fat.

Limitation of Study

This study was a short-term study, which were conducted at the Malaysian Football Association's Complex at Kelana Jaya and Kajang, Selangor Darul Ehsan for the non-active group. There were several limitations for the present study as below:

1. Sample Size

   The sample size of this study was small therefore, it may not be extrapolated for the over-all population

2. Sample

This present study consists of Malays subjects only. Therefore, the result may not be applied to other races. In addition to that, only male subjects were involved in the study, so the results were only applicable to males population.

3. Food Intake and Physical Activity

Information obtained from the subjects has to be accurate and honestly written during the recording of food intake and physical activity for 7-day dietary record. Accuracy, honesty and cooperation are very subjective and do influence the outcome of the study. A 7-day record of dietary intake and physical activity can be somewhat burden and the compliance may become difficult unless of course the subjects are immensely co-operative and committed.

4. Anthropometric measurements

Anthropometric measurements performed in this study were limited to height, weight, waist-hip circumference and thickness of skin-fold of the subjects. There were carefully measurements to avoid errors. Hence, all measurements were performed by a single person to avoid operator variability.

RESULTS

Subjects Biodata

Table 3: Biodata of the Subjects

Variable	Active	Non-Active
Race	Malay	Malay
N	10	10
Age (years)	18.5 ± 0.17	18.6 ± 0.16

Table 3 presents the biodata of the subjects in two groups. There was no significant difference in the mean age values for the two groups. Ages of subjects ranged from 18 to 19 years old.

Anthropometry Measurements of Subjects

Table 4: Mean Anthropometry measurements of Active and Non Active Subjects

Physical Characteristics	Subjects	
	Active	Non Active
Height (m)	1.75 ± 0.018	1.68 ± 0.017
Weight (kg)	65.38 ± 2.10	61.12 ± 3.53
Body Mass Index (kg/m^2)	21.44 ± 0.41	21.69 ± 1.04

Mean anthropometry measurements of the two groups are presented in Table 4. Active subjects were significantly taller than the non-active subjects ($p < 0.01$) Body weight and BMI values were however not significantly different between the two groups.

Waist-Hip Ratio

No significant differences were found for the mean values for the waist and hip circumference between the two groups. Similarly, analysis also found that there was no significant differences mean values of WHR between the groups.

Table 5: Waist – Hip Measurements of the Study Population

Physical Characteristics	Subjects	
	Active	Non Active
Circumference (mm)		
Waist	73.25 ± 1.12	73.25 ± 2.81
Hip	92.51 ± 1.13	92.83 ± 2.20
Waist Hip Ratio (WHR)	0.7 ± 0.0055	0.79 ± 0.015

Body Fatness of the Subjects

Table 6: Body Fat Percentage of the two groups

Parameter	Subject (Group)	
	Active	Non Active
% Body Fat	11.48 ± 0.88	14.49 ± 1.60

Mean body fat percentages of active and non-active groups are summarized in Table 6. Percentage body fat was lower in the active group when compared to the non-active group. This difference however did not reach statistical significant.

Biochemical Parameter Measurements of Subjects

Serum Lipid Profile

Mean serum lipid profiles of the subject are summarized in Table 7 and Figure 7.

Total Cholesterol (TC)

As shown in Table 7, the mean TC levels were comparatively lower in active group when compared to the non-active group subjects. However, this difference was statistically significantly different.

Triglyceride (TG)

Triglyceride (TG) concentration was within the normal range in both groups. Mean TG level however was significantly higher in the non-active or control group ($p<0.026$).

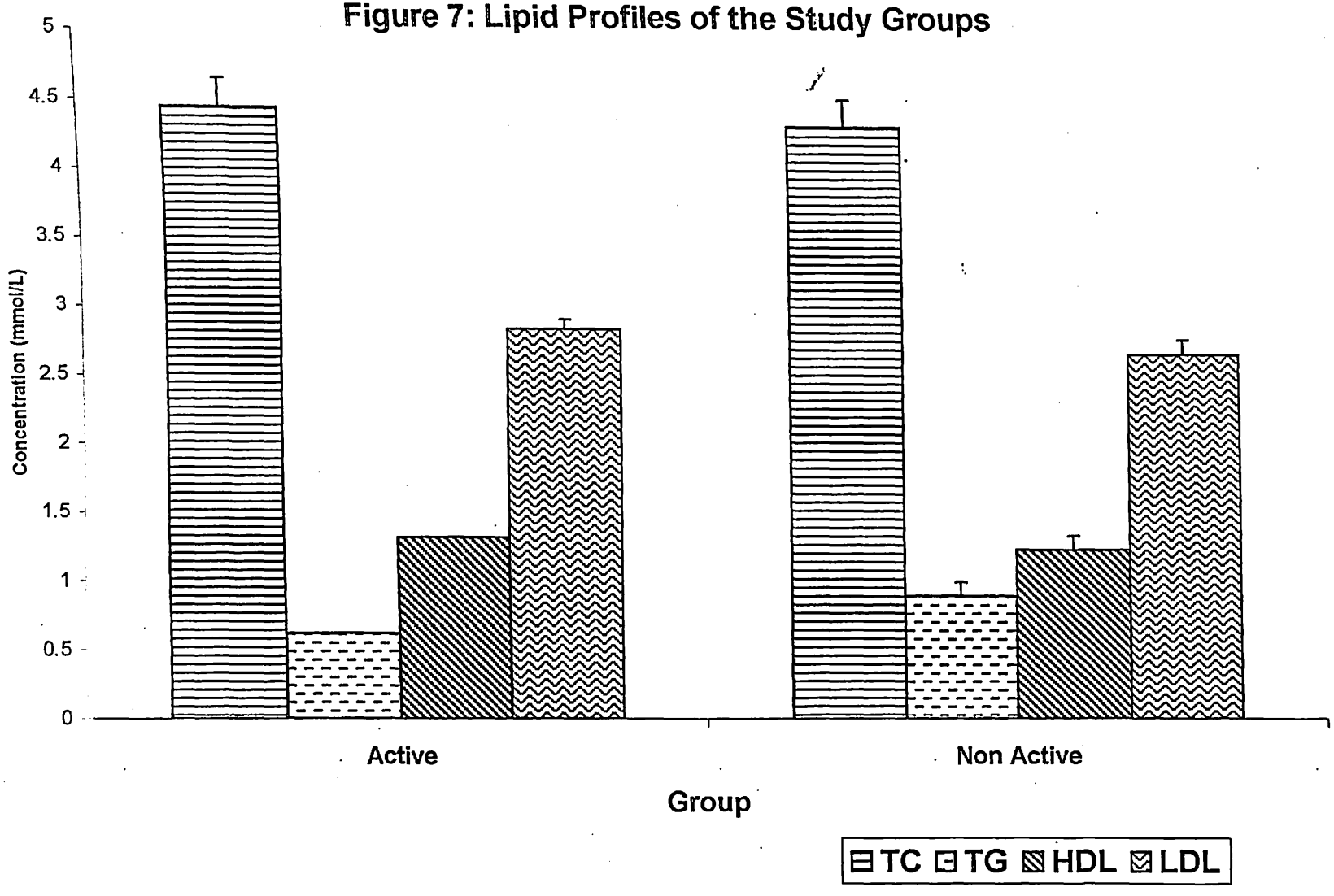
Table 7: Mean Blood Lipid Profiles of the Group

Blood Profile (mmol/L)	Group	
	Active	Non Active
Total Cholesterol (TC)	4.44 ± 0.21	4.32 ± 0.20
Triglyceride (TG)	0.62 ± .0043	0.90 ± 0.10
Low Density Lipoprotein (LDL)	2.84 ± 0.22	2.67 ± 0.18
High Density Lipoprotein (HDL)	1.32 ± 0.071	1.24 ± 0.11

High Density Lipoprotein (HDL) & Low Density Lipoprotein (LDL)

No significant difference was observed in HDL or LDL values between the two groups. All the values ranged within the normal range.

Figure 7: Lipid Profiles of the Study Groups



Plasma leptin concentration

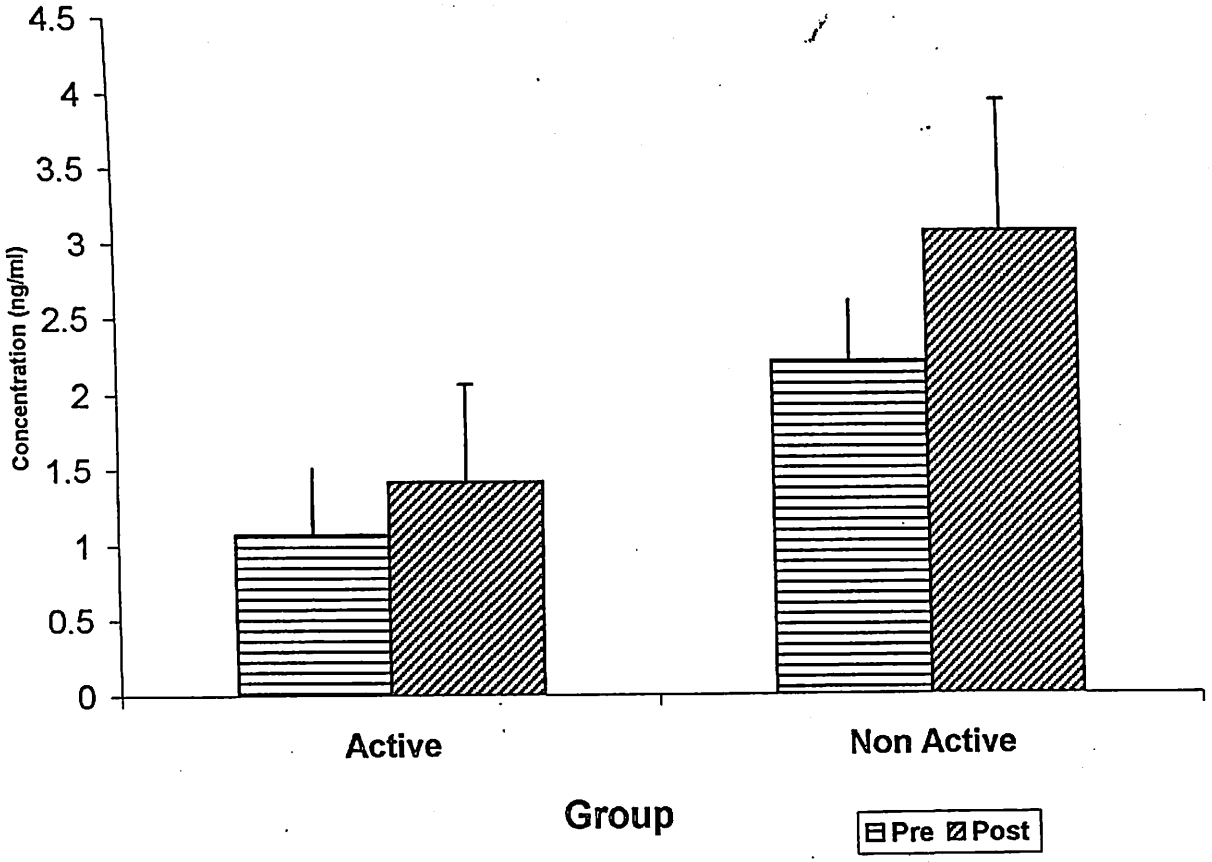
Mean values of plasma leptin concentrations are presented in Table 8. As shown in Figure 8, that acute exercise had detectable effects on plasma leptin concentration in these subjects. The present study showed there is an increased level of leptin concentrations in both groups after completing the 45 – minute of exercise.

Table 8: Mean Leptin Concentration Levels of Pre and Post Exercise among the Subjects

Parameter	Active		Non Active	
	Pre	Post	Pre	Post
Leptin (ng/ml)	1.07 ± 0.44	1.42 ± 0.40	2.22 ± 0.65	3.08 ± 0.88

Before exercise, plasma leptin concentrations in the active group were lower than those in the non-active group. However, statistical analysis did not show any significant difference between the means of the two groups. Similarly, mean plasma leptin concentrations after exercise were slightly lower in the active group. The difference however did not reach statistical significance also. When compared to plasma leptin concentration levels within the groups before and after exercise, statistical analysis still did not show any significant different between the means.

Figure 8: Effect of Exercise on Plasma Leptin Among the Study Groups



Nutrient Intake from 7-Day Records

Analysis of food intake by the subjects after a 7-day dietary record was done by using Nutrical software Ver 1.01 (Professional User) for the determination of total energy intake of the subjects. This was later compared with Malaysian RDA values (Teoh, 1975). According to Malaysian RDA, the estimated recommended energy requirements for a male in the age range of 18 to 19 years old was 2580 Kcal.

Energy intake of the subjects are summarized in Table 9. Mean energy intake values of the active group were higher than that of non-active group. However, statistical analysis showed that there were no significant differences in mean energy intakes for both groups in the present study. It is good to mention here that, the mean energy intake of the active group met the recommended energy requirements. However, the mean energy intake of the non-active group was lower than the recommended energy requirement for that age group.

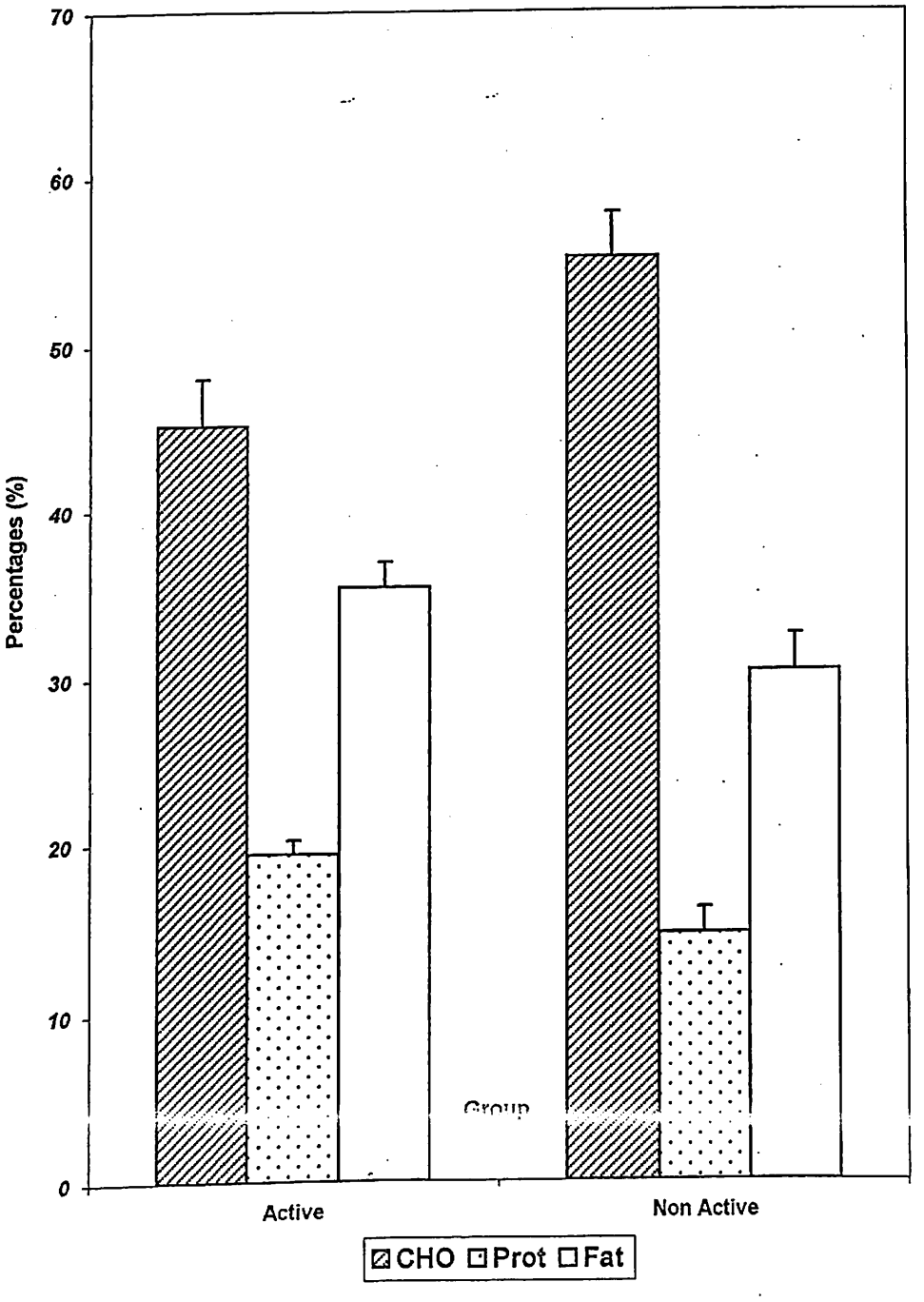
Energy intake is derived from three main macronutrients namely; carbohydrate, protein and fat. Statistical analysis revealed a significant difference in mean protein calorie intakes between both groups ($p<0.01$). However, there was no significant difference of mean values for carbohydrate and fat intake found between the two groups in the present study.

Table 9: Energy Intakes and Contribution of Carbohydrates, Protein and Fat to Energy Intake

Group	Energy (Kcal)	CHO		Protein (Kcal)		Fat (Kcal)	
		(Kcal)	%	(Kcal)	%	(Kcal)	%
Active							
Mean	2682.62	1213.07	45.22	517.66	19.40	947.70	35.38
SEM	± 152.71	± 81.11	0.76	± 30.90	0.84	± 52.56	1.51
Non Active							
Mean	2459.40	1356.05	54.42	362.02	14.73	769.43	30.85
SEM	± 195.63	± 113.66	3.17	± 44.52	1.49	± 88.93	2.17

Figure 9 shows the percentage of calorie intakes from the main three sources of energy in this study population. Carbohydrate was the main source of energy intake, contributing about 45.22 % of the total calorie intake, followed by fat 35.38 % and protein 19.40 % for the active group subjects. While in the non-active group, the contributions of carbohydrate, fat and protein were 54.42 %, 30.85 % and 14.73 %, respectively.

Figure 9 : Contribution of Carbohydrates, Protein and Fat to Energy Intakes of the Study Groups



Energy Expenditure

The calculations for energy expenditure in the present study were based on a factorial method, which was published by Bouchard et al. (1983). Energy expenditure was calculated based on the record of daily physical activity of the subjects for 7-days.

Table 10: Mean Total Energy Expenditure among the Groups

Energy Expenditure (Kcal)	Group	
	Active	Non-Active
Mean	2718.34 ± 98.99	2764.00 ± 195.63

Mean energy expenditures (EE) of the two groups were provided in Table 10. Independent t-test analysis found that there was no significant difference between the groups in terms of daily energy expenditure.

Energy Balance

Energy balance for all subjects in the present study are shown in Table 11. It was found that there were negative energy balances in both groups. The range of negative energy balance was higher in the non-active group when compared to the active group. However statistically, no significant differences were observed between the two groups.

Table 11: The Energy Balance (Kcal) of The Study Groups

	Active			Non Active		
	Energy Intake	Energy Expenditure	Energy Balance	Energy Intake	Energy Expenditure	Energy Balance
Mean	2682.62	2718.34	-35.72	2459.40	2764.00	-304.61
SEM	± 152.71	± 98.99	± 191.01	± 195.63	± 213.02	± 256.92

± SEM – Standard Error Mean

Leptin and correlation

Table 12 shown the relationship between leptin and other parameter in this study. Only body fat had a significant relationship between plasma leptin levels before exercise. Other parameters computed from Pearson correlation test however did not reach the statistical significant correlation level.

Table 12: Correlation between Leptin Pre-Exercise and the selected parameters Among the Study Group

Parameters	Active Group		Non-Active Group	
	Corr. Coeff (r)	Significance level (p)	Corr. Coeff (r)	Significance level (p)
WHR	0.331	0.351	-0.312	0.381
BMI	0.607	0.063	-0.052	0.886
% Body Fat	0.774	0.009**	-0.121	0.738
Energy Intake	-0.523	0.121	-0.369	0.294
Energy Expenditure	0.263	0.463	-0.110	0.762
Energy Balance	-0.555	0.096	-0.190	0.599
TG	0.059	0.871	-0.074	0.839
TC	0.258	0.472	-0.260	0.468
HDL	-0.568	0.087	-0.102	0.780
LDL	0.427	0.218	-0.210	0.334
Leptin Post Exercise	0.552	0.098	0.398	0.254

** Significance level $p<0.01$

DISCUSSION

Not many studies have been conducted to investigate the acute effects of physical activity on plasma leptin levels in humans. In particular, studies comparing the effects of physical activity on plasma leptin levels in active and non-active individuals are non-existent. The aim of this study therefore was to determine and compare the effects of acute exercise on plasma leptin concentration in active, fit individuals and non-active sedentary individuals. To minimize the possible influence of other variables known to influence plasma leptin concentration, subjects in both the groups were closely matched for age, race, sex, percentage body fat and body mass index (BMI).

Anthropometry

All the subjects in the study were from the Malay race and were closely matched for age. Subjects in the active group were taller than the non-active subjects. This difference was statistically significant ($p<0.01$; Table 4). Similarly, the active subjects also had a higher body weight than the non-active subjects, although this difference in body weight did not reach statistical significance. Mean height and mean body weight of the active subjects in this study were higher than those of the general Malaysian population with a matching age range (Chee et al., 1997; Ismail & Zawiah, 1988; Ismail et al., 1998). This was expected, as active subjects in this study were members of the national junior football team and representatives at this level of sport can be expected to have physical attributes that are

somewhat higher or greater than the average population. Non-active subjects were selected to match some of these physical characteristics of the active group, particular the body weight and body mass index, as these two parameters have been shown to correlate positively to plasma leptin concentrations (Gippini et al., 1999). Hence the mean height and body weight of the non-active subject was also higher than the average population (Chee et al., 1997, Ismail & Zawiah et al., 1988). One of the objectives of this study was to examine the effect of chronic physical activity on plasma leptin concentration. To ensure that we had subjects who have been physically active for over a period of a few years, we selected the national junior footballers. Most of them had been training and playing competitive football at one level or another for at least six or more years. They therefore were good representatives of a group to study the effect of long-term physical activity on plasma leptin levels.

Although mean height and mean body weight were slightly higher in the active group, the body mass index however was not significantly different between the two groups. The BMI of the subjects was within the normal population range (WHO, 1995) and compares well with those reported for other local footballers (Reeves et al., 1999) and some Asian footballers in general (Chin et al., 1992). BMI is commonly used as an indicator for the determination of nutritional status of an individual. However it is well known that BMI may not always correlate with percentage body fat and therefore may not always represent a true index of percentage of body fat or obesity. Since BMI may not always correlate well with percentage body fat, particularly in athletes who have a higher muscle mass, it may therefore also correlate very poorly with plasma leptin concentration.

Correlation of height, body weight and BMI to serum plasma leptin concentrations did not reveal any significant correlation between these parameters and leptin (Table 12). This is in contrast to what has been generally reported in the literature although one other study also failed to find any correlation between BMI and plasma leptin (Gippini et al., 1999). However significant positive correlation has been observed between plasma leptin concentration and BMI (Garcia-Mayor et al., 1997; Considine et al., 1996; Campfield et al., 1995). The reason for this discrepancy is unclear. The small number of subjects in this study and close matching of body weight and BMI between the two groups may have somehow obscured this correlation. It is possible that a correlation could become evident if the sample size had been larger and/or if the body weight and BMI of the subjects had been over a broader range.

Waist Hip Ratio (WHR)

Recent research suggests that waist circumference may have certain advantages over other measurements of adiposity in predicting the risk of obesity-related diseases (Strauss, 1995). Waist circumference is a convenient and simple measurement, which is unrelated to height and has been shown to correlate closely with BMI and WHR (Lean et al., 1995). It is also an approximate index of intra-abdominal fat mass. Over the last 10 years or more, a high WHR (WHR > 1.0 in men & > 0.85 in women) has been accepted as the clinical method of identifying patients with abdominal fat accumulation (James, 1996; WHO, 1997).

Mean WHR values obtained was not significantly different between the two groups. The WHR values of subjects in this study were within the normal range, i.e. is below 1.00. Analysis did not reveal a significant correlation between these WHR and leptin. However, this finding is once again in contrast to previous reports, where plasma leptin concentrations have been significantly correlated with WHR (Liuzzi et al., 1999; Hickey et al., 1997). Leptin levels increase with increasing WHR ratio. In this study, WHR did not correlate with plasma leptin probably because of the small number of subjects in each group and narrow range of values. It has also to be remembered that waist and hip circumference changes are not so obvious in young growing adults but become more significant in fully-grown adults. The age of the subjects in this study was in the range of 18 to 19 years.

Body fat

Skinfold measurements represent the thickness of a double layer of skin and the underlying subcutaneous fat. According to Sanborn (1991), the utility of the skinfold measurement is threefold. Firstly, the measurement is used for estimating percentage of body fat. Secondly, skinfold thickness is used in determining regional distribution of subcutaneous adipose tissue. Thirdly, skinfold measurements, along with circumference, have been used to estimate muscle and bone changes.

Specific athletic events require different body types and weights for maximal performance. Generally, the range for body fat levels among the athletes range from 5 to 12 % for males and 10 to 20 % for females (Position of The American Dietetic Association & The

Canadian Dietetic Association, 1993). However, ideal body composition may vary according to the sports or event in which the person involves. The body fat levels in active subjects in this study (Table 6) were within the normal range and similar to those reported in local footballers (Reeves et al., 1999). However when compared to footballers in Hong Kong, the percentage body fat of the active group in this study was higher (Chin et al., 1992). The reason for this is unclear. It may be racial, as the subjects in the study of Chin et al. (1992) were mainly Chinese.

According to Katch FI and McArdle (1983), the acceptable body fat levels for general population males and females is in the range of 15 to 18 % and 20 to 25 % respectively. Values above 25 % and 30 % of body fat for males and females respectively is classified as a clinical indicator for obesity and a risk of having chronic diseases (American Dietetic Association, 1987; Sjostrom, 1992). The present study revealed that the mean percentage body fat of non-active group was within the range of a normal Malaysian poupulation (Ismail & Zawiah, 1988). Although mean percentage body fat was lightly higher in the non-active group, the difference however did not reach statistical significance. Correlation of percentage body fat with plasma leptin concentration in this study, revealed a positive correlation in the active group only ($r = 0.77$, $p<0.01$; Table 12). The reason as for this is unclear. All skinfold measurements were made by the same observer using the same apparatus. Most studies report that plasma leptin concentrations in humans correlated closely and positively with indexes of body fat mass (Considine et al., 1995; Lonnqvist et al., 1995; Caro et al., 1996; Considine et al., 1996). The reason as to why a significant correlation between percentage body fat and serum plasma leptin concentration was not evident in the non-active group remains unclear.

Lipid profiles

No significant differences were evident in mean serum Total Cholesterol (TC), Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) between the active and non-active groups. However, mean Triglyceride (TG) was significantly higher in the non-active subjects when compared to the active group. The overall lipid profiles of all subjects' were within the normal range (US National Cholesterol Education Program (NCEP) (1993)).

Correlation of leptin to lipid profiles did not show any statistical significant relationship between these parameters in both groups (Table 12). This is in contrast to what has been observed in a number of the other studies (Ho et al., 1999; Haluzik et al., 1998; Liuzzi et al., 1999). Caro (1998) where leptin concentrations reflect the amount of TG stored in the body as adipose tissue. When energy intake (EI) and energy expenditure (EE) of an individual are equal or in balance stage. Haluzik et al (1998) reported that serum leptin concentrations positively correlated with BMI, body fat and LDL concentrations in their sedentary subjects whilst, another study reports of a direct association between serum leptin and HDL cholesterol (Liuzzi et al., 1999). The precise reason for the absence of any of these correlation in the present study is not evident. Further larger scale studies may provide a better and more conclusive picture.

Energy (Intakes, Expenditures and Balance)

A nutritional intake of an athlete is a critical determinant of performance (Economos et al., 1993). Therefore, it is necessary for an athlete or the normal person to monitor his/her nutritional status frequently to optimize performance and maintain normal growth and development. Circulating leptin has been shown to regulate food intake and energy expenditure in animal models (Wisse et al., 1999) and in humans (Salbe et al., 1997). Leptin has been hypothesized to be a hormonal signal of body fat stores to the central nervous system whereby it may act to limit food intake and increase energy expenditure and eventually regulate adiposity (Rohner-Jeanrenaud & Jeanrenaud, 1996). In the central nervous system, leptin may interact with central signals involved in food intake regulation such as neuropeptide-Y (Schwartz et al., 1996; Stephens et al., 1995), glucagon-like peptide-1 (Turton et al., 1996) or another peripheral signal, insulin (Schwartz et al., 1996) (Figure 4). Systemic administration of leptin in the leptin-deficient ob/ob mouse results in normalization or reduction of food intake and an increase in energy expenditure (Campfield et al., 1995) and lower plasma insulin and glucose concentrations (Pelleymounter et al., 1995; Schwartz et al., 1996) in ob/ob mice. In addition, leptin also influences energy homeostasis through central regulation of thermogenesis (Collins et al., 1996).

The results of the present study show that there was no significant difference in the mean energy intakes, mean energy expenditures and mean energy balance between the two groups. Mean energy intakes in both the groups were within the recommended norms (Teoh, 1975). However, when compared to Malaysian male students the mean energy

intake values were similar to those reported (Ismail & Zawiah, 1988). Similarly, the energy expenditures were also within the normal range in both groups when compared to the student's data (Ismail & Zawiah, 1988).

Energy expenditures in the two groups in this study were also very similar to that reported for national athletes (Ismail et al., 1995). However, subjects in both groups and subjects in the study of Ismail et al. (1995) it was found that all subjects were having negative energy balance. The results highlighted a nutritional problem among the subjects in both two groups. However, it should be noted that, there may be a possibility of under-reporting of food intake and also physical activity, which may affect the energy balance calculation for the subjects'. This is a well-known flaw of dietary record studies. This requires a record of 7-days of dietary intake and physical activity. Subjects may feel burdened or extra work added to their daily routine, that may cause under-reporting. Therefore it is possible that the actual energy intake and energy expenditures may not be obtained accurately unless we assume full compliance from the subjects. In estimating energy expenditure of any individual, errors arise when determining the correct time spent on physical activity during a given period. Therefore, the subjects in this present study may have also made errors in recording their physical activity.

Although the subjects in the present study were in negative energy balance, it may not mean that they are in chronic energy deficiency (CED) as this study only measured the current status of energy intake and expenditure. Furthermore, this study is a cross-sectional study for determining the effect of physical activity and energy balance on leptin concentrations and it is not a long-term study to evaluate the energy consumptions of the

subjects. However, it should be remembered that negative energy balance may have an effect on body weight regulation in humans. In this regard an individual with CED may lose weight whereby the average energy intakes are below the estimated requirements. This may not mean that they have a negative energy balance (Ismail et al., 1995). This is because the individuals have the capability to adapt to low energy intakes for a limited period (WHO/UNU/FAO, 1985).

Recommended percentage for protein intakes are 12 to 15 % of total calories during training, 10 to 12 % of total calories in pre-competition period, while during competition protein percentage should be below 14 % of total calories (Economous et al., 1993). When compared to these recommended values, the subjects in the active group in the present study, who were in centralized training, consumed protein equivalent to 19.4 % of their total calorie intake (Table 9). Protein intake was above the recommended level. Carbohydrate intake, in term of percentages, were below the recommended level, while fat intake of the active group in this study was above the recommended level (Economous et al., 1993). However, in the non-active group, the contributions of protein and carbohydrates to total energy intake of the subjects corresponded well to healthy eating guidelines of 10-15% for protein, 25-30% for fat and 60-70 % for carbohydrates (Ismail et al., 1995), however, energy contribution from fat was above the recommended levels.

Correlation of plasma leptin and mean energy intake, energy expenditure and energy balance did not show any statistical significant relationships, in either of the two groups. However, the results do not support the previous findings (Salbe et al., 1997; Pagano et al., 1999; Van Aggel-Leijssen et al., 1999) where significant correlations have been observed

between serum leptin and energy intake and energy expenditure. Increased plasma leptin seems to have a potential to elevate the energy expenditures in young children (Salbe et al., 1997). However this has not been confirmed in the adult population. The reason as to why there wasn't any significant correlation between serum leptin and enrgy balance in this study is unclear. Sample size in this study may be one of the reasons. Another reason may be because of closely matched anthropometric measurements especially body weight, BMI and % body fat between the two groups, which may have influenced the correlation of energy intake, energy expenditure and energy, balance with plasma leptin

Plasma Leptin Concentration

Although, mean fasting plasma leptin concentration at rest was slightly higher in the non-active group, the difference however was not statistically significant. Mean fasting plasma leptin levels in this study were slightly lower than those reported in the literature (Hickey et al., 1996; Van Aggel-Leijssen et al., 1999; Boden et al., 1996; Banerji et al., 1999). Fasting causes a decrease in the serum leptin levels, and our subjects were fasted overnight and this may possibly explain the slightly lower plasma leptin level. Serum leptin levels have been shown to decrease (Hickey et al., 1997; Gutin et al., 1999) or remain unchanged (Perusse et al., 1997) following a long-term or chronic exercise. It is possible that the decreased plasma leptin concentration that follows chronic exercise may secondary to the associated decrease in percentage body fat. A bout of physical activity lasting of 45-minutes in this study showed a slight increase in mean plasma leptin concentration in both the active subjects and the non-active subjects. These changes in plasma leptin concentration immediately following exercise were however not statistically significant. Data on this in the literature is

to be equivocal. Serum leptin has been shown to decrease (Duclos et al., 1999) or remain unchanged (Hickey et al., 1996; Perusse et al., 1997; Racette et al., 1997; Torjman et al., 1999) immediately following exercise. Although statistically our data did not reveal a significant difference in plasma leptin levels before and after exercise in both the groups, there however appears a trend that certainly needs further study as mean plasma leptin concentrations were slightly higher in post-exercise in both the groups. Exactly how an acute bout of exercise influences the plasma leptin levels is unclear and its role in exercise induced suppression of appetite remains unknown. The slightly higher levels of leptin post exercise may be due to its reduced renal clearance during exercise as renal blood flow decline during exercise. It also possible that the hemo-concentration that occurs during exercise may be responsible for the rise in serum or plasma leptin during exercise.

Catecholeamines are also known to influence the leptin production however catecholeamines were not measured in this study. Circulating epinephrine and nor-epinephrine increase exponentially during maximal exercise and linearly during prolonged moderate exercise (Torjman et al., 1999). In resting rodents, catecholeamines inhibit leptin production (Gettys et al., 1996; Kosaki et al., 1996) but their role in humans is unclear. The fall of plasma leptin levels post-exercise in some studies (Duclos et al., 1999) may be due to the suppression effect of circulating catecholeamines. It is therefore, possible that in the present study, catecholeamines may have affected the changes in leptin production.

In conclusion, it appears that plasma leptin concentration is not significantly different between active and non-active young males after adjusting for age, BMI and % body fat. An acute bout of exercise does not decrease plasma leptin concentration if anything there

appears to be a trend towards a slight rise in plasma leptin. This may be a consequence of it's a decreased clearance during exercise. Similarly, energy intake or balance did not significantly alter plasma leptin levels in both the active and non-active young individuals. However, further studies are required to confirm these findings.

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