PLASMA LIPID VARIATIONS

IN RESPONSE TO DIET AND EXERCISE

By

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B.Sc.(P.E.), University of Guelph, 1970

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PHYSICAL EDUCATION

in the School

of

Physical Education

and

Recreation

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1972

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ABSTRACT

The purpose of this study was to determine the plasma lipid
variations during periods of low calorie diet and low calorie diet
plus increased physical activity. Four male graduate students, with
above normal activity levels, volunteered for the 10 week study which
was divided into five experimental periods. The first, or control
condition involved a two week period during which the subjects re-
ceived a regular diet of normal foods equivalent to approximately
3600 calories per day. During this period 'normal' activity was
maintained.

The second treatment condition involved a 10 day period of
a low calorie diet, with continued 'normal' activity. The low calorie
diet was equivalent to approximately 1800 calories daily, of natural
foods, plus one multiple vitamin pill.

The third experimental period was similar to the control
period; a two week period during which the subjects received approx-
imately 4000 calories, per day, of the regular diet. Again, 'normal'
activity was maintained.

The fourth treatment condition was indentical to the second
experimental period with the additional requirement of increased daily
energy expenditure, per subject, of 500 calories.

The final experimental period consisted of a two week period
of the regular diet with 'normal' activity.

Blood samples were taken, following an overnight fast, twice during each experimental period: once mid-way through the period and again at the end. Plasma triglyceride and free fatty acid concentrations were measured in duplicate in each sample.

The results of orthogonal comparisons among treatment means showed a statistically significant increase in the plasma free fatty acid concentration during the low calorie diet and the low calorie diet plus exercise treatment conditions. Increased mobilization of free fatty acids from adipose tissue triglycerides in response to the insufficient dietary supply of substrates for metabolism was cited as the mechanism responsible for the rise in free fatty acid concentration. Neuman-Keuls method was used to examine the effect of the increased physical activity during the low calorie diet periods; the results showed that the increased physical activity had no significant effect on the plasma free fatty acids.

Similar statistical procedures applied to the plasma triglyceride values showed a significant decrease in the plasma triglyceride concentration during the low calorie diet and the low calorie diet plus increased physical activity periods. The stress of the low calorie diet on the habitually active subjects was responsible for the decreased levels. The lipid and carbohydrate content of the normal and the low calorie diets, as well as increased peripheral uptake of triglycerides, were suggested as possible explanations for the plasma triglyceride changes. The additional daily output of 500 Calories during the second stress condition was not of sufficient magnitude to elicit a further decrease in plasma triglyceride concentration.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

STATEMENT OF THE PROBLEM

Introduction

This study represented a portion of a larger investigation undertaken by the Division of Human Nutrition, School of Home Economics, U.B.C., and the School of Physical Education and Recreation, U.B.C., during the summer of 1971. The purpose of the entire study was to investigate work performance on a semi-defined, low calorie diet during daily periods of light and moderately heavy physical activity, and to measure various physiological and biochemical parameters deemed relevant.

Since stress conditions such as insufficient caloric intake, physical activity, and hormonal and nervous variations, increase the tissue requirements for lipids (Carlson and Pernow, 1961; Carlson et al. 1965; Renold and Cahil, 1965; Gollnick et al. 1970); measures of lipid metabolism were included in this investigation and constitute the main effort in this thesis. Generally, in response to stress, the triglycerides stored in adipose tissue are hydrolyzed and the liberated fatty acids are transported in an albumin-free fatty acid complex to the tissues (Fredrickson and Gordon, 1958 (b); Olson and Vester, 1960). The plasma free fatty acids have been shown to be a major fuel source for oxidative metabolism during exercise and times of insufficient caloric intake (Dole, 1956; Gordon and Cherkes, 1956; Laurell, 1956; Basu et al. 1960; Rodahl, 1964; Kuel, 1970; Pruett, 1970; Horstman, 1971; Misbin et al. 1971). On the basis of this relationship, the measurement of triglycerides and free fatty acids were made.

Purpose of the Study

The purpose of this study was to determine the variations in plasma triglyceride and free fatty acid levels during a low calorie diet period and a low calorie diet plus exercise period.

Delimitations

1. This study of lipids was confined to the triglycerides and free fatty acids contained in blood plasma, and their changes during the length of the experimental periods.

Limitations

1. The subjects involved in this study were volunteers and highly active and therefore not representative of a random sample.

Hypotheses

A. Free Fatty Acids

1. The level of plasma free fatty acids increases during the low calorie diet.

2. The level of plasma free fatty acids increases during the low calorie diet plus exercise condition.

B. Triglycerides

1. There is no change in the level of plasma triglycerides during the low calorie diet.

2. During the low calorie diet plus exercise period the level of plasma triglycerides is decreased.

Significance of the Study

The principal value of the study was to determine the changes in plasma lipids during the experimental conditions stated previously, thus expanding the knowledge in this area. Plasma lipid variations have been extensively investigated in sedentary subjects or subjects with some form of pathological disorder. This study is unique in that the plasma triglyceride and free fatty acid concentrations are measured in subjects who are habitually active and involved in approximately two hours of daily physical activity.

This study of plasma lipid variations during diet and exercise may also be of use to researchers in the area of coronary heart disease and atherosclerosis. There is considerable evidence implicating abnormal lipid metabolism in the development of coronary heart disease and atherosclerosis. In addition, lipid mobilization in obesity is also of interest as this condition certainly is one of the more common ailments in our society today and its presence has been correlated with many cardiovascular disorders.

Definition of Terms

Triglycerides. (TG) Generally defined as compounds in which each of the alcohol groups of glycerol is esterified with a fatty acid (Masoro, 1968).

Fatty Acid. Any monobasic aliphatic acid containing only carbon, hydrogen, and oxygen and made up an alkyl radical attached to the carboxyl group. The saturated fatty acids have the general formula $C_nH_{2n}O_2$; there are also several series of unsaturated fatty acids having one or more double bonds.

Free Fatty Acids. (FFA) Fatty acids not in ester or amide linkage (free carboxyl group). Also termed unesterified fatty acids (UFA), or non-esterified fatty acids (NEFA).

Hydrolysis. Generally defined as the splitting of a compound by the addition of water. For the purposes of this study, it refers to the breakdown of the triglyceride molecule to glycerol and free fatty acids.

Lipolysis. The splitting up, or chemical decomposition of fat.

Lipase. An enzyme that catalyzes the hydrolysis of ester linkages between the fatty acids and glycerol of the triglycerides.

Lipogenesis. The formation of fat; the transformation of non-fat food materials into body fat.

Esterification. For the purpose of this thesis it may be defined as the synthesis of the triglyceride molecule, basically the reverse of hydrolysis.

Lipemia. The presence of an abnormally high concentration of fat or lipid in the blood.

CHAPTER II

REVIEW OF THE LITERATURE

Introduction

The published material on lipids is enormous and an effort has been made to confine the literature review solely to those areas that pertain directly to the problem. Reports on the effects of restricted caloric intake and increased caloric expenditure on plasma triglycerides and FFA are extensively reviewed, while possible neural and hormonal mechanisms involved in these relationships are more generally recorded.

Related Study

There is only one study analogous to the present investigation. Carlson and Froberg (1967) observed blood lipid and glucose levels during a ten day period of low calorie intake and exercise. Data were obtained on 12 men, aged 20 - 50, who walked 50 km. each day, for ten days. Daily caloric intake was estimated to be about 200 calories; vitamins and minerals were supplied as tablets, in doses slightly above minimum requirements. Levels of glucose, FFA, glycerol, cholesterol, phospholipids and TG in serum, were measured, after an overnight fast, immediately prior to the study, and on the third, sixth, and tenth day of the walk.

The level of blood glucose decreased significantly until the sixth day, but by the tenth day the level had risen above the initial standard. The levels of FFA and glycerol paralleled each other. They were increased during the first period of the study but had decreased by day ten, such that the concentration of glycerol was still significantly

5

elevated above the initial value, but the FFA concentration was only slightly above the initial measurement. The serum cholesterol concentration decreased progressively from the first day. The decrease was statistically significant by day three. The concentration of the phospholipids decreased until the third day, remained about the same until day 6, and then decreased further. The plasma triglyceride level was decreased markedly during the walk; however, this decrease was confined to the very low density lipoproteins.

In their interpretation of these results, Carlson and Froberg suggest that the metabolic events changed in the middle of the study. As an example, they cite the biphasic changes in the concentrations of plasma FFA and blood TG. They also explain that the increase in plasma glycerol, accompanied by the increase in FFA, demonstrated the increased mobilization of FFA by means of increased lipolysis of adipose tissue triglycerides.

The decrease in plasma TG was attributed to the effects of the walk rather than the problem of malnutrition. They believe that this decrease may be due to: 1) diminished hepatic synthesis of plasma lipoproteins, which may have occurred as a result of low availability of fatty acids;

2) increased uptake of TG by peripheral tissues due to increased perfusion of these tissues; and/or

3) increased amounts of lipoprotein lipqse.

With regards to the plasma cholesterol levels, the authors believe that exercise per se, or fasting, does not decrease the plasma

cholesterol level; however, "when weight loss occurs in connection with physical training, there are indications that the cholesterol level in plasma falls." Carlson and Froberg. (1967:630).

Nutritional Influence on Plasma TG and FFA

Keys and his colleagues (1950) published a comprehensive treatise on human starvation and undernutrition which has since become a classic reference to studies in this area. Thirty-two subjects volunteered for the study (the Minnesota Experiment) which was divided into four experimental periods: a control period of 12 weeks with approximately 3500 Calories per day; a semi-starvation period of 24 weeks with approximately 1600 Calories per day; a 12 week period of restricted rehabilitation; and an 8 week period of unrestricted rehabilitation. During the 168 days on the low calorie diet the subjects lost an average of 64% of body fat; in spite of this change in body fat content, there was very little change in any of the lipid fractions studied. Total plasma lipids, the phospholipids, and the serum cholesterol were measured and although all three lipid fractions decreased, only the results on serum cholesterol were statistically significant. Keys offers a partial explanation to the absence of any lipemia on the basis that the total fat metabolism during the semi-starvation period was approximately 57% of the value for the control period.

The effect of undernutrition on the level of plasma FFA is well known. The concentration of plasma FFA increased due to increased mobilization from adipose tissue.

Gordon (1957), measured the arteriovenous unesterified fatty acid (UFA) differences in adipose tissue and skeletal muscle of the forearm in normal, fasting subjects. He reported that the fatty acids were entering the circulation from the adipose tissue and that the myocardiam and skeletal muscle tissues were capable of extracting the U.F.A. This study has been supported by other researchers who also have concluded that the adipose tissue TG represents the major source of circulating FFA (Laurell, 1956; Gordon and Cherkes, 1956; Carlson and Froberg, 1967).

The effects of a low calorie diet on the plasma TG are not so well defined. Kartin et al. (1944) studied serum lipid changes during fasting and conditions of undernutrition. His results showed only a small insignificant alteration of the plasma TG concentration. On the other hand, Rubin and Aladjem (1954) have shown that plasma TG are significantly elevated as a result of fasting. Carlson and Wadstrom (1956) investigated glyceride levels in four healthy young individuals, two females and two males, fasted for 63 hours. Their results showed an increase in the triglyceride level only, there was no change in the concentration of the di and monglycerides. This increase in the triglyceride level was attributed to increased lipid moblilization to meet the energy requirements during caloric restrictions. R.J. Havel, in response to this paper, commented that he had found considerably reduced quantities of glycerides in fasting individuals.

When glucose or sucrose was administered to previously fasted men, the plasma triglyceride level decreased (Bragdon et al. 1957; Baker et al. 1968). These lowered levels have been shown to persist up to 8 to

11 hours (Havel, 1957). It is probable that this reduction of plasma triglyceride concentration by glucose ingestion is mediated by the stimulation of insulin secretion.

Dole (1965) and Gordon and Cherkes (1956) suggested that one of the probable ways which glucose acutely interferes with the plasma triglyceride levels is through the lowering of the concentration and the turnover rate of FFA. Miller (1967) showed that the total amount of plasma FFA and glycerol converted into liver triglycerides and their release into the plasma was decreased during a glucose load. Also, since the endogenous synthesis of hepatic fatty acids was still depressed during the first few hours after administration of glucose (Baker et al. 1968), the formation of liver and plasma triglyceride ceases simply as a result of the lack of the available precursor (FA).

Effects of Exercise on Plasma TG and FFA

During exercise, energy is derived both from fats and carbo-hydrates (Astrand and Rodahl, 1970). The plasma FFA have been shown to be an important source of energy during exercise (Basu et al. 1960; Havel et al. 1963; Miller, Issekutz and Rodahl, 1963; George and Vallyathan, 1964; Havel et al. 1964; Keul, 1970; Horstman et al. 1971). During the first 10 to 15 minutes of exercise the plasma concentration of FFA decreases due to increased efflux of FFA from the plasma to the peripheral tissues (Friedberg et al. 1960 (a); Havel et al. 1963). Carlson and Pernow (1961) have suggested that the increased efflux of plasma FFA is due to the increased amount of FFA perfusing muscular tissue per unit time. If the exercise is continued, the FFA level is increased as a result of enhanced mobilization of FFA from adipose

tissue (Friedberg et al. 1963; Havel et al. 1964.) At the cessation of exercise cardiovascular adjustments return more rapidly to basal levels than do the metabolic changes, thus the plasma concentration of free fatty acids first increases rapidly, then slowly returns to basal levels (Carlson and Pernow, 1961; Friedberg et al. 1963).

Lipolysis during exercise is commonly thought to be controlled by the adrenergic system (Gollnick, 1970). Havel (1963:1060) suggested that: " ... mobilization of fatty acids during exercise may result from augmented activity of sympathetic nerves in adipose tissue with consequent local liberation of norepinephrine, a potent activator of triglyceride hydrolysis in this tissue".

Havel and Goldfien (1959) and Vendsal (1960) have shown that the level of circulating catecholamines during exercise is elevated and the fact that both norepinephrine and epinephrine stimulate lipolysis (Horstman et al. 1971; Rudman, 1963) support the hypothesis that the adrenergic system controls lipolysis during exercise. However, numerous nonadrenergic substances have been shown to induce lipolysis and these, too, may exert some control over lipolysis during exercise.

Rudman (1963) has shown that such nonadrenergic hormones as ACTH, growth hormone, glucagon and several other pituitary polypeptides possess adipokinetic activity. Of these, ACTH would seem to be the most important during exercise as it is a rapid stimulant of lipolysis, and it has been shown to increase in concentration during physical activity (Hunter et al. 1965).

Hunter and Sukkar (1968) have shown that the plasma insulin concentration falls during exercise. This fact, combined with the knowledge that in small concentrations, insulin inhibits fat mobilization (Dole, 1956; Havel and Goldfien, 1959) may indicate that this fall in plasma insulin assists the mobilization of FFA during exercise.

It is a well known principle that during heavy exercise, the blood lactate concentration increases. Miller et al. (1963) and Issekutz (1964) have shown that lactate inhibits lipolysis and they suggest that the buildup of lactate during exercise may supress the plasma FFA concentration.

Rodahl (1964) has demonstrated that the plasma FFA concentration depends upon the intensity and duration of exercise. During heavy, short work bouts the FFA concentration was shown to drop, possibly due to the rapid increase of the blood lactic acid level. When the workload was such that it could be continued for several hours, the blood lactate remained practically unchanged and the plasma FFA concentration rose.

These changes in plasma FFA are caused by the acute effects of exercise. These alterations are important to the over-all picture of FFA mobilization however, as the blood sampling period was immediately after an over-night fast, it is the chronic effects of exercise on FFA that pertains more directly to this study.

Carlson and Permow (1961) studied the plasma FFA concentration during and after exercise. Immediately after exercise the FFA level increased rapidly, then slowly decreased in concentration; 30 minutes

after exercise the values were only slightly greater than the resting measurements.

Havel et al. (1964) investigated the change in plasma FFA during and after two hours of moderate exercise. During the exercise period the plasma FFA values rose as expected. At the cessation of exercise, again, the FFA values rose rapidly and then fell, until the resting levels two hours after exercise showed no difference when compared to the pre-exercise values.

In view of the fact that FFA are the most metabolically active of the plasma lipids (Harper, 1971), these results are not suprising. Thus it is quite probable that the acute effects of exercise during the days prior to the blood sampling period would not be reflected in the resting values the next morning.

Experiments pertaining to the acute effects of exercise on the concentration of plasma TG have indicated a decrease in this lipid fraction and although all the factors involved have not been clearly established, a summary of the known mechanisms contributing to the reduction in plasma TG concentration is presented. Since the major site of plasma TG producation is the liver (Carlson and Ekelund, 1963; Nikkila, 1969), the decreased plasma TG level could be a result of reduced hepatic production of TG. FFA are important precursors to the liver, and therefore, the plasma TG (Havel and Goldfien, 1961: Friedberg, 1961; Havel et al, 1962), and although it is known that during physical activity the mobilization of the FFA is increased, Hagenfeldt and Walren (1971) have demonstrated a linear increase in the uptake of FFA if increased blood flow to the

hepatic region is significantly reduced. These facts would suggest that the uptake of FFA by the liver, would be decreased during exercise, which would therefore result in a decreased production of liver TG. To support this hypothesis, Havel et al (1964) have shown a reduction in the amount of labelled FA incorporated into plasma TG during exercise as compared to resting values.

There is an hypothesis that during exercise the hepatic production of lipoprotein peptides (LP) may be decreased (Carlson, 1967) and thus the vehicle used to transport the TG in plasma may not be available. This could be a limiting factor in hepatic TG release (Nikkila, 1969). Still another factor to be considered in the reduction of plasma TG due to exercise, is the role of lipoprotein lipase activity (VLPL). Nikkila et al. (1963) have shown that the activity of this enzyme system is increased in the myocardium as well as in skeletal muscle during exercise. As this enzyme is responsible for the hydrolysis of circulating TG it may well be an important factor in the decrease of plasma TG.

Holloszy et al. (1964) studied the chronic effects of exercise on the serum cholesterol, phospholipid, and triglyceride levels of middle-aged men. Two groups of subjects were involved in the study. Group A consisted of 15 men, all of whom had led sedentary lives for three or more years. They participated in a progressively more strenuous program of endurance calisthenics and distance running (2 to 4 miles) on an average of 3.35 times per week for six months. Five nonexercising control subjects were included in this group to serve as a check on the seasonal variations in serum lipid values. Group B was made up of 12 men, also having led sedentary lives for a number of years. They participated in a program of distance running geared to their individual capacity.

Three fasting blood samples were obtained on each subject over a seven day period prior to the exercise program to establish baseline values. Thereafter, fasting blood samples were obtained once a month until the last week of the study, when three samples were again taken over a seven day period. Total serum cholesterol levels were determined once a month, while phospholipids and triglycerides were measured every other month.

The serum triglycerides were measured in 14 subjects in Group A. The mean value for this group decreased from 208 ± 127 mg% to 125 ± 78 mg% during the six month period. At the completion of the exercise program several men with initially high triglyceride levels were asked to remain sedentary for five or six days. Fasting levels were determined at the end of this period and then the men were instructed to run three miles. Triglyceride values were measured at 2, 3, 20, and 44 hours following this run.

After the five or six days of inactivity the fasting triglyceride level had increased considerably over the final values determined at the end of the exercise program (an average of 150 mg%). Within two or three hours following the three mile run a reduction was evident and this reduction persisted for the 44 hour period. The authors suggest that the effect of exercise may be cumulative. Certain subjects had lower fasting serum triglyceride levels after a number of days of exercise program than they did following a single period of exercise consisting of a three mile run.

Thus exercise does produce a chronic effect on the plasma triglyceride concentration.

Hormonal Regulation of Plasma TG and FFA

Hormones may have a facilitating or permissive action on lipid metabolism (Carlson et al. 1965). In 1958, Laurell and Christensson conducted an experiment to determine the effect of a single dose of various hormones on the plasma FFA, and where indicated, the plasma TG and Phospholipids. The hormones injected were: growth hormone (GH), prolactin (LH), adrenocorticotropic hormone (ACTH), noradrenaline, adrenaline and glucagon. They concluded that ACTH and GH had no significant effect on plasma FFA; LH and glucagon had a similar reducing effect on plasma FFA; noradrenaline increased the FFA concentration to roughly the same extent as a corresponding dose of adrenaline. No changes in plasma TG or phospholipids were observed in this experiment. There appears to be some conflict between these conclusions and those of more recent investigation.

Growth hormone. Henneman and Henneman (1960) have shown that intravenous administration of GH results in a prompt rise of plasma FFA and that continued daily doses of GH sustain the rise in plasma FFA. In a similar study Rabinowitz et al. (1965) demonstrated not only that GH increases the release of FFA from adipose tissue but also that GH enhances FFA uptake by muscle tissue.

Recently, Felig (1971) conducted experiments which support the findings of Rabinowitz. He also noted that the increase in plasma FFA, with administration of GH, was paralleled by a rise in blood glycerol, thus supporting the theory that the increase in FFA was due to lipolysis in adipose tissue.

Possibly the most influential study on GH was conducted by Roth et al, (1963). They have shown that the rate of secretion of GH was markedly stimulated by hypoglycemia, fasting, interference with glucose utilization, and by muscular exercise. In view of the fact administration of GH results in rapid release of fatty acids from · adipose tissue (Raben and Hollenberg, 1958; Henneman and Henneman 1960), they interpreted their results as follows:

> ... endogenous plasma growth hormone concentrations are strikingly increased in a variety of physiologic and experimental conditions known to be associated with high concentrations of unesterified fatty acids in plasma. Thus, hypoglycemia, exercise, fasting and interference with glucose utilization by means of deoxyglucose are all followed by secretion of growth hormone, a response that provides for increased availability of a monocarbohydrate source of oxidizable substrates, namely fatty acids. Roth et al. (1963:579)

Glucagon. Unger and Eisentrout (1964) have identified glucagon in the efferent plasma of the pancreas and their demonstration of alterations of its secretion, induced by changes in blood glucose concentration, supported the view that glucagon is a true hormone with a major role in blood glucose regulation. Glucagon secretion has been shown to rise during all forms of glucose need (Unger et al. 1962; Unger et al. 1963). Thus, Unger and Eisentrout (1963:1031) consider glucagon, "... a hormone of glucose need, the function of which is to maximize hepatic glucose production when food is not available, thereby serving to maintain the flow of glucose to the brain".

Lipsett et al (1960) examined the effects of glucagon on plasma FFA. They injected glucagon intravenously and analyzed the blood glucose and plasma FFA concentrations at 0, 1, 2, 4, and 6 hours after injection.

One hour after injection the plasma FFA concentration was significantly decreased; by the second hour the FFA level had returned to the initial value. However, at the fourth and sixth hours, the levels were significantly increased above the base-line value. In view of these results the authors stated: "A reasonable hypothesis is that glucagon effects the release of unesterified fatty acids from fat depots." Lipset et al. (1960:352).

Steinberg et al (1959) conducted studies on rat adipose tissue in vitro and demonstrated an eight fold increase in the release of unesterified fatty acids in the presence of glucagon. They also administered glucagon to a fasting dog, which caused a initial fall in plasma FFA during the hyperglycemic phase, followed by a sustained rise (2 to 10 hours) after blood glucose had returned to normal.

Lipsett, Engel and Bergenstal (1959) suggest that glucagon possesses activity apart from its effects on glycogenolysis and glucose utilization. Intravenous injection of glucagon during fasting resulted initially in a slight fall of plasma FFA. Three to six hours later, when the blood sugar concentration was normal, the plasma FFA concentration had increased two to three fold. Control injections of saline were followed by slight increases in plasma FFA due to the continued fast. Thus, they concluded that glucagon affects FFA metabolism independently of carbohydrate metabolism.

Insulin. The permeability of the cell membrane for glucose depends on the plasma insulin concentration. If an overnight fast is continued from 8:00 p.m. to 10:00 a.m., subjects at rest show a steady fall in plasma insulin concentration (Sukkar et al. 1967).

Insufficient caloric intake is associated with increased mobil-
ization of plasma FFA from adipose tissue stores and increased utilization
of fat for oxidative metabolism. Dole (1956) has shown that in very small
concentrations, insulin inhibits fat mobilization and therefore the fall
in plasma insulin during malnutrition may assist fat mobilization.

Bierman, Schwartz and Dole (1957) studied the effect of insulin
on the release of fatty acids from adipose tissue stores. Using C^{14} -
labelled palmitic acid, they were able to show that insulin decreased the
release of fatty acids from tissue stores but it did not accelerate the
removal of fatty acids from blood.

This inhibitory effect of insulin of the release of fatty acids
and glycerol from adipose tissue was investigated by Jungas and Ball (1963).
They interpretated the function of insulin, with regards to FFA, in three
manners:

1) Insulin may have a direct or indirect inhibitory effect on the adipose
tissue lipase.

2) Insulin could conceivably accelerate the steps whereby monoglyceride
or diglyceride recombines with free fatty acids.

3) Insulin may accelerate the conversion of glycerol to glycerophosphate
by activating the latent glycerokinase enzyme. This would permit a re-
synthesis of diglyceride by way of phosphatidic acid. In either of the
last two cases a re-esterification of free fatty acids would be promoted.

Jones and Arky (1965) reported that in normal humans a single
insulin injection did not change the serum triglyceride level but a pro-
longed insulin infusion produced a marked fall in all subjects. Haahti
(1959) has described a nondiabetic patient with hyperlipemia, in which

insulin treatment effectively reduced all plasma lipids.

Epinephrine and Norepinephrine. Studies in vivo in man (Laurel and
Christensson, 1958; Havel and Goldfin, 1959), and in animals (Spitzer
and Hohenleitner, 1961) have all shown increased levels and increased
output of FFA after injection of catecholamines.

Carlson and Oro (1963) have studied the effects of norepine-
phrine on the release of fatty acids from adipose tissue and their results
showed that the main effect of norepinephrine was on the lipolysis in
adipose tissue and not on the re-esterification process. Rizack (1961) has
shown a similar lipolytic response of adipose tissue when exposed to epine-
phrine.

White and Engel (1958) studied the effects of epinephrine and
norepinephrine on rat adipose tissue in vitro. They concluded that both
suprarenal hormones stimulate production of nonesterified fatty acids from
rat adipose tissue, presumably by stimulating the hydrolysis of neutral
fats within the tissue.

Autonomic Control of Fat Mobilization

The autonomic nervous system is concerned principally with internal
adjustments of the organism and it has been shown that this system is in-
volved with the responses of those endocrine glands that receive a nervous
supply (Morgan, 1965).

Beznak and Hasch (1937) were the first investigators to suggest
that mobilization of fat from adipose tissue involved the autonomic system.
This hypothesis has been confirmed by other researchers (Havel and Goldfin,
1959; Confalonieri et al. 1961).

Two types of adrenergic receptors have been demonstrated by Ahlquist (1948), the alpha- and the beta- adrenergic receptors. Both receptors have been shown to exist in human adipose tissue, where the alph-adrenergic receptors inhibited lipolysis while the beta-adrenergic receptors activated lipolysis in the adipose tissue (Efendic, 1970; Fredholm and Karlsson, 1970; Ostman and Efendic, 1970).

Adipose tissue contains appreciable stores of the sympathetic transmitter substance, norepinephrine, and these stores have been shown to be depleted after cutting the autonomic nerve supply (Sidman et al, 1962; Stock and Westerman; 1963). Conversely, Correll (1963) has shown that direct stimulation of the autonomic nerve supply can result in rapid release of fatty acids from adipose tissue.

Chapter Summary

Figures 1 and 2 are presented as a summary of the available literature on the fat transport cycle as it pertains to plasma FFA and TG. At rest (Figure 1), there is an equilibrium existing between the FFA hydrolyzed and re-esterified within the adipose tissue; the circulating TG hydrolyzed to FFA by the vascular lipoprotein lipase (VLPL); and the FFA incorporated into plasma TG in the liver. Thus the plasma concentrations of TG and FFA remain constant.

Figure 2 represents the fat transport cycle during times of insufficient caloric intake. There is considerable evidence that the plasma concentration of FFA is increased during stress of this nature and this increase has been shown to be a result of lipolysis of the adipose tissue triglycerides. Changes in the circulating hormone levels of GH, glucagon and insulin mediate this response. The fate of the plasma TG during caloric
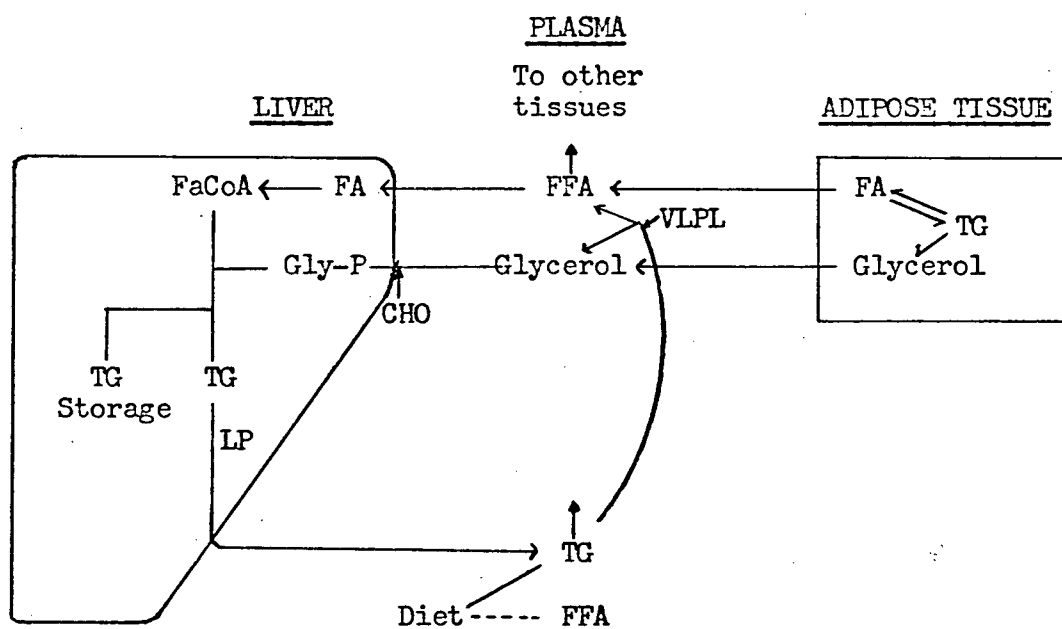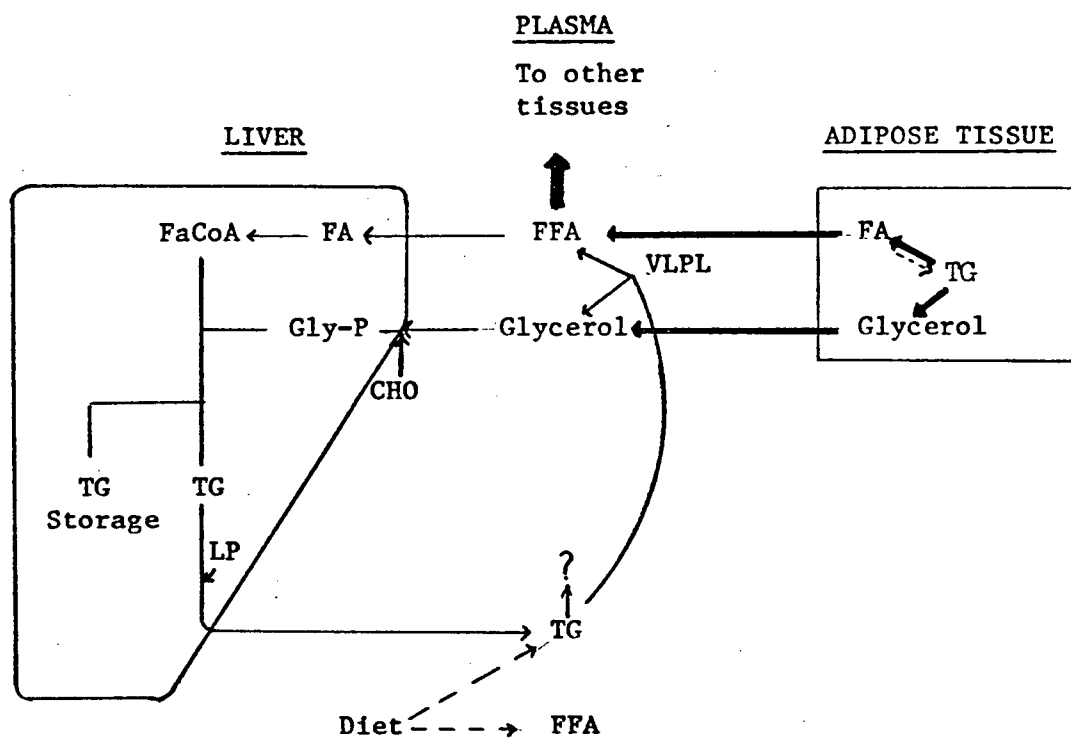
FIGURE 1. FAT TRANSPORT CYCLE : AT REST

FIGURE 2. FAT TRANSPORT CYCLE : CALORIC INSUFFICIENCY

insufficiency however, is open to controversy, and no concrete theories have been made.

During exercise the plasma FFA concentration is increased due to lipolysis of the adipose tissue triglycerides. This process is controlled, to a large extent, by the adrenergic system, although numerous non adrenergic mechanisms such as increased amounts of GH, ACTH, glucagon; decreased plasma insulin concentration, and the production of lactic acid, have been shown to exert some control over lipolysis during exercise. However, these acute effects of exercise on plasma FFA are diminished within 2 hours after the cessation of the activity and therefore these changes in plasma FFA are not reflected in the blood samples taken after an overnight fast.

Thus there are no chronic effects of exercise on plasma FFA. In addition, there is no evidence present to suggest what effects habitual exercise has on the endocrine secretions that regulate the plasma FFA and TG levels.

The actue effects of exercise on plasma TG are well documented; there is a decrease in the circulating levels dur to a number of factors. The blood flow to the hepatic region is reduced during exercise, which, accompanied by the increased peripheral uptake of the FFA, accounts for reduced quantities of precursor for the endogenous TG. Also, the activity of the vascular lipoprotein lipase is increased during exercise causing hydrolysis of some of the circulating TG. In addition, there is evidence that the hepatic synthesis of the lipoprotein peptides (LP) may be decreased during exercise as well as the factor of increased peripheral uptake of the circulating TG by the working muscles.

These acute effects remain for at least 44 hours and there is strong evidence to suggest that habitual exercise produces a cumulative decrease in plasma TG concentration.

CHAPTER III

METHODS AND PROCEDURES

Subjects

Four male graduate students, aged 23 to 27, from the University of British Columbia, volunteered for the ten week study which began May 17, 1971. Prior to the first treatment condition the subjects were given a medical examination. This was done to ensure that all biochemical and physiological parameters fell within the normal ranges. None of the subjects was considered obese or overweight.

The subjects had higher than 'normal' activity levels. Three of the subjects were involved in competitive athletics during the summer and spent approximately two hours per day in training; the other subject supplemented his 'normal' activity with swimming and jogging in order to closely approximate the levels of activity for all subjects.

Experimental Periods

The subjects were housed together on campus for the entire summer, May - September, 1971. The weights of the subjects were recorded daily, upon rising, for the duration of the investigation. Each meal was prepared by a qualified dietician, and the food weighed so that each subject received the same amount of food, with the same caloric content. An additional serving of each meal was prepared, blended and frozen for the analyses of the constituent nutrients.

There were five experimental periods; these were performed consecutively. In the first, or control period, the subjects received approximately 3600 Calories per day of a regular diet which consisted of normal foods which met or surpassed those nutritional requirements recommended in the Dietary Standard for Canada. During this two week control period 'normal' activity was maintained; periodically, daily records of activity were recorded to ensure that a 'normal' level was being continued.

The second treatment condition involved a 10 day period of a low calorie diet with continued 'normal' activity. The low calorie diet amounted to approximately 1800 Calories of a semi-defined diet consisting of natural foods which, combined with one multiple vitamin pill per day, met or surpassed those nutritional requirements recommended in the Dietary Standard for Canada. The entire allotment of food for the day, in the form of eight biscuits or a loaf, plus 21 grams of margarine, was given to the subjects early in the morning to be consumed, as they wished, prior to 12 o'clock midnight. One vitamin pill per day was also given to the subjects and an unlimited supply of tea or coffee (without cream or sugar) was also available. There were no restrictions as to the amount of water consumed.

The third experimental period was identical to the control period; a two week period during which the subjects received approximately 4000 Calories, per day, of the regular diet. Again, 'normal' activity was maintained.

The second stress situation, the fourth treatment condition, was similar to the second experimental period with the additional requirement of increased daily energy expenditure, per subject, of 500 Calories. In order to achieve this goal, the subjects had to jog six miles in approximately 50 minutes or swim continuously for 40 minutes. This was generally broken into two exercise sessions.

The final experimental period was identical to the third treatment condition; a regular diet with 'normal' activity.

Blood samples were taken twice during each experimental period, once mid-way through the period and again at the end. After an overnight fast, approximately 25 milliliters of blood were drawn from the antecubital vein of each subject. Approximately three ml were stored with various reagents or used immediately for the determination of hematocrit, hemoglobin concentration, vitamin C and glucose analyses. Of the remaining whole blood, roughly one half was allowed to clot and the serum removed and frozen; the rest of the whole blood was stored using heparin as the anticoagulant. Later, upon thawing, the serum was removed from this portion also. The plasma was stored in a freezer for approximately 18 weeks prior to the analyses.

The free fatty acids were analyzed in duplicate by the colorimetric, micro-method of Laurell and Tibbling, Clinica Chimica Acta. 16:57-62, 1967, using the serum stored without heparin.

The heparinized serum was used in the duplicate determination of the plasma triglycerides by the enzymatic method of Schmidt and von Dahl, Z. klin. Chem. 6, 156-159, 1968, obtained in a kit form from the Boehringer Mannheim Company, Biochemical Department.

Experimental Design

The purpose of this investigation was to determine the plasma lipid variations during diet and exercise. Thus, the lipids contained within the plasma, specifically the triglycerides and free fatty acids, represented the dependent variable, while the diet and level of physical activity, depicted the independent variables, or the variables manipulated during the study.

This study is representative of a single group, repeated measures design with the first treatment condition acting as a control for the remaining four experimental periods. See Table I

Statistical Analyses

The data obtained on the triglyceride and free fatty acids were analyzed statistically and represented graphically. A planned, rather than post-hoc, comparison was chosen to test the hypothesis. Dunnett's method (Winer, 1962) for comparing all means with a control mean was used. Theoretically, as this is a planned comparison, an analysis of variance between means was not necessary, however, as the denominator of Dunnett's method require the MS error term, an analysis of variance to test the significance between means was calculated.

TABLE I

EXPERIMENTAL DESIGN

TREATMENT CONDITIONS

	1	2	3	4	5
	Control	Stress Situation 1	Recovery	Stress Situation 2	Recovery
Blood Sample	1 2	3 4	5 6	7 8	9 10
Day	7 14	6 10	7 14	5 10	7 14
	Regular Diet	Low Calorie Diet	Regular Diet	Low Calorie Diet	Regular Diet
	+	+	+	+	+
	Normal Physical Activity	Normal Physical Activity	Normal Physical Activity	Increased Physical Activity	Normal Physical Activity

CHAPTER IV

RESULTS AND DISCUSSION

Results

The means and standard deviations of the plasma free fatty acid and triglyceride levels determined for each blood sampling period are presented in Table II.

TABLE II

MEANS AND STANDARD DEVIATIONS

BLOOD SAMPLE	FFA mmoles/l	TRIGLYCERIDES mg/100 ml
1	0.373 ± 0.073	115.63 ± 25.97
2	0.307 ± 0.121	110.27 ± 18.96
3	0.592 ± 0.258	87.71 ± 5.40
4	0.540 ± 0.168	77.33 ± 7.49
5	0.325 ± 0.132	94.51 ± 12.37
6	0.297 ± 0.119	105.97 ± 19.28
7	0.688 ± 0.130	70.88 ± 10.22
8	0.500 ± 0.187	56.56 ± 10.81
9	0.267 ± 0.092	98.45 ± 25.73
10	0.228 ± 0.115	104.89 ± 20.96

These values are also presented graphically in Figures 3 and 4. Visual inspection of the data suggested a possibility of heterogeneity of variance, which would render the analyses of variance and orthogonal comparisons invalid. However, Bartlett's test (Edwards, 1964) was applied to the data and the results concluded that the variances were homogeneous.

Statistical Analyses

As the recovery values of the FFA and TG did not return to the control values, Dunnett's method for comparing all means with a control could not be used to test the hypotheses.

Orthogonal comparisons of treatment means (Edwards, 1964) were chosen as an alternate method to serve this purpose. Although there were two blood samples per experimental period, the most appropriate values to use to test for a significant difference were the final means for each condition, that is, the values immediately prior to the experimental period as compared to the final values for the treatment condition. Therefore, to test the changes in the plasma lipids due to the low calorie diet, the final lipid values of condition 1 were compared to the final values of condition 2. Similarly, the final lipid values of condition 3 were compared to those of condition 4 in order to examine the effects of the low calorie diet plus increased physical activity on the plasma lipids. The following Tables summarize the results of these comparisons.
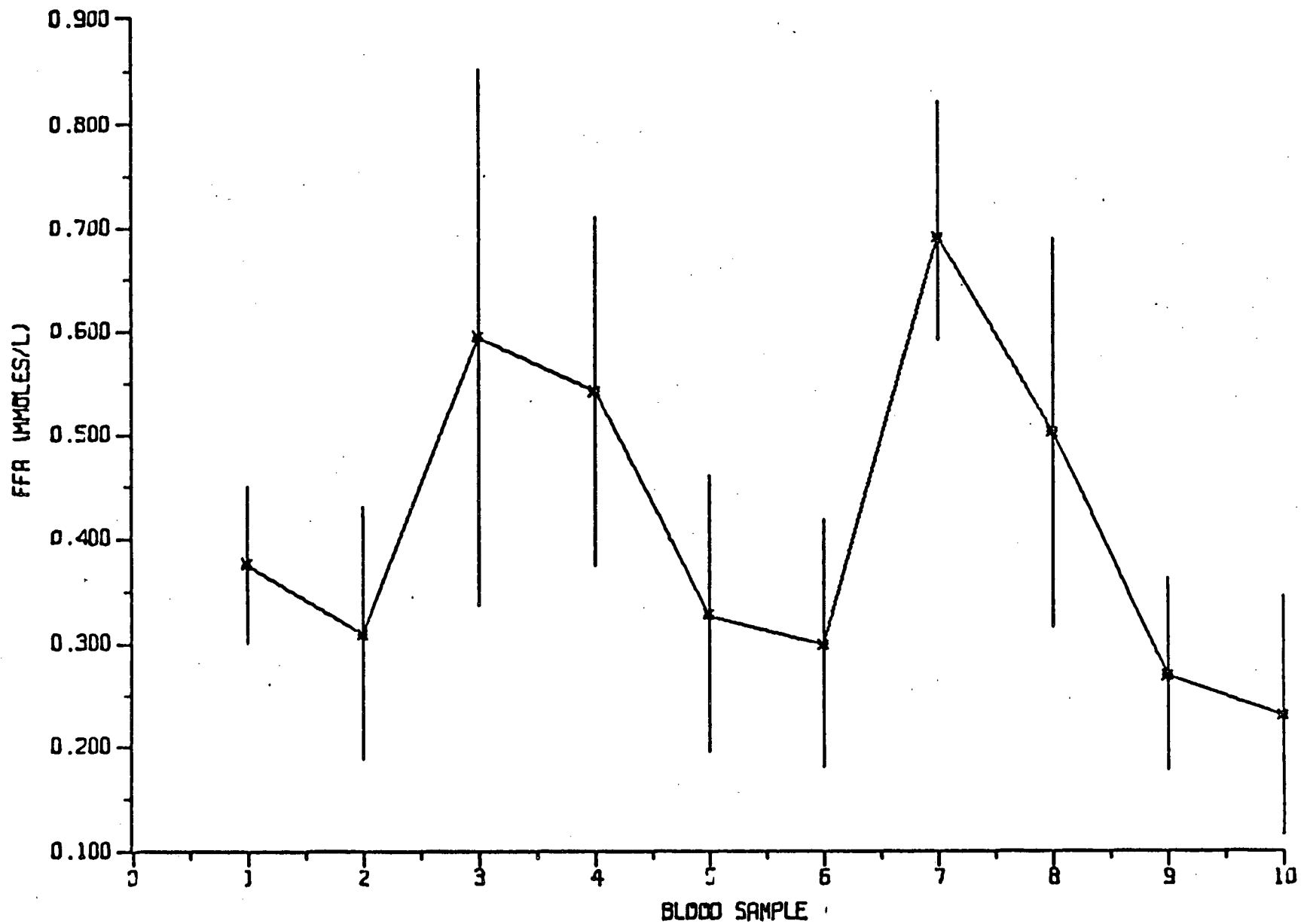
FIGURE 3. PLASMA FREE FATTY ACID VARIATIONS
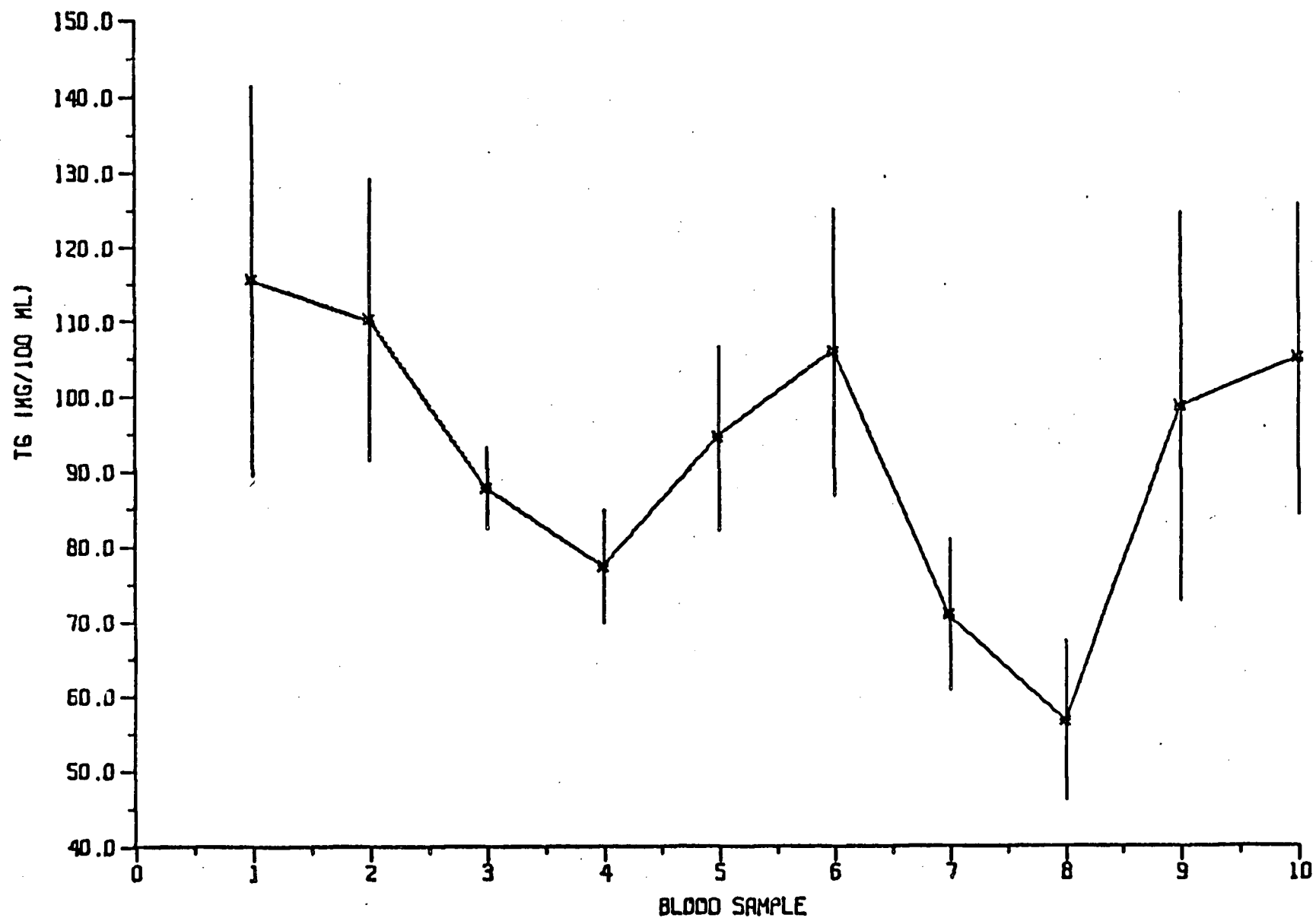
FIGURE 4. PLASMA TRIGLYCERIDE VARIATIONS

TABLE III

ORTHOGONAL COMPARISONS:

FREE FATTY ACIDS

COMPARISON	df	Sd	t	P
CONDITION 1 VS. CONDITION 2	27	0.083	2.81	$< .01$
CONDITION 3 VS. CONDITION 4	27	0.083	2.45	$< .05$

TABLE IV

ORTHOGONAL COMPARISONS:

TRIGLYCERIDES

COMPARISON	df	Sd	t	P
CONDITION 1 VS. CONDITION 2	27	10.75	3.06	$< .01$
CONDITION 3 VS. CONDITION 4	27	10.75	4.69	$< .01$

Subsidiary issues concerning the experimental periods, such as the effect of increased physical activity during the low calorie diet condition, were considered relevant to the study and post-hoc analyses of these comparisons were carried out. As this was a post-hoc examination, an analysis of variance among the means for both the FFA and the TG was necessary prior to any further comparisons. The facilities of The University of British Columbia Computing Centre were used and the results are summarized as follows.

TABLE V

SUMMARY OF ANOVA:

FREE FATTY ACIDS

SOURCE	df	MEAN SQUARE	F	P
SUBJECTS	3	0.096		
CONDITIONS	9	0.098	7.18	< .01
ERROR	27	0.014		
	39			

TABLE VI

SUMMARY OF ANOVA:

TRIGLYCERIDES

SOURCE	df	MEAN SQUARE	F	P
SUBJECTS	3	888.339		
CONDITIONS	9	1438.330	6.23	< .01
ERROR	27	231.011		
	39			

Neuman - Keuls method (Winer, 1962) of examining the nature of the differences among treatment means was used following the significant over-all F ratios of the FFA and TG. The results of this procedure used in the discussion section of this chapter are presented below.

TABLE VII

NEUMAN – KEULS ANALYSES:

PLASMA LIPIDS

COMPARISON	PLASMA LIPID	q.95 (r;27)	cal.q.95	RESULT
CONDITION 2 VS. CONDITION 4	FFA	.169	.040	N.S.
	TG	26.68	20.77	N.S.

Discussion

The subjects had a higher activity level than "normal" and although the daily recommended allowance for the subjects (3600 Calories) was observed for the first treatment condition, the subjects showed a gradual loss of weight, indicating that a daily intake of 3600 Calories was not sufficient to maintain caloric balance. Therefore, you would expect the low calorie diet, which contained fifty percent of the recommended calorie allowance, to be a considerable stress on the caloric equilibrium of the body and this stress to be further compounded by the effect of the increased physical activity.

Free Fatty Acids. The results of the orthogonal comparisons have shown that the plasma FFA level was significantly elevated ($p < .05$) during both the

low calorie diet and the low calorie diet plus exercise conditions. This is not surprising as it is well known that the plasma FFA are related to the nutritional state of the subject (Dole, 1956; Gordon and Cherkes, 1956). The low calorie diet initiated a negative calorie balance of approximately 1800 Calories per day per subject. In addition, during the low calorie diet plus exercise period, daily increased physical activity accounted for an additional 500 Calories creating a total deficit of approximately 2300 Calories per day for this period. Therefore, as the diet was not providing sufficient substrates to supply the metabolic processes of the body, the source of these substrates must have been of an endogenous nature.

It is an accepted principle that the plasma FFA serve directly, along with glucose, as the major substrates for metabolism (Andres et al, 1956; Carlson and Pernow, 1959; Friedberg et al, 1960 a; Friedberg et al, 1960 b). In addition, Gordon and Cherkes (1956), Carlson and Oro (1963), Baker et al (1968) and others have shown that there is a definite relationship that exists between carbohydrates and lipids in substrate utilization. If the nutritional requirements are satisfied by carbohydrates, the plasma FFA concentration drops to a low level, probably due to the secretion of insulin, which is a known inhibitor of FFA release (Dole, 1956 a). Conversely, if glucose is not available in sufficient amounts to satisfy the cellular requirements, the level of FFA in plasma rises. Statistical analysis of the serum glucose level [1] showed a significant decrease ($p < .05$) in this variable during the diet and the diet plus exercise conditions, indicating that the glucose stores were being depleated (Issekutz et al, 1966). Therefore, the rise in plasma FFA

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(1) Variables, other than lipids taken during this study are available
     from the Division of Human Nutrition, School of Home Economics, UBC.

concentration, during the low calorie diet and the low calorie diet plus exercise periods, followed a logical and previously demonstrated pattern.

The rise in plasma FFA was a result of increased mobilization of FFA from the triglycerides stored as adipose tissue (Gordon and Cherkes, 1956; Fredrickson and Gordon, 1958 a; Fredrickson and Gordon, 1958 b; Basu, 1960; Carlson and Oro, 1963). The contribution of the short chain fatty acids (C<10), absorbed directly from the gastrointestinal tract, can be considered very minor (Fredrickson and Gordon, 1958 a), especially considering the small amount of fat in the low calorie diet. The only other source of FFA is the circulating plasma TG; however, the activity of the vascular lipoprotein lipase, which acts on the TG-lipoprotein complex to liberate the FFA, would not contribute significantly to the plasma FFA level at rest (Monkhouse et al, 1961; Persson et al, 1970). Considering the data obtained from the anthropometrical measurements, which showed a decrease in various skin fold thicknesses during the low calorie diet and the low calorie diet plus exercise periods, and the accompanying decrease in the percentage of body fat, as calculated from the body density measures, a decrease in the size of the adipose tissue during these periods was indicated and supports the fact that hydrolysis of adipose tissue triglyceride was responsible for the elevated plasma FFA level.

Neuman - Keuls method was used to examine the differences between the lipid values observed during the low calorie diet and the low calorie diet plus increased physical activity periods. The results showed no significant difference (P>.05) between the final FFA values observed during each period, nor was there statistical significance between the samples taken at the mid-points of these experimental conditions.

Therefore the increased caloric expenditure, during the low calorie diet, had no statistically significant effect on the plasma FFA level as measured during this study. Certainly all the literature concerning plasma FFA levels and exercise has indicated that these levels are increased significantly during exercise; however, as the FFA are known to be the most metabolically active of the plasma lipids (Harper, 1971), it is probable that the acute effect of exercise was diminished prior to the blood sampling.

The graph of the plasma FFA variations (Figure 3) shows that the highest values of the FFA were attained at the mid-point of each stress situation, rather than at the termination of each condition as would be expected. Similar findings have been reported by Carlson and Froberg (1967) who studied 12 men during a 10 day period of very low calorie diet and exercise. The plasma FFA level increased during the first six days but had decreased by the tenth day to a concentration only slightly above the initial value. The authors did not attempt to explain this phenomenon and merely proposed that the metabolic events changed during the middle of the study. However, the changes in the plasma FFA were paralleled by the plasma glycerol levels which indicates that the FFA increase was due to lipolysis of the adipose tissue TG (Carlson and Oro, 1963). This would suggest that at the end of the 10 day period the adipose tissue was mobilizing less FFA than at the mid-point; therefore, it is possible that other substrates were being used to satisfy the cellular requirements, thus decreasing the demand for FFA.

Triglycerides. The plasma triglyceride level was significantly decreased
(P <.01) during the low calorie diet and the low calorie diet plus in-
creased physical activity periods; a finding which contradicts the hypo-
thesis that the plasma TG concentration would not change during the low
calorie diet period.

There was no significant change in the plasma TG concen-
tration during the control period (blood samples 1 and 2), indicating that
any effects of habitual exercise on the plasma TG levels had plateaued.
Also, Neuman - Keuls method of comparing treatment means showed no statis-
tically significant difference (P >.01) between the TG values obtained
during the low calorie diet and the low calorie diet plus increased physical
activity treatment conditions. Therefore the decrease in plasma TG con-
centration during the two stress situations is attributed solely to the
effects of the low calorie diet, quite a unique finding.

In addition, it is evident that an additional daily energy
output of 500 Calories does not affect the plasma TG concentration of
these subjects. This additional output represents an increase of 12.5%
above the daily caloric requirement (approximately 4000 Calories), and
apparently this increase is not of sufficient magnitude to elicit a further
decrease in TG concentration.

There are two possible explanations for the decreased plasma
triglyceride concentration. Ballard et al. (1960) and Gousios et al. (1963)
have demonstrated that peripheral tissues can utilize esterified fatty acids
as a source of energy. In agreement with these findings, Hollenberg (1960)
has also reported a significant increase in the lipoprotein lipase activity
in the myocardium and diaphragm during fasting, a factor which would account
for the hydrolysis of some circulating triglycerides. Therefore, if the

low calorie diet represents a stress, such that the increase in plasma FFA can not supply sufficient substrates, it is possible that the plasma TG could be utilized.

The amount of carbohydrate and lipid in the diets could also be responsible for the change in the circulating TG. With the essential fatty acids providing the only source of fat in the low calorie diet, the level of circulating chylomicrons would be insignificant. However, the normal diet contained approximately 35 to 40 per cent fat; it is possible that the change in fat content of the diets alone might be responsible for a reduction in circulating TG.

Dietary carbohydrates supply much of the "raw materials" needed in the synthesis of long-chain fatty acids (Wakil and Barnes, 1971). Glucose is broken down via the glycolytic pathway to pyruvate, which is then oxidized to acetyl - CoA. Acetyl - CoA is condensed with oxaloacetate to form citrate, which diffuses out of the mitochondria. Citrate is then cleaved by an extramitochondrial enzyme to oxaloacetic acid and acetyl - CoA, which can be used for fatty acid synthesis and as the source of C_2 units for elongation after conversion to malonyl - CoA.

The low calorie diet did not provide sufficient substrates for metabolism and it is probable that all the carbohydrate in the diet was oxidized rather than converted to glycogen or long-chain fatty acids. As these fatty acids are the precursors of plasma TG, it is apparent that the level of carbohydrate in the diet plays an important role in altering the concentration of plasma TG.

CHAPTER V

SUMMARY AND CONCLUSIONS

Summary

The purpose of this study was to determine the plasma lipid variations during periods of low calorie diet and low calorie diet plus increased physical activity. The study was divided into five consecutive experimental periods; the first, or control period, was similar to the recovery periods, which followed each stress situation. During these three periods the subjects maintained 'normal' physical activity and consumed a regular diet containing 3600 Calories per day (4000 Calories for the recovery periods). The first stress situation involved a ten day period on a semi-defined, low calorie diet (1800 Calories per day) with continued 'normal' physical activity. Following the fourteen day recovery period, the second stress situation began; it was identical to the first stress condition with additional daily energy expenditure of 500 Calories above the established 'normal' activity. This was approximately equivalent to a six mile run in 50 minutes. Blood samples were taken, following an overnight fast, twice during each period, once mid-way through the period and again at the end.

Plasma triglycerides and free fatty acids were measured in the samples taken from four male graduate students with a high initial level of activity. The results of orthogonal comparisons among treatment means showed a statistically significant rise in plasma free fatty acids during the low calorie diet and the low calorie diet plus increased physical

activity periods. This was attributed to increased mobilization of free fatty acids from adipose tissue triglycerides in response to the insufficient dietary supply of substrates for metabolism. Neuman - Keuls method was used to examine the effect of the increased physical activity during the low calorie diet periods; the results showed that the increased caloric expenditure had no significant effect on the plasma free fatty acids.

Similar statistical procedures applied to the plasma triglyceride values showed a significant decrease in the plasma triglyceride concentration during the low calorie diet and the low calorie diet plus increased physical activity periods. The stress of the low calorie diet on the habitually active subjects was responsible for the decreased levels. The lipid and carbohydrate content of the normal and the low calorie diets, as well as increased peripheral uptake of triglycerides were suggested as possible explanations for the plasma triglyceride changes. The additional daily output of 500 Calories during the second stress condition was not of sufficient magnitude to elicit a further decrease in plasma triglyceride concentration.

Conclusions

1. The plasma free fatty acids were significantly increased in active male subjects during a low calorie diet and a low calorie diet plus increased physical activity.

2. During the same treatment conditions mentioned above, the plasma triglyceride concentration was significantly reduced.

3. The increase in physical activity during the second stress condition had no significant effect on the plasma lipids.

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APPENDIX

RAW SCORES

<u>Free Fatty Acids</u>   (m moles / 1)

<u>Subject</u>

Blood Sample	1	2	3	4
1	.333	.299	.395	.464
2	.280	.405	.395	.549
3	.549	.537	.949	.333
4	.449	.687	.675	.348
5	.200	.349	.500	.253
6	.240	.309	.457	.181
7	.836	.739	.532	.647
8	.405	.692	.616	.287
9	.174	.244	.393	.257
10	.127	.233	.387	.164

Triglycerides   (mg / 100 ml)

Subject

Blood Sample	1	2	3	4
1	148.93	103.10	121.72	88.78
2	124.58	85.92	121.02	104.54
3	80.19	88.78	88.78	93.08
4	83.06	67.30	75.90	83.06
5	100.24	77.33	94.51	105.97
6	131.61	94.51	94.51	100.24
7	63.01	67.30	85.92	67.30
8	41.53	67.30	58.71	58.71
9	110.26	87.35	127.45	68.74
10	128.88	85.92	115.99	88.78