AN ABSTRACT OF THE THESIS OF

HYUN SUH PAIK for the degree of DOCTOR OF PHILOSOPHY

in FOODS AND NUTRITION presented on July 22, 1977

Title: INFLUENCE OF DIETARY FAT AND MEAL FREQUENCY ON LIPOPROTEIN LIPASE AND HORMONE-SENSITIVE LIPASE IN RAT ADIPOSE TISSUE

Abstract approved by: Elisabeth S. Yearick

Activities of lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) in adipose tissue, accumulation of carcass fat, and serum triglyceride have been determined in meal-fed (MF) and ad libitum-fed (AD) rats. At each feeding frequency, the animals received diets providing total fat as 15% or 30% of calories and polyunsaturated fatty acids (PUFA) as 2.5% or 11% of calories.

The food intake of the MF rats was 75% of that consumed by the AD rats but MF rats utilized their food more efficiently, as evidenced by weight gain per 100 Kcal consumed. Meal feeding, as contrasted to ad libitum feeding, resulted in greater activities of both LPL and HSL. This suggested a higher turnover of fat in the adipose tissue of MF rats. In AD rats, body fat was significantly correlated with LPL and the ratio of LPL:HSL. Meal feeding significantly increased the ratio of LPL:HSL, indicating a greater capacity for energy storage and fat deposition in the MF rat.

However, at the limited caloric intake, MF rats failed to realize this potential; there was no significant difference in percentage of body fat at the two feeding frequencies.

Body fat deposition was greater in rats fed the 30% fat diet, as compared with the 15% diet, regardless of the rate of food ingestion. This was coupled with a higher ratio of LPL:HSL. The significant correlation of serum triglycerides with body fat and with the ratio of LPL:HSL in AD rats suggests that LPL activity and fat deposition may be controlled by the concentration of circulating triglycerides. Both serum triglycerides and adipose LPL activity were significantly reduced when the diet contained high levels of PUFA. The percentage of body fat was also lower in animals whose intake of PUFA was high.

Influence of Dietary Fat and Meal Frequency on Lipoprotein Lipase and Hormone-Sensitive Lipase in Rat Adipose Tissue

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed July 22, 1977

Commencement June 1978

APPROVED:

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in charge of major

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Date thesis is presented July 22, 1977

Typed by Lora Wixom for Hyun Suh Paik

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to Dr. Elisabeth S. Yearick for her guidance in producing this thesis. I am also grateful for her knowledge, wisdom and special friendship which encouraged me most during my studies.

I especially thank Dr. Margy J. Woodburn for her strong support during my research, particularly with regard to financial arrangements. I also thank Dr. Lorraine T. Miller, Gil-Won Song-Cusack and Janet Wozenski who shared my ups and downs and tolerated my Korean "Irish Temper."

My warm gratitude is extended to my mother-in-law for her loving care of my sons, Gene and Sung.

I am very grateful to my husband, Young H. Paik, for his support throughout my struggles toward a significant goal in my life.

I also wish to thank the Oregon State University Foundation Fellowship for their awards which allowed me to continue my studies over the last two years.

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I. INTRODUCTION

Laboratory rats, allowed access to food for a single two-hour period daily (meal-feeders), grow at a rate similar to that of ad libitum-fed rats (nibblers) although ingesting fewer calories (Leveille & Hanson, 1965). Rats, force-fed an amount of food equal to that ingested by ad libitum-fed rats, accumulate more body fat and less protein and water than the controls with similar weight gains (Cohn et al., 1965). Thus, meal-feeding alters body metabolism so as to increase the capacity for energy storage.

The increased capacity for energy storage by meal-fed rats consuming high carbohydrate diets seems to be related to an adaptive increase in the levels of enzymes associated with de novo lipogenesis, primarily those of adipose tissue (Leveille, 1967b). If the total caloric intake is sufficient, an increase in body fatness results.

It has been demonstrated that lipogenic activity of adipose tissue is inversely related to the amount of fat in the diet of meal-fed as well as nibbling rats (Leveille, 1967a). Although the capacity for de novo lipogenesis is reduced by fat feeding, fat deposition appears to be enhanced. Thus, the increase in fat deposition which accompanies high fat feeding must be due to a mechanism other than de novo lipogenesis.

Fat deposits in adipose tissue are dependent, not only on the

capacity for lipogenesis, but also on the balance between mobilization of fatty acids from the tissue into the circulation and uptake by the tissue of fatty acids derived from the circulating triglycerides (TG). Lipolysis in adipose tissue, a function of hormone-sensitive lipase (HSL), results in the output of non-esterified fatty acids into the blood. On the other hand, lipoprotein lipase (LPL) facilitates the uptake of TG fatty acids from circulation by adipose and other extrahepatic tissues. It is possible, therefore, that meal feeding a high fat diet may alter the balance between these two enzymes, resulting in increased fat accumulation.

It is proposed that LPL in adipose tissue may play a significant role in controlling fat deposition. LPL activity of the adipocyte is found to be increased in genetically obese mice and rats and also in nutritionally-induced obesity.

When polyunsaturated fat is ingested, there is an increase in LPL activity and a decrease in lipolytic capacity of adipose tissue. Thus, decreased mobilization of adipose tissue TG and increased uptake of circulating TG could result from a diet that is high in unsaturated fat.

It seems that fat accumulation in animals could be influenced by (1) consumption of large, infrequent meals, (2) a high intake of total fat, and (3) an increase in unsaturated dietary fat. All three of these conditions have their counterpart in human feeding patterns. Fábry et al. (1964) have reported finding an inverse relationship between the incidence of overweight and the frequency

of meals. Food consumption statistics indicate that the use of dietary fat has increased over the past 60 years. Particularly noteworthy is the increased consumption of polyunsaturated fat.

Therefore, the present experiment was designed to determine:

(1) the effect of feeding frequency on the relative activity of LPL and HSL in adipose tissue of the rat,

(2) the relation of feeding frequency to body fatness in rats fed diets supplying two levels of total dietary fat and two levels of polyunsaturated fat,

(3) the effect of quantity and unsaturation of dietary fat on the relative activity of LPL and HSL in adipose tissue of the rat,

(4) the influence of dietary fat on body fat accumulation,

(5) the relationship between the relative activity of LPL and HSL in adipose tissue and accumulation of fat in the body.

II. REVIEW OF LITERATURE

Feeding Frequency

The rat, given free access to food, eats small amounts at frequent intervals; this manner of feeding is often called nibbling. If the same amount of ration is administered by stomach tube, the feeding can be limited to twice daily; such animals are force-fed. Another method of reducing feeding frequency is by training the animal to consume its daily ration within a two to three-hour period; such rats are usually designated as meal-fed.

Cohn and Joseph (1959) compared the weight gain of force-fed rats with that of animals given isocaloric diets ad libitum. Although both groups gained the same amount of weight, the composition of the newly-formed tissue was dependent on the rate of ingestion of the diet. Carcasses of the tube-fed animals contained less water and protein but 35% more fat than did those of the ad libitum group. When force-fed rats received only 80% of the calories eaten by the ad libitum group, they deposited less water, fat, and protein. However, the relative amount of fat accumulated by the underfed animals was not greatly different from the quantity gained by the rats eating ad libitum.

Leveille and Hanson (1965) demonstrated that meal-fed rats consumed less food than did nibbling control animals. The meal-fed rats lost weight initially, but following this initial adjustment period, gained weight at the same rate as the control animals.

Because they achieved a similar rate of weight gain while ingesting only 75-80% as much food as the controls, it was postulated that the meal-fed rats utilized their food more efficiently. However, Leveille and O'Hea (1967) showed that this greater feed efficiency was due to a reduction in physical activity rather than to a change in postabsorptive metabolic rate.

Leveille and Chakrabarty (1968) showed that the adapted meal-fed rat absorbed nutrients more rapidly from the digestive tract. Moreover, glucose administered either orally or intraperitoneally was removed from the circulation more rapidly in the meal-fed rat than in the ad libitum-fed rat. These investigators proposed that insulin plays an important role in the improved glucose tolerance of the meal-fed rat. Wiley and Leveille (1970) reported that plasma insulin levels were higher in meal-fed than in nibbling rats, regardless of whether the rats were in the fed or fasted state.

Tepperman and Tepperman (1958a) demonstrated increased lipogenesis by livers of rats trained to ingest their daily ration in one hour, or fed a high carbohydrate diet following two days of fasting. In this study, lipogenesis was measured by in vitro incorporation of acetate-1-^{14}C or glucose-u-^{14}C into liver lipid. Since that time, there have been numerous reports of increased lipogenesis by adipose tissue of meal-fed rats compared with that of ad libitum-fed animals (Cohn and Joseph, 1967; Hollifield and Parson, 1962; Leveille and Hanson, 1965). Cohn and Joseph (1967) compared the lipogenesis in adipose tissue when both the amount and the timing

of food ingestion were controlled. Subnormal amounts of a 60% carbohydrate diet were presented to the undernourished rats, either all at once (1-2 hour feeding), or hourly in 24 small aliquots. They found that the in vitro incorporation of acetate-1-^{14}C into lipid was increased in the group of rats eating the limited amount of food in a short time, compared with those eating 1/24th of the restricted diet every hour.

Leveille and O'Hea (1967) demonstrated in the meal-fed rats that for eight hours after the initiation of the daily meal, the respiratory quotient (RQ) was in excess of unity. This indicated that glucose was serving as the major oxidative fuel and that lipogenesis was proceeding at a rapid rate. From eight to fourteen hours following meal initiation, the RQ decreased to 0.85, which suggested that carbohydrate, probably glycogen, and lipid were serving as the oxidative fuel. Finally, from 14 hours after the start of the meal until the initiation of the next meal, the RQ of 0.73 indicated lipid to be the major source of energy. Leveille (1967b) proposed that adipose tissue of the meal-fed animal was responsible for virtually all of the lipid formed during and immediately following meal-ingestion, and that liver was quantitatively unimportant.

Adipose tissue of meal-fed rats shows an adaptive increase in lipogenic enzyme activity (Tepperman and Tepperman, 1958b; Cohn and Joseph, 1960; Hollifield and Parson, 1962; Leveille and Hanson,

1966). NADPH, formed in the pentose pathway, favors fatty acid synthesis. Flatt and Ball (1964) demonstrated that the two NADPH-generating enzymes, glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6-PGDH), could produce about 50% of the NADPH required for the high rates of lipogenesis in adipose tissue. Although both enzymes are increased in adipose tissue of the meal-fed rat, the increase in 6-PGDH activity is less than that of G6PDH (Leveille and Hanson, 1966). The significance of the increase in malic enzyme activity with meal-feeding was first noted by Tepperman and Tepperman (1964). Oxaloacetate derived from citrate cleavage could be converted to malate, then to pyruvate via malic dehydrogenase and malic enzyme, thereby effecting a transfer of hydrogen from NADH to $NADP^+$ in order to produce NADPH for fatty acid synthesis.

Initially, it appeared that lipogenesis occurred in response to the increased activity of the related enzymes. However, Leveille (1966) showed that the lipogenic capacity of adipose tissue increased before any change in enzyme activity was observed. Whereas, after five days of meal feeding, lipogenesis had increased significantly, the activity of the pentose pathway dehydrogenases and malic enzyme was actually below that of the control animals. Enzyme activity did not increase above the control levels until after nine days of meal feeding. Leveille proposed that an accelerated rate of lipogenesis would oxidize an increasing amount of NADPH; the increasing

concentration of $NADP^+$ in the presence of other necessary subtrates would increase the flux through these enzymatic steps and thereby trigger an increase in enzyme activity.

The composition of the diet influences the effects of changes in feeding frequency. Cohn (1963) and his colleagues (1963) studied the effect of dietary protein levels on the body composition of force-fed and ad libitum-fed rats. On a protein-free diet, neither group thrived and no differences in body composition were noted. However, as the protein was increased from 0 to 67% of the diet, the fat content of the force-fed animals became progressively greater, relative to that of the ad libitum group. Potter and Ono (1961) reported no increase in hepatic dehydrogenase activity when rats were refed a 2% protein diet after 72 hours of starvation. However, as the dietary protein was increased to 60%, there was a stepwise increase in these enzymes. From these observations, the increase in lipogenic enzymes appears to be dependent on the presence of protein in the diet.

The capacity for lipogenesis is reduced by fat feeding. Hausberger and Milstein (1955) observed that the feeding of a high-fat low-carbohydrate diet was associated with a reduced capacity of isolated adipose tissue to synthesize fatty acids from acetate. These findings were confirmed by other investigators (Leveille and Hanson, 1966; Leveille, 1967a; Wood and Reid, 1975). Hill et al. (1960) showed that the ability of liver to synthesize fat from acetate was dramatically reduced by the administration of a single dose of

oil to the carbohydrate-fed rat. They postulated that the inhibition of lipogenesis resulted in a decreased oxidation of NADPH and that this could lead to a reduction in G6PDH, 6-PGDH, and malic enzyme activities. Leveille and Hanson (1966) were unable to demonstrate any increase in lipogenesis or enzyme activity in adipose tissue from rats that were meal-fed a high-fat diet. Moreover, they found that the feeding of a high-fat diet completely abolished the adaptive increase in fatty acid synthesis that had accompanied the high-carbohydrate diet. Leveille (1967a) investigated the effect of increasing levels of dietary fat on the response to meal-feeding (two hours per day). The diets supplied fat up to levels that would approximate the percentage of fat calories in human diets. Male rats consumed rations containing 10, 20, and 30% fat (equivalent to 21, 36, and 52% of calories, respectively). At the 10% and 20% fat levels, the meal-fed rats surpassed the ad libitum-fed animals in fatty acid synthesis from acetate-1-^{14}C or glucose-u-^{14}C and in activities of G6PDH, 6-PGDH, and malic enzyme. However, the capacity for lipogenesis and the enzyme activity in adipose tissue of both groups decreased as the levels of dietary fat were increased. With the 30% fat diet, the effects of meal-feeding were abolished. The authors suggested that the inability to demonstrate a response to meal-feeding in human subjects may be due to the high level of fat in typical human diets.

Although the capacity for de novo synthesis is reduced by fat

feeding, fat deposition appears to be enhanced. Cohn et al. (1965) reported that when rats were force-fed a high-carbohydrate low-fat diet, the body fat accumulated at a greater rate than when the same diet was given ad libitum. As the carbohydrate of the diet was lowered and the fat increased, the relative differences in body fat became greater. Animals that were force-fed a high-fat carbohydrate-free diet for 57 days deposited about 75% more fat than did those that were given the same diet ad libitum. Wood and Reid (1975) studied the effects of dietary fat and feeding frequency on the body fat of rats that were restricted to 80% of normal calories. Isocaloric diets, providing either 28% or 2.3% of calories as fat, were given either as a single four-hour feeding (meal-eater) or in six spaced meals (nibbler). The rats given the high fat diet deposited more body fat per day than did those consuming the low fat diet. The effect of dietary fat on body fat deposition was significant but the effect of feeding frequency was not. At the higher fat intake, dietary fatty acids were incorporated into adipose tissue and lipogenesis from glucose was low. Thus, the increase in fat deposition which accompanies high fat feeding appeared to be due to a mechanism other than de novo lipogenesis.

Other investigators have explored the effects of triglyceride fatty acids on fat synthesis. In vitro rates of lipogenesis in liver and adipose tissue of rats receiving long-chain triglycerides were significantly depressed, relative to the rates in tissues of rats consuming a low-fat diet (Wiley and Leveille, 1973). Activities

of G6PDH, 6-PGDH, malic enzyme and citrate-cleavage enzume in liver were also depressed. Medium chain triglycerides (MCT) containing chiefly C_8 and C_{10} fatty acids, were markedly less effective in depressing fat synthesis and enzyme acitivity. The lack of effect of MCT on lipogenesis was attributed to its portal absorption and the rapid uptake and oxidation by the liver.

Lipoprotein Lipase and Hormone-Sensitive Lipase

Adipose tissue contains at least two distinct triglyceride lipases: hormone-sensitive lipase and lipoprotein lipase (Khoo and Steinberg, 1975). These two enzymes play a large part in the control of the uptake and output of fatty acids by adipose tissue; both have the ultimate effect of making lipid energy available to the peripheral tissues.

Hormone-sensitive lipase (HSL) is responsible for the hydrolysis of triglycerides stored in adipose tissue. Most of the fatty acids released by HSL action are circulated to the peripheral tissues where they serve as a major energy source. Some fatty acids are esterified by the liver and returned to the blood as very low density lipoprotein (VLDL) triglycerides. Since adipose triglycerides are not released to the circulation, it is chiefly through the action of HSL that the stored fat is mobilized. Activity of HSL is stimulated by epinephrine, glucagon, and other fat-mobilizing hormones; it is inhibited by insulin (Steinberg, 1972). Hormonal regulation of the enzyme is mediated through cylic AMP.

The main physiological function of lipoprotein lipase (LPL) is to facilitate the uptake of triglyceride fatty acids (TGFA) from the circulation by adipose and other extrahepatic tissues (Robinson, 1970). This function is thought to be exercised through the hydrolysis of triglycerides in the chylomicrons and the VLDL, sequestered at the luminal surface of the capillary endothelial cells of the extrahepatic tissues (Blanchette-Mackie and Scow, 1971). Fatty acids produced by LPL action are taken up by the tissue, either for oxidation or storage as triglycerides (Robinson, 1963).

Although these two lipases are clearly distinct enzymes and have many properties that differentiate one from the other, they are sufficiently similar that assays carried out on a mixture of the two will probably reflect some of each under most conditions. The optimum pH for LPL is pH 8.0-8.4 (Korn, 1955a) while that of HSL is pH 6.8-7.0 (Huttunen et al., 1970; Okuda and Fujii, 1973). However, assays carried out at an alkaline pH will include some of the activity of HSL and vice versa. One can use the differential effects of activators and inhibitors to help distinguish the two activities in a mixture (Krauss et al. 1973a; 1974), but it is difficult, if not impossible, to obtain complete dissociation except by physical resolution of the two enzymes.

LPL requires the presence of a serum apolipoprotein as a cofactor (Krauss et al., 1973b) for substrate activation in order to form an enzyme-substrate complex with fat emulsions (Korn, 1955b; Havel et al., 1970; Whayne and Felts, 1970). Lipoprotein lipase is inhibited by

1 M sodium chloride and is selectively inactivated by protamine sulfate (Krauss et al., 1974; Bimpson and Higgins, 1969; Korn and Quigley, 1957). In contrast, HSL requires no apolipoprotein as cofactor for its activity, is not inhibited by 1 M sodium chloride, and is not inactivated by protamine sulfate (Krauss et al., 1974; Huttunen et al., 1970).

LPL is found in muscle, heart, adipose tissue, spleen, lung, kidney medulla, aortic wall tissue, diaphragm and lactating mammary glands (Korn, 1955a); its release into plasma is induced by the mucopolysaccaride heparin. Because it facilitates the uptake of TGFA from the blood, LPL has been called the clearing factor.

It has been suggested that LPL activity of adipose tissue may play a significant role in controlling fat deposition in adipose tissue. Cryer et al. (1976) reported a positive correlation between the LPL activity of rat epididymal adipose tissue and the incorporation of chylomicron TGFA into the fat cells. Huttunen et al. (1976) found that, in normal humans, the fractional removal rate of endogenous triglyceride from plasma was positively correlated with the post-heparin lipolytic activity (PHLA). However, Krauss et al. (1974) reported no correlation between serum TG concentration and the activity of extrahepatic LPL in post-heparin plasma in spite of the good agreement of the assay method with Huttunen et al. (1976). Likewise, Nilsson-Ehle (1974) found LPL activity in post-heparin plasma was not correlated to LPL activity in adipose tissue of normal subjects. The widely different results obtained in these studies may be partly due to the use of various doses of heparin and to different

conditions for the assay of PHLA.

The LPL of adipose tissue is low in insulin deficit, untreated hyperglycemic diabetic subjects (Pykalisto et al. 1975) and in alloxan-diabetic rats (Kessler, 1963). LPL activity of adipose tissue is correlated to the insulin concentration in plasma when both parameters are increasing (Cryer et al. 1976). Nikkilä and Pykälistö (1968b) found a significant correlation between the degree of LPL induction and the inhibition of free fatty acid (FFA) release. However, the finding is not always supported by other studies (Nikkilä and Pykälistö, 1968a; Persson, 1970). The question of whether the effect of insulin on LPL activity is mediated through cyclic AMP directly or indirectly remains unsettled at the present time.

The activity of both HSL and LPL appears to be enhanced in the obese animal. Compared with the normal rat, the genetically-obese Zucker rat had a greater output of free fatty acids from adipose tissue, greater muscle consumption of fatty acids for energy, and a greater return of fatty acids to the adipose storage depots (Zucker, 1972). This suggested a more active cycle of fat transport between liver and adipose tissue. In the young obese rat, the mobilization of fat per cell, in response to epinephrine, was greater than normal. In the older obese rat, the output per cell was near normal but the total fat-mobilizing capacity of the older animal was well above normal because of the greater number of fat cells. De Gasquet and Pequignot (1972; 1973b) reported that the LPL activity

per adipocyte was abnormally high in obese hyperglycemic mice, genetically-obese Zucker rats, and also in nutritionally-induced obesity. However, when the enzyme activity was related to adipocyte surface area (LPL/cm^2), there was no difference between the obese and the lean animals. These findings were confirmed by Bjorntrop et al. (1975) who reported a higher LPL activity and an increased rate of uptake of exogenous fatty acids by large fat cells, in contrast to small fat cells. Enser (1972) found that the LPL activity of epididymal fat pads decreased rapidly in both lean and obese mice during a 24-hour period of starvation but the activity in the obese mice remained higher than in lean mice. Plasma triglyceride concentrations did not differ greatly between the two groups.

Several investigators have reported that adipose LPL was decreased by fasting (Hollenberg, 1959; Robinson, 1960). After a fast of 24-48 hours, LPL activity of rat adipose tissue had declined to a level that was only 5-20% of that found in the fed animal. On refeeding, the activity was rapidly restored. Thus, in situations of energy excess, the enzyme activity in adipose tissue was high and a large proportion of the plasma TGFA was taken up into fat stores (Bezman et al., 1962; Garfinkel et al., 1967). On the other hand, in energy-deficit situations (during starvation, exercise, or cold exposure), LPL of heart and skeletal muscle increased and the uptake of plasma TGFA was diverted to these tissues (Borensztajn and Robinson, 1970; Rogers and Robinson, 1974; Borensztajn et al., 1975). The increased LPL activity of heart during fasting was

sustained for about 24 hours, after which time it gradually declined (Borensztajn and Robinson, 1970).

Food deprivation is not always accompanied by a decrease in adipose LPL activity. Reichl (1972) proposed that increased LPL activity in adipose tissue may be a function of increased plasma concentrations of insulin and glucose. In rats that had been adapted to a 14-hour feeding period every 48 hours, feeding produced a concomitant increase in adipose LPL and plasma insulin. During the fast that followed, LPL activity declined but rose a second time as the fasting was prolonged. This peak coincided with a moderate elevation in plasma insulin and glucose concentrations. In the ad libitum-fed rat, neither LPL nor insulin increased during fasting. Likewise, Persson (1970) reported that the adipose LPL activity of obese humans decreased during the first week of a total fast but tended toward recovery during the second week. Throughout the experimental period, the serum non-esterified fatty acid concentrations remained high and the serum triglycerides were essentially unchanged.

The release of free fatty acid from adipose tissue is greatly reduced in the intact animal fed with glucose or injected with insulin (Gordon et al. 1957). Conversely, starvation, which stimulates the release of fatty acid from adipose tissue, inhibits the uptake of glucose. Rizack (1961) has reported that extracts prepared from adipose tissue of starved animals contained significantly higher levels of lipolytic activity than tissues from normally fed animals.

A significant increase in LPL activity of epididymal fat of rats was observed by Pawar and Tidwell (1968b) after feeding a polyunsaturated fat (20% corn oil) for six weeks, as compared with a more saturated fat (20% lard). These investigators also showed that the release of free fatty acids and glycerol from adipose tissue was less in rats fed polyunsaturated fat than in rats fed lard (Pawar and Tidwell, 1968a). Furthermore, the rate of in vitro incorporation of labelled fatty acids into adipose triglycerides was greater in the corn oil-fed rat. Incubation of adipose tissue with prostaglandin also inhibited lipolysis and increased the uptake of fatty acids. It was suggested that the response of adipose tissue to corn oil may be mediated through the formation of prostaglandin from the polyunsaturated fatty acids ingested. In humans, diets high in polyunsaturates produced a significant increase in post-heparin LPL activity of plasma and a decrease in the plasma triglyceride concentrations (Engelberg, 1966; Bagdade, 1970). Engelberg proposed two mechanisms by which the high PUFA diet might reduce plasma TG concentrations: (1) The major chemical change that occurs is an increased unsaturation of the circulating TG and cholesterol esters. The more highly unsaturated cholesterol esters are apt to form more soluble lipoproteins than are the saturated esters. Furthermore, they may modify the solubility of other lipids. (2) The increased negative charge resulting from the increased unsaturation produces a finer particle dispersal. Thus, both increased substrate solubility and better dispersal would enhance substrate-

enzyme contact. Theoretically, the steric effects of the unsaturated fatty acid would also tend to facilitate substrate-enzyme contact.

Naismith and Khan (1971) reported that adipose LPL activity was greater in rats that were consuming a high sucrose diet than in those on a starch-rich regimen. On the other hand, Vrána et al. (1974) showed that the chronic administration of sucrose or fructose produced significantly greater LPL activity in heart and diaphragm than did glucose or starch but adipose LPL was lower with sucrose or fructose feeding. The hypertriglyceridemic effect of dietary fructose was also shown in this study. This phenomenon has been attributed to the increased synthesis and output of triglycerides from the liver (Nikkilä, 1969). The failure of adipose LPL to rise with sucrose or fructose feeding was confirmed when equicaloric solutions of glucose, fructose, or sucrose were administered by tube to fasted rats (Cryer et al., 1974). Only glucose produced an increase in adipose LPL. Plasma triglycerides and non-esterified fatty acids rose after sucrose or fructose feeding but adipose LPL activity remained at the low fasting levels. These same investigators demonstrated that adipose LPL activity was significantly correlated with the ability of tissue to incorporate ^{14}C-labelled (TGFA) of injected chylomicrons into the fat-cell lipids, regardless of the form of carbohydrate that was administered.

The response of adipose LPL to the various sugars was associated with their capacity to elicit a rise in plasma insulin (Cryer et al., 1974). When fructose or sucrose was administered to fasted rats there

was no increase in either plasma immunoreactive insulin or adipose LPL activity. Glucose administration, on the other hand, caused the plasma insulin concentration to rise to values characteristic of rats fed ad libitum and the adipose tissue LPL activity increased two-fold to approximately 50% of that found in animals fed ad libitum. It has been repeatedly reported that the in vivo activity of LPL in rat adipose tissue is positively correlated with the plasma insulin concentration (Borensztajn et al., 1972; De Gasquet and Pequignot, 1973a; Cryer et al., 1974 and 1976; Garfinkel et al., 1976). However, Cryer et al. (1974 and 1976) found that the correlation between these two parameters was significant only when both the plasma insulin concentration and the tissue enzyme activity are increasing, as in starved rats given glucose. When adipose tissue of starved rats was incubated with insulin, in vitro, the characteristically low LPL activity increased toward that of fed animals (Robinson and Wing, 1970). On the other hand, Cryer et al. (1976) reported that plasma insulin concentrations were negatively correlated with LPL activity of diaphragm, cardiac and skeletal muscle.

However, insulin is not the only hormone that affects the activity of the enzyme in adipose tissue in vivo. The reciprocal changes in LPL activity of adipose tissue and heart were apparent with other hormonal treatments (Borensztajn et al., 1972; De Gasquet et al., 1975). For example, when starved rats were fed glucose, the LPL activity of adipose tissue was increased while that of heart was decreased. Glucagon or glucocorticoid administration to these

animals at the time of glucose feeding prevented the decline in heart LPL activity but had no effect on the adipose tissue enzyme. When glucagon was administered to fed rats, the heart LPL activity was increased to levels found in starved animals but there was no change in the adipose tissue enzyme. These investigators suggested that the reciprocal LPL activities in heart and adipose tissue of fed and fasted animals may be regulated by the circulating plasma insulin and glucagon concentrations.

Lipoprotein lipase activity in adipose tissue was also influenced by plasma estrogen levels (Hamosh and Hamosh, 1975). Ovariectomy resulted in increased LPL of adipose tissue. Administration of 17-β-estradiol to male rats or to ovariectomized female rats significantly lowered the adipose LPL. However, progesterone administration did not affect the enzyme in adipose tissue of either male or ovariectomized rats.

Zinder et al. (1974) reported that the secretion of prolactin from the anterior pituitary gland controlled LPL activity in both mammary gland and adipose tissue. During lactation, LPL activity was high in mammary gland and low in adipose tissue. However, by the 6th day after hypophysectomy, this situation was reversed. Prolactin injections restored the previous activity levels.

Although many physiological and pathological factors have been shown to influence LPL activity, the ultimate regulatory mechanism remains unknown. The effect of diet may be secondary to changes in the circulating hormones and regulation of the enzyme activity may be achieved mainly by hormonal controls.

III. EXPERIMENTAL PROCEDURE

Experiment Design

The study was planned to observe the effects of feeding frequency and dietary fat on LPL and HSL activity in adipose tissue and body fatness of rats fed diets supplying different levels of total fat and of polyunsaturated fatty acids (PUFA). The experimental conditions compared were:

1. Feeding frequency:
    a. Ad libitum feeding (AD). Rats were allowed unlimited access to food.
    b. Meal feeding (MF). Rats were allowed access to food for 3-1/2 hours daily.
2. Dietary fat: At each level of feeding frequency, rats were assigned to one of four diets:
    a. Diet A provided 15% of calories as total fat and 2.5% of calories as PUFA.
    b. Diet B provided 15% of calories as total fat and 11% of calories as PUFA.
    c. Diet C provided 30% of calories as total fat and 2.5% of calories as PUFA.
    d. Diet D provided 30% of calories as total fat and 11% of calories as PUFA.

Thus, there were eight experimental groups, as shown in Table 1. The experimental conditions were applied for 35 days following an adjustment period.

Table 1. Design of experiment.

Group	Feeding Frequency	Dietary Fat (percent of Calories)	
		Total Fat	Polyunsaturated
AD-A	ad libitum	15%	2.5%
AD-B	ad libitum	15%	11.0%
AD-C	ad libitum	30%	2.5%
AD-D	ad libitum	30%	11.0%
MF-A	meal feeding	15%	2.5%
MF-B	meal feeding	15%	11.0%
MF-C	meal feeding	30%	2.5%
MF-D	meal feeding	30%	11.0%

Animals

Male Wistar rats, about five weeks old and weighing 90-120 gm, were purchased[1] over a period of three weeks. They were kept in quarantine for two days before delivery to the animal laboratory of the Department of Agricultural Chemistry. The rats were housed in individual cages with raised wire floors situated in a temperature-regulated room (22°C) which was isolated from other animal rooms. They were exposed to alternating periods of light (7:00-20:00 hours) and dark (20:00-7:00 hours). Water was freely available to all rats. Food was available at all times to the ad libitum group. The meal-feeders were fed only 3-1/2 hours daily (17:30-21:00 hours). During the experimental period, the animals were weighed twice weekly, at 15:00 hours. Food cups were weighed three times a week, at 14:00 hours.

The animals were started on the dietary regimes according to a staggered schedule; this permitted the sacrifice of one rat from each of the eight groups on alternate days at the end of the experiment. Every other day, three rats were randomly assigned to each of the four experimental diets. One of the three was fed ad libitum and two were put on the meal-feeding regimen. This distribution was adopted because a pilot study had shown that some meal-fed rats failed to thrive. In the present experiment, rats that were poorly-adapted to meal-feeding were discarded after 2-3 weeks. At completion, there

[1] Purchased from Simonsen Laboratory, Inc., Gilroy, California.

were eight rats in each of the experimental groups, representing four diets and two feeding frequencies.

Dietary Program

Four experimantal diets were used in this study: diets A, B, C, and D. As shown in Table 2, the protein (as casein) provided 24% of calories in all four diets. For diets A and B, the low fat regimes, 61% of the calories was derived from carbohydrate (as corn starch), and 15% from fat (as a mixture of safflower and palm oils). One gram of the low-fat diet supplied 4.17 kilocalories. For diets C and D, the high fat regimes, total fat was increased to 30% of total calories at the expense of carbohydrate. One gram of the high fat diet contained 4.56 kilocalories. All diets provided identical amounts of vitamins and minerals per calorie.

The proportions of safflower oil and palm oil in the diets were adjusted according to their calculated content of polyunsaturated fatty acids (PUFA) so that diets A and C provided 2.5% of calories as PUFA and diets B and D provided 11% of the calories as PUFA. To protect the PUFA in the rations from rancidity, the calculated natural tocopherol of the oils was supplemented with dl-α-tocopherol acetate to give a total activity of 5 mg d-α-tocopherol per gram of PUFA in the diet. The final adjusted level of d-α-tocopherol activity for each diet is shown in Table 2. Vitamins A and D were dissolved in 95% ethanol and the aliquot was mixed into the dietary oils before they were added to the diet mix.

Table 2. Composition of experimental diets.

Composition	Diet A	B	C	D
	gm/1000Kcal			
Casein, vitamin-free	60.0	60.0	60.0	60.0
Corn starch	152.2	152.2	114.7	114.7
Oil[a]				
Palm oil	14.45	0.15	33.12	18.82
Safflower oil	2.25	16.55	0.28	14.58
HMW Salt Mixture[b]	7.68	7.68	7.68	7.68
Zinc Mixture[c]	1.92	1.92	1.92	1.92
Vitamin Mixture[b]	1.58	1.58	1.58	1.58
Distribution	% of Kcal			
Protein	24.0	24.0	24.0	24.0
Carbohydrate	60.9	60.9	45.9	45.9
Total Fat	15.0	15.0	30.0	30.0
PUFA	2.5	11.0	2.5	11.0
d- α-Tocopherol(mg) activity[d]	13.9	61.1	13.9	61.1

[a]Vitamin A & D added to oil to give 3 ppm and 1.5 ppm respectively, in the finished diet.

[b]Composition given in Appendix Table i.

[c]Zinc mixture: $Zn(C_2H_3O_2)_2 \cdot 2\ H_2O$ (1.67 gm) mixed with 1 kg cerelose.

[d]Total activity expressed as d-α-tocopherol.

The total amount of feed required for the experiment was prepared at the same time and kept frozen until it was needed for use.

Sacrifice Schedule

The experimental period lasted five weeks. One rat from each of the eight experimental groups was sacrificed on alternate days. Food cups were removed at 21:00 hours from the rats that were to be sacrificed the next morning at 8:00 hours (11 hours fasting). The rats were weighed before they were killed. Each rat was decapitated after being exposed to CO_2 inhalation for 10 sec in a gas chamber. Blood from the carcass was drained into a centrifuge tube and allowed to coagulate. Both epididymal fat pads were removed immediately and each pad was placed in a separate pre-weighed vial for assay of LPL and HSL. The vials were held in ice and each fat pad was weighed as quickly as possible. Serum was separated by centrifugation at 1200 rpm for 30 min and the red cells were added to the carcass. Serum and carcass were held at -20°C until needed. Five minutes were allowed for the sacrifice of one rat so that there was a 35 min time difference between the killing of the first rat and the eighth rat. In order to have an even distribution of the time difference between groups, the order of sacrifice was rotated on each killing day.

IV. METHODS OF ANALYSIS

Lipoprotein Lipase

Preparation of enzyme:

The enzyme was extracted directly from fresh adipose tissue rather than from the customary acetone powder since it was difficult to recover sufficient acetone powder from the small samples. However, the buffer commonly used for extraction of the acetone powder (Garfinkel et al., 1976) was also used in this investigation. A preliminary study indicated that the specific activity of LPL, extracted directly from adipose tissue, was slightly greater than or the same as that of the enzyme extracted from acetone powder. This study also showed that the enzyme in solution was stable for several hours after extraction but had lost 5-10% of its activity by the 4th hours.

The specific activity of LPL was found to be the same in both epididymal fat pads by the direct extraction method. Therefore, one of the epididymal fat pads was homogenized in 2.5 parts (v/w) of 0.05 M Tris-HCl (pH 8.0) buffer containing 1.0 M ethylene glycol (Garfinkel et al., 1976). The homogenization was carried out in a Virtis glass homogenizer, equipped with an ice-water bath and operated at medium speed for 30-40 sec. The homogenate was centrifuged at 2000 rpm for 10 min in a refrigerated Sorvall centrifuge. The bulk of the fat was removed with a glass rod and the supernatant was recentrifuged in a fresh centrifuge tube at 15000 rpm for 30 min.

The clear infranatant was used for the assay of LPL.

In the preliminary study, the LPL preparation by direct extraction from adipose tissue was found to be more inhibited by 1 N sodium chloride than by protamine sulfate (800 ug/ml). Accordingly, the enzyme extract was preincubated with an equal volume of 4 N sodium chloride solution at 2°C. A second portion of the enzyme extract was diluted with an equal volume of extraction buffer to give the total lipolytic activity. This procedure was adopted since the enzyme activity varied with dilution of preparation. The NaCl-inhibited LPL activity of adipose tissue was calculated as the difference between total lipase activity (incubated without NaCl) and NaCl-resistant lipase activity (incubated with NaCl).

LPL activity was determined as the capacity of the NaCl-inhibited LPL to release labelled oleic acid from triolein-1-^{14}C which had been activated by serum lipoprotein.

LPL assay reagents:

1. Extraction buffer: 0.05 M Tris-HCl containing 1.0 M ethylene glycol, adjusted to pH 8.0.
2. 0.2 M Tris-HCl buffer: pH 8.0
3. Boric acid-potassium carbonate buffer: 0.1 M boric acid and 0.1 M potassium carbonate, adjusted to pH 10.5.
4. Triolein[1] (non-labelled): 2.5% in benzene, stored under nitrogen at 4°C.

[1]Purchased from Sigma Chemical Company, St. Louis, Missouri.

5. Labelled triolein: Labelled triolein was purchased[1] as triolein-1-^{14}C. When this preparation was stored for any length of time, even at very low temperatures, it showed a progressive increase in level of labelled fatty acid (FFA). To obtain a low background, it was necessary to pass a stock solution of triolein-1-^{14}C in benzene, with non-labelled triolein as carrier, through a column of Silica gel (60-200 mesh) with Florisil (60-100 mesh) layered on top to remove the contaminating FFA. After this treatment, the stock solution of triolein-1-^{14}C was made up in benzene to give a radioactivity of 55×10^6 dpm/ml. When stored at -20°C in a tightly screw-capped tube under nitrogen, this preparation could be used for two weeks without an important increase in ^{14}C-FFA background.
6. Bovine serum albumin: A 1% solution of bovine serum albumin[2] (fraction V), fatty acid-free, was prepared in 0.2 M Tris-HCl buffer (pH 8.0), stored at -20°C.
7. Lysolecithin[2]: 0.3% in chloroform, stored at 4°C.
8. Inactivated rat serum: Blood was collected from rats that had been fasted 12 hours to reduce the triglyceride concentration. Serum was separated from the pooled blood and heated for 10 min at 60°C to inactivate the lipases. The treated serum was stored at -20°C.

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[1] Purchased from Applied Science Laboratory, Inc., State College, Pa.

[2] Purchased from Sigma Chemical Company, St. Louis, Missouri.

9. Triolein substrate emulsion: The substrate emulsion for LPL assay was prepared according to a modification of the method of Nilsson-Ehle (1974). Triolein (labelled and non-labelled) was emulsified with lysolecithin in Tris-HCl buffer (pH 8.0) and activated by rat serum. Bovine serum albumin served as the fatty acid acceptor in the reaction mixture. The final concentration of triolein was 0.283 umole per assay. A 3-ml batch of the substrate, sufficient for at least 28 assays, was prepared fresh daily. When additional quantities of the substrate emulsion were needed the process was repeated. Preparation was as follows:

   a. 100 ul lysolecithin (0.3%), 300 ul non-labelled triolein (2.5%), and 100 ul labelled triolein (55×10^6 dpm/ml) were pipetted into a small plastic vial and evaporated to dryness under a stream of nitrogen.
   b. 400 ul bovine serum albumin (1%) and 2.1 ml Tris-HCl buffer (0.2 M, pH 8.0) were added to the vial.
   c. The substrate was sonicated[1] for a total of 4 min, alternating a 30 sec sonication with a 30 sec pause. To maximize the reproducibility of the assay results, the sonication procedure was performed in a standardized manner. The vial containing the substrate was suspended in a beaker of ice. The flat tip of the sonifier was centered and

[1] Branson Sonifier Model S 75, Branson Instruments, Inc., Danbury, Connecticut.

inserted below the surface to a point 3/8ths of the depth of the solution. The force exerted at the tip of the sonifier was maintained constant at 1 gm[1].

d. 500 ul inactivated rat serum were added to the emulsified substrate. The whole was mixed by inversion and held at room temperature for at least 10 min before use.

LPL assay procedure:

The LPL activity was assayed by a modification of the method of Nilsson-Ehle (1974).

1. 100 ul aliquots of each enzyme preparation (with and without NaCl), in triplicate, were incubated with 100 ul of the triolein substrate emulsion for 20 min in a shaking incubator at 37°C.
2. A reagent blank of 100 ul extraction buffer (0.05 M Tris-HCl-1.0 M ethylene glycol) was treated similarly.
3. The reaction was terminated by the addition of 3.25 ml of chloroform:methanol:heptane in the proportions of 1.25:1.41:1.00, by volume (Belfrage and Vaughan, 1969).
4. The released oleic acid was immediately isolated by a liquid:liquid partition procedure described by Nilsson-Ehle et al. (1972). The pH was increased by the addition

[1]Technical advice and assistance were provided by Professors M. C. Schotz and A. S. Garfinkel of the Lipid Research Laboratory, University of California, Los Angeles, California.

of 1.05 ml boric-potassium carbonate buffer (0.1 M, pH 10.5) and the mixture was mixed vigorously in a Vortex mixer for at least 15 sec and centrifuged for 10 min at 2000 rpm.

5. 1 ml of the upper phase was transferred to a counting vial, 10 ml scintillation cocktail (Instagel:toluene, 1:1) were added and counted in a Liquid Scintillation Counter[1]. The counting efficiency was 91% by External Standard Channels Ratio (ESCR).
6. For the dose assay, 25 ul of substrate emulsion were counted with 1 ml upper phase from the reagent blank tube.

Calculation of LPL activity:

1. The recovery of the released oleic acid was 75%.
2. The total volume of the upper phase was 2.45 ml but only 1 ml was counted.
3. $$\frac{0.25 \text{ mg triolein/assay}}{0.884 \text{ mg/umole triolein}} = 0.283 \text{ umole triolein/assay}$$

   If it is assumed that 1 umole triolein released 3 umoles fatty acids, then 0.849 umole FFA were released per assay.
4. Dose assay = 25 ul substrate emulsion counted

   Total dpm per assay = dose assay (d.a.) x 4

[1] Beckman Model LS-3133P. Beckman Instruments, Inc., Irvine, California.

5. One milliunit of enzymatic activity represents the production of 1 nmole of fatty acid per min.

$$\text{Enzyme unit} = \frac{\text{dpm in sample} \times 0.849 \times 10^3 \times 2.45 \times 100}{(\text{d.a.}) \times 4 \times \text{time incubation (min)} \times 75}$$

$$= \frac{\text{dpm in sample} \times 693}{(\text{d.a.}) \times \text{min}}$$

6. Specific activity is expressed as nanomoles of fatty acid released/min/mg of protein. Protein in the diluted enzyme preparation of LPL was determined by the method of Lowry et al. (1951).

Hormone-Sensitive Lipase

Preparation of enzyme:

The enzyme was extracted directly from fresh tissue by a modification of the procedure described by Khoo and Steinberg (1975). The specific activity of HSL was found to be the same in both epididymal fat pads extracted by sucrose buffer. Therefore, one of the epididymal fat pads was homogenized in 2.5 parts (v/w) of 0.25 M sucrose buffer (pH 7.4) containing 1 mM EDTA and 10 mM Tris. The procedure followed that described for LPL.

LPL was selectively inactivated by pre-incubation of enzyme extract with protamine sulfate (Krauss et al., 1973a). The protamine-resistant enzyme was prepared by incubating 0.6 ml of the enzyme extract with 0.2 ml protamine sulfate (12.8 mg/ml) for 30 min at 2°C.

A second aliquot of the enzyme extract was incubated with 0.2 ml sucrose buffer (0.25 M, pH 7.4) to give total lipolytic activity. The enzyme in solution was stable for three hours after extraction but had lost 5-10% of its activity by the 5th hour.

In a preliminary study, the activity of protamine-resistant lipase was consistently higher in rats that had fasted 50 hours than in rats fasted 10 hours. This finding lent confidence to the assumption that the method described did, indeed, isolate the lipolytic enzyme.

HSL activity is determined as the capacity of the protamine-resistant lipase to release labelled oleic acid from triolein-1-^{14}C.

HSL assay reagents:

1. Extraction buffer: 0.25 M sucrose buffer containing 1 mM EDTA and 10 mM Tris, adjusted to pH 7.4.
2. Sodium phosphate buffer: 0.2 M, pH 6.8
3. EDTA (ethylenediamine tetraacetic acid): 0.3 M, pH 6.8
4. Triolein (non-labelled): 2.5% solution of triolein in benzene, stored under nitrogen at 4°C.
5. Labelled triolein: The triolein-1-^{14}C solution (55×10^6 dpm/ml), as prepared for the LPL assay.
6. Bovine serum albumin: A 10% solution of bovine serum albumin (fraction V), fatty acid-free, was prepared in 0.1M sodium phosphate buffer (pH 6.8), stored at -20°C.
7. Gum arabic (acacia): 5% in redistilled water

8. Triolein substrate emulsion: The substrate emulsion for HSL assay was prepared by a modification of the method of Khoo and Steinberg (1975). The final concentration of triolein was 1.041 umoles per assay. A 15-ml batch, sufficient for 24 assays was prepared fresh daily. When the additional quantities of the substrate emulsion were needed, the process was repeated. Preparation was as follows:

   a. 0.9 ml non-labelled triolein (2.5%) and ∿90 ul labelled triolein (55×10^6 dpm/ml) were pipetted into a small plastic vial and evaporated to dryness under a stream of nitrogen.

   b. 2.5 ml gum arabic solution (5%) was added to the vial and sonicated as described in the LPL assay.

   c. The emulsion was transferred quantitatively to a large centrifuge tube. To it were added 5 ml bovine serum albumin (10%), 5 ml sodium phosphate buffer (0.2 M, pH 6.8), and 2.5 ml EDTA (0.3 M, pH 6.8). The contents of the tube were mixed gently by inversion.

HSL assay procedure:

The activity of HSL was assayed by a modification of the method of Khoo and Steinberg (1975).

1. 0.6 ml substrate emulsion was added to 0.2 ml of each enzyme preparation (with and without protamine sulfate). Triplicate samples were incubated for 30 min in a shaking incuba-

tor at 37°C.

2. A reagent blank of 0.2 ml sucrose buffer was treated similarly.
3. The reaction was terminated by the addition of 3 ml of chloroform:methanol:benzene in the proportions of 2:2.4:1, which contained 0.3 umole nonradioactive oleic acid as carrier.
4. The released oleic acid was isolated by the addition of 0.1 ml NaOH (1 N). The mixture was mixed vigorously in a Vortex mixer for at least 15 sec and centrifuged for 20 min at 2500 rpm.
5. 1 ml of the upper phase was transferred to a counting vial. 10 ml of scintillation cocktail (Instagel:toluene, 1:1) were added and counted in a Liquid Scintillation Counter. The counting efficiency was 88-90% by ESCR.
6. For the dose assay, 50 ul substrate emulsion were counted with 1 ml upper phase from the reagent blank.

Calculation of HSL activity:

1. The recovery of the released oleic acid was 75%.
2. The total volume of the upper phase was 2.1 ml but only 1 ml was counted.
3. $$\frac{0.92 \text{ mg triolein/assay}}{0.884 \text{ mg/umole triolein}} = 1.041 \text{ umoles triolein/assay}$$

   If it is assumed that 1 umole triolein released 3 umoles fatty acids, then 3.123 umoles FFA were released per assay.

4. Dose assay = 50 ul substrate emulsion counted

   Total dpm per assay = dose assay (d.a.) x 12

5. One milliunit of enzymatic activity represents the production of 1 nmole of fatty acid per min.

$$\text{Enzyme unit} = \frac{\text{dpm in sample} \times 3.123 \times 10^3 \times 2.1 \times 100}{(\text{d.a.}) \times 12 \times \text{time incubation(min)} \times 75}$$

$$= \frac{\text{dpm in sample} \times 729}{(\text{d.a.}) \times \text{min}}$$

6. Specific activity is expressed as nanomoles of fatty acid released/min/mg of protein. Protein in the diluted enzyme preparation of HSL was determined by the method of Lowry et al. (1951).

Carcass Analysis

Preparation of lipid extract:

A modification of the method of Mickelsen and Anderson (1959) was used to prepare the carcasses for analysis of total lipids and triglycerides. The frozen carcass was weighed to the nearest hundredth of a gram and thawed in the refrigerator overnight. It was then placed in a 1-liter beaker, covered with a watch glass, wrapped in aluminum foil, and autoclaved for 60 min at 15 lb. pressure. The hot carcass was placed in an Osterizer[1] and the blending was begun at low

[1]Made by Oster Corporation, Milwaukee, Wisconsin.

speed. During the blending, redistilled water was added slowly and in small portions. After the whole carcass was reduced to small pieces, it was homogenized at medium speed for 3-4 min to produce a homogeneous liquid. The homogenate was quantitatively transferred to a sieve (425 um mesh size) and the glass container and lid were washed three times with small amounts of water. Filtration was accomplished by pressing the homogenate with a rubber spatula until no more water emerged. The residue, consisting of hair and small pieces of bone, was resuspended in water and incubated 5-10 min in a hot water bath at 80°C to facilitate the removal of fat. The total volume of water used in preparing the homogenate was 2.5 times the carcass weight. All of the filtrates from one carcass were pooled and the volume was recorded. The filtrate was mixed well and a 100-ml sample was transferred to a polyethylene bottle and stored at -20°C.

For lipid extraction, the method of Bligh and Dyer (1959) was used by following the phase diagram[1]. According to the phase diagram, the water content of the sample should be within 15% to maintain the monophasic state after the addition of the chloroform:methanol (1:1) solvent. In order to change from the monophasic to the biphasic state, the water content of the final system should be 28%. Therefore, 10 ml of the well-mixed filtrate were pipetted into a separatory

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[1]The assistance of Robert Lowry in adapting the phase diagram is gratefully acknowledged.

funnel containing 51 ml of chloroform:methanol (1:1). The mixture was mixed well and allowed to rest for 10 min. Then, 10.8 ml water were added to the same separatory funnel. The funnel was shaken well and allowed to stand for at least 3 hours until a clear separation of phase was obtained. The coagulated residue collected in the interface between the aqueous-methanol and the chloroform layers.

The chloroform layer was drawn out into a graduated 100-ml cylinder and the upper layer was reextracted with 25.5 ml fresh chloroform. The two chloroform extracts were pooled and the volume was adjusted to 60 ml with chloroform. Preliminary tests of the procedure showed that there was a good duplication between samples and that more than 99% of the lipid was extracted.

Total lipids:

The carcass lipid was determined gravimetrically. Twenty millilitres of the chloroform extract were pipetted into an aluminum weighing pan which had been dried and preweighed. The sample was dried at 45-50°C and weighed to the nearest tenth of a milligram.

An aliquot of the carcass lipid extract was reserved for triglyceride analysis. Five millilitres of the chloroform extract were dried under nitrogen in a small vial and stored under nitrogen at -20°C until all of the samples had been extracted.

To obtain an estimate of total body fat, the calculated lipid of fat pads was added to that of the carcass. It was assumed that the previously removed fat pads contained 85% fat, as obtained in a preliminary study and confirmed by De Bont (1975). Body fat was

then expressed in terms of total lipid as percent of body weight (BW) or triglyceride as percent of body weight.

Triglycerides:

The concentrations of triglyceride in serum and in lipid extracts from carcass homogenates were determined by the method of Van Handel and Zilversmit (1957) with the following modifications: a) phospholipids were removed by adsorption onto activated silicic acid (100-200 mesh) instead of Florisil, and b) the volumes of the final colored reaction mixture were equalized with redistilled water prior to measurement of absorbance. In this assay, triglycerides are hydrolyzed by alkali and the released glycerol is oxidized to formaldehyde by periodic acid. The colored complex formed by reaction of formaldehyde with chromotropic acid is measured photometrically at 570 nm. The concentration of triglyceride is calculated from the absorbance of tripalmitin as a standard.

Triglyceride analyses were carried out in triplicate. Five hundred microlitre samples of serum were used for triglyeride analysis. The dried lipid extract from carcass was diluted with chloroform and triglyceries were determined in an aliquot representing 0.1 to 0.2 ml of the carcass extract.

Statistical treatment:

The data were analyzed statistically by multiple regression. Each parameter was regressed on feeding frequency, total dietary fat

level and PUFA level in the diet. Analysis of variance and regression analysis were done with the SPSS package through the Cyber system of Oregon State University Computer Center.[1] Differences between treatment groups were tested for statistical significance by the student's t test. Correlation coefficients between parameters were also obtained.

[1] Theresa Trackwell of the Department of Statistics at Oregon State University was the statistical consultant and Harvey Lipman was the programmer.

V. RESULTS AND DISCUSSION

Body weight and energy consumption:

The average intake of calories per week for each experimental group is shown in Figure 1. Mean weekly caloric intakes are recorded in Appendix Table ii. These data clearly show that the MF rats consumed less food than did the nibbling control animals. The difference was statistically significant ($p<0.001$). Initially the average caloric intake of all MF rats was only 53% of that of the control animals but their food consumption increased with time to a maximum of 75% that of the controls. This failure of meal-fed rats to eat as much as ad libitum-fed animals has been reported by other investigators (Leveille and Hanson, 1965; Leveille and O'Hea, 1967). The weekly caloric intake of the rats that received the 2.5% PUFA diets (A and C) was significantly ($p<0.004$) greater than that of rats receiving the 11% PUFA diets (B and D).

The changes in body weight over the experimental period are shown in Figure 2. Mean weight gains for each group appear in Appendix Table iii. Although MF rats lost weight initially, following this initial adjustment period they gained weight at a rate similar to that of the control animals. The final body weight of AD rats was significantly ($p<0.001$) higher than that of the MF animals. However, weight gain was not significantly influenced by diet, despite the greater caloric intake associated with the low PUFA diets. Thus, the weight gained per 100 kcal ingested was lower with the low PUFA

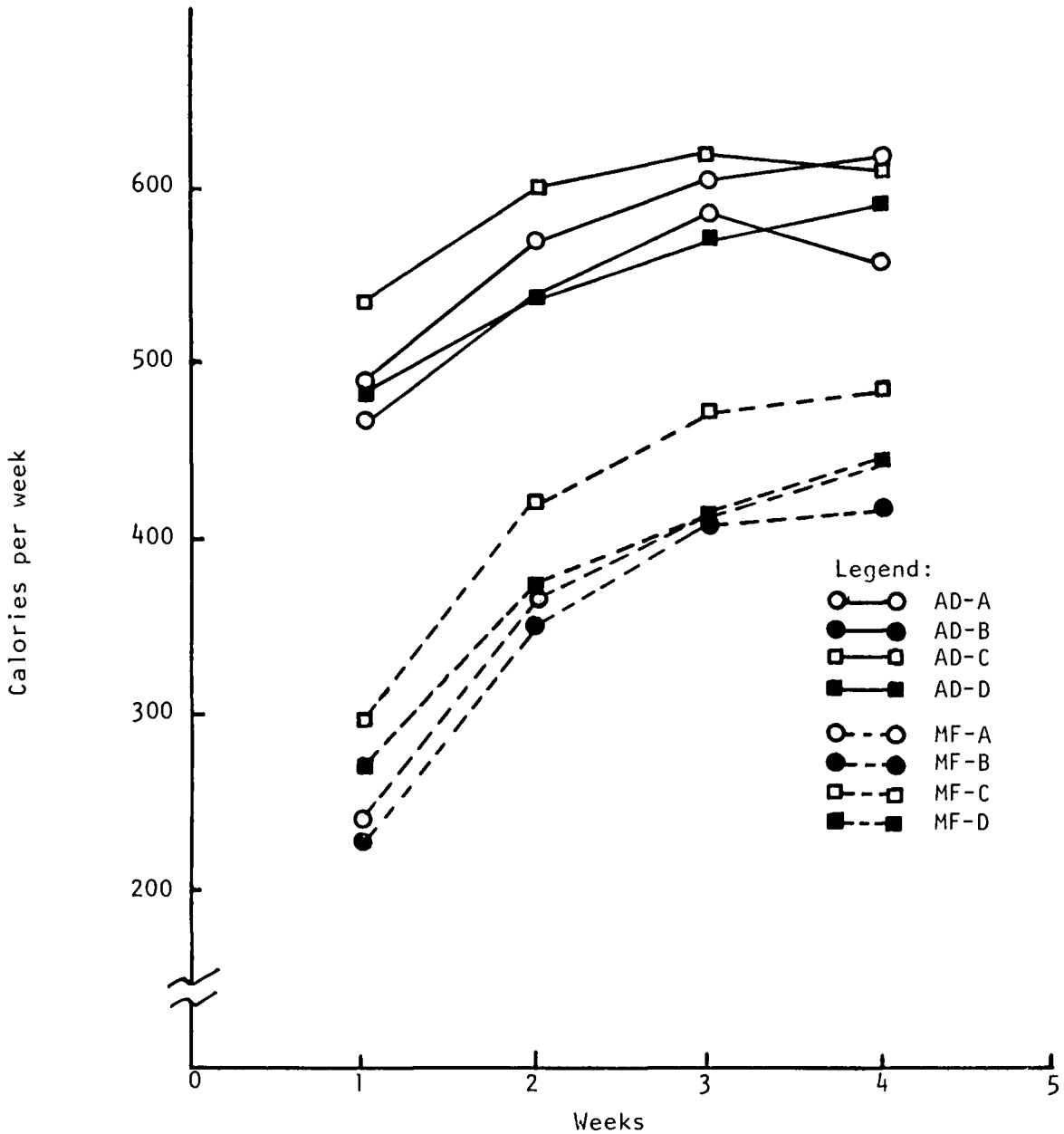

Figure 1. Caloric intake per week by ad libitum (AD) and meal-fed (MF) rats on diets A, B, C and D.

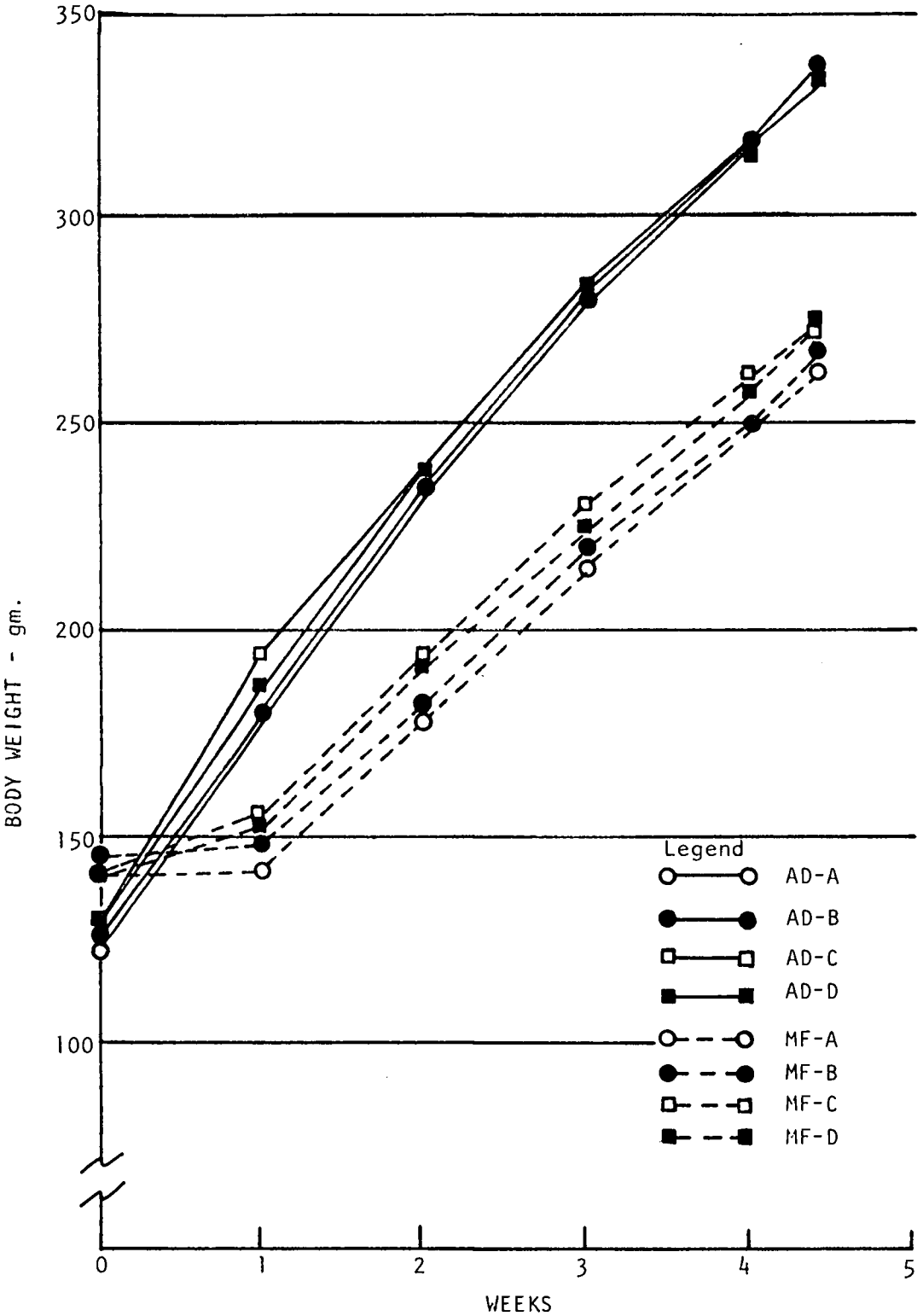

Figure 2. Weight gain of ad libitum (AD) and meal-fed (MF) rats on diets A, B, C and D.

diets (Appendix Table iv).

Since a similar rate of weight gain was achieved by the MF rats while ingesting only 75% as much food as the control animals, the MF rat must have utilized its food more efficiently than did the control; this is shown in Figure 3. This observation is in agreement with the results of Leveille and Hanson (1965) who showed that meal-fed rats had an increased capacity for energy storage.

Responses to feeding frequency:

A pilot study was conducted to determine whether or not LPL activity in adipose tissue would be affected by the time of day selected for feeding, fasting, and sacrifice. Since adipose tissue LPL is influenced by the nutritional state, all parameters were measured at fasting, i.e., 9-11 hours following the last feeding. The rat eating ad libitum is normally a nocturnal feeder. Therefore, the LPL activity of adipose tissue of AD rats was compared with that of MF rats under two conditions. One group was meal-fed for 3 hours in the morning and a second group was meal-fed for 3 hours in the evening. Both AD and MF rats were fasted prior to sacrifice. Under both conditions, LPL activity was greater in adipose of the MF than in that of the AD animals. Differences in activity between the morning and evening meal-feeders were not statistically significant. Therefore, the meal-feeders in the present study were fed from 17:30 to 21:00 hours and the lighting period was maintained from 7:00 to 20:00 hours.

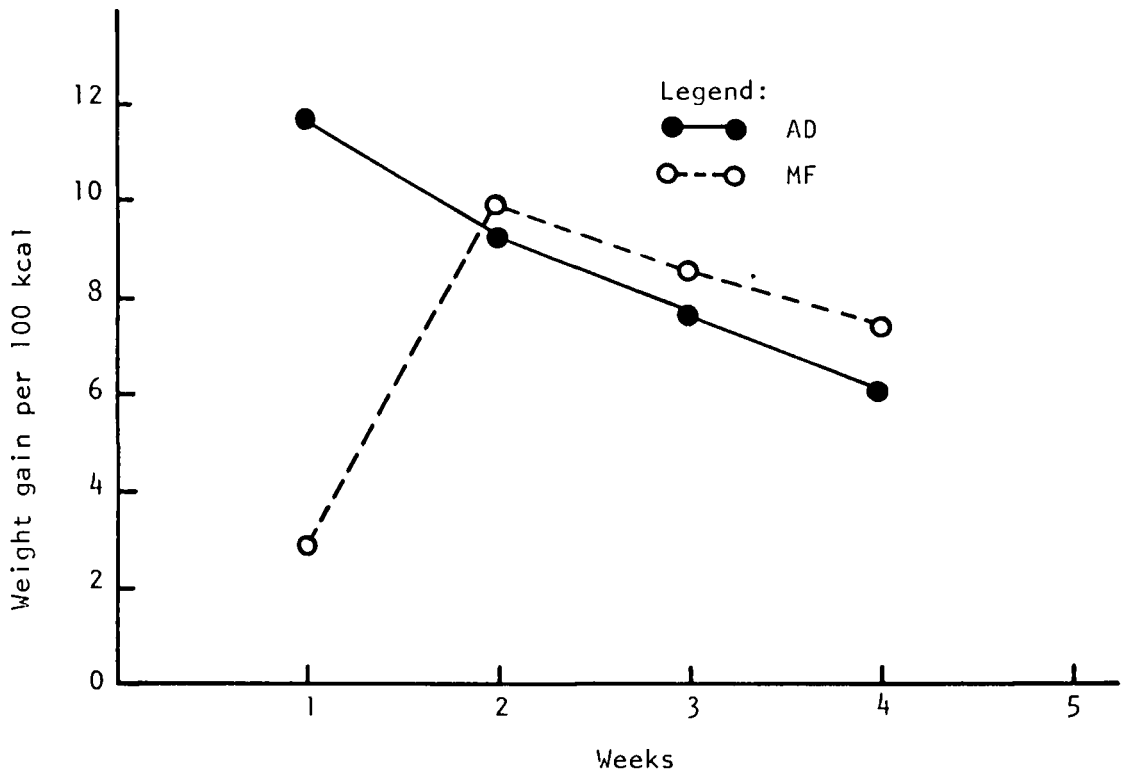

Figure 3. Weight gain per 100 kcal by ad libitum (AD) and meal-fed (MF) rats.

The effects of meal frequency on enzyme activities and tissue lipids are shown in Table 3. The specific activity of NaCl-inhibited LPL in adipose tissue of the MF rats was significantly ($p<0.001$) greater than that of the AD animals, at both levels of dietary fat and at both levels of dietary PUFA. Activity of the protamine-resistant lipase, HSL, was also significantly higher in the MF rats under all dietary conditions. For both feeding frequencies, there was a positive correlation ($p<0.05$) between LPL and HSL (Table 4). These findings were unexpected. LPL is associated with the uptake by adipose tissue of plasma TGFA, while HSL is associated with the release of adipose fatty acids into the circulation. The finding that both enzymes were elevated by meal-feeding contrasts with the posutlate of Robinson and Wing (1972) that there may be a single control mechanism affecting the two lipases differentially. On the one hand, it has been shown that the activity of adipose LPL is positively correlated with the plasma insulin (Borensztajn et al., 1972; De Gasquet and Péquignot, 1973a, Garfinkel et al., 1976). Plasma insulin levels were not measured in the present study but Wiley and Leveille (1970) reported that the plasma insulin was higher in meal-fed rats than in nibbling rats, regardless of whether the animals were in the fed or fasted state. Reichl (1972) observed concomitant increases in adipose LPL activity and plasma insulin levels and proposed that the rise in plasma insulin and glucose may be prerequisite to the rise in adipose LPL. On the other hand, lipolysis is known to be inhibited when the plasma insulin concentra-

Table 3. Effects of feeding frequency on enzyme activities and tissue lipids. (means and standard deviations)

Feeding Frequency	Diet	Adipose			Serum	Body	
		LPL	HSL	LPL:HSL	TG	Lipid	TG
AD	15% fat	11.98 ± 2.93	6.76 ± 1.74	1.83 ± 0.53	67.01 ± 45.59	13.85 ± 3.25	9.58 ± 3.13
MF	15% fat	19.30[a] ± 6.30	9.01[a] ± 1.51	2.15 ± 0.63	73.35 ± 35.10	13.11 ± 3.44	9.06 ± 2.70
AD	30% fat	14.20 ± 2.65	6.87 ± 1.48	2.13 ± 0.47	76.26 ± 55.17	15.80 ± 2.14	11.20 ± 2.06
MF	30% fat	19.54[a] ± 2.91	8.60[b] ± 2.61	2.50 ± 0.97	107.74[b] ± 48.91	15.65 ± 3.39	11.33 ± 3.58
AD	2.5% PUFA	14.49 ± 2.96	6.80 ± 1.17	2.17 ± 0.49	98.58 ± 56.15	15.46 ± 3.30	11.34 ± 3.15
MF	2.5% PUFA	20.24[a] ± 5.37	9.04[a] ± 1.41	2.25 ± 0.53	93.37 ± 38.24	14.33 ± 4.10	10.24 ± 4.04
AD	11% PUFA	11.87 ± 2.45	6.83 ± 1.94	1.82 ± 0.49	44.03 ± 18.39	14.27 ± 2.42	9.55 ± 2.06
MF	11% PUFA	18.60[a] ± 4.24	8.56[b] ± 2.66	2.40[b] ± 1.06	87.73[a] ± 53.69	14.43 ± 3.16	10.14 ± 2.56
AD	All groups	13.09 ± 2.97	6.82 ± 1.59	1.99 ± 0.52	71.30 ± 49.52	14.82 ± 2.88	10.39 ± 2.73
MF	All groups	19.42[a] ± 4.83	8.80[a] ± 2.11	2.33[b] ± 0.83	90.55 ± 45.38	14.38 ± 3.60	10.19 ± 3.32

[a] MF significantly greater than AD ($p<0.01$)

[b] MF significantly greater than AD ($p<0.05$)

Table 4. Correlation coefficients for enzyme activities and tissue lipids of ad libitum-fed and meal-fed rats(n = 32 for AD or MF).

Feeding Frequency	Parameter	Adipose		Serum	Body	
		HSL	LPL:HSL	TG	Lipid	TG
AD	LPL	0.39[d]	0.48[b]	0.29	0.21	0.32[d]
MF	LPL	0.36[d]	0.35[d]	-0.08	-0.04	-0.12
AD & MF	LPL	0.55[a]	0.44[a]	0.17	-0.01	-0.01
AD	HSL		-0.60[a]	-0.24	-0.34[d]	-0.25
MF	HSL		-0.63[a]	-0.19	-0.21	-0.20
AD & MF	HSL		-0.42[a]	-0.08	-0.25[d]	-0.20
AD	LPL:HSL			0.50[b]	0.47[b]	0.47[b]
MF	LPL:HSL			0.18	0.12	0.08
AD & MF	LPL:HSL			0.31[c]	0.20	0.19
AD	Serum TG				0.50[b]	0.51[b]
MF	Serum TG				0.22	0.26
AD & MF	Serum TG				0.32[c]	0.35[b]
AD	Body lipid					0.95[a]
MF	Body lipid					0.95[a]
AD & MF	Body lipid					0.95[a]

[a] $p<0.001$

[b] $p<0.005$

[c] $p<0.01$

[d] $p<0.05$

tion is high. Furthermore, in vitro experiments have shown that the catecholamines, glucagon, and other hormones that inhibit the rise of LPL activity in adipose tissue enhance the activity of HSL (Scow and Chernick, 1970). Moreover, insulin is able to prevent such activation. The enzyme response of meal-fed rats in the present study may indicate a more active cycle of fat transport between liver and adipose tissue, i.e., there may be a greater output of FFA from adipose tissue to supply energy for muscle during the semistarvation period, a greater synthesis and output of TG by liver, and a return of TGFA to the adipose depots. This phenomenon has been observed in obese rats (Zucker, 1972).

It would seem that the deposition of body fat would be a function of the relative activities of LPL and HSL. The ratio of LPL:HSL in adipose tissue of MF rats was higher than that of the AD rats under all dietary conditions (Table 3); the overall ratio was significantly higher at the 5% level. This suggested that meal-feeding, by inducing a relatively greater activity of LPL, could promote the storage of fat in adipose tissue. However, the two feeding frequencies did not produce significantly different proportions of carcass fat (Table 3). Correlation coefficients in Table 4 show that body fat and body TG were positively correlated at both feeding frequencies. For the AD animals, fat deposition was positively correlated with LPL and negatively correlated with HSL activity of adipose tissue; the proportions of body fat and triglycerides were significantly ($p<0.005$) correlated with the LPL:HSL ratio.

This was not true of the MF rats. Even though meal feeding produced high ratios of LPL:HSL, the caloric intake of the MF animals appeared to be insufficient to promote the expected fat deposition. This finding is in accord with that of Cohn and Joseph (1959). When force-fed rats received only 80% of the calories consumed by ad libitum animals, the relative amount of fat accumulated by the underfed rats was not significantly different from the quantity gained by the ad libitum-fed rats. Only when the force-fed rats received dietary calories equal to those of the ad libitum group did they deposit more body fat (Cohn and Joseph, 1959).

The importance of using isocaloric conditions in comparing the effects of feeding frequency was known from the start of the investigation. In a pilot study, nibbling rats were pair-fed with the meal-feeders. It was found that the nibbling rats, thus underfed, consumed more than half of their daily ration in 1-2 hours, simulating the meal-feeding condition. Therefore, pair-feeding was not considered feasible without an automatic dispenser to dispense food evenly throughout the day and night. Force-feeding the MF rats by stomach tube was also not feasible because such a large number of animals was involved. As a result, the meal-fed rats in this study were underfed and did not have sufficient calories to deposit as fat, even under conditions that were favorable for increased fat deposition. It can be speculated that if the MF rats had consumed as much food as the AD animals, the higher ratio of LPL:HSL in the MF rats might have favored the conservation of energy resulting in

greater fat accumulation.

Responses to level of dietary fat:

The influence of dietary fat (as percent of calories) on the parameters measured is presented in Table 5. Rats receiving the 30% fat diet had significantly greater proportions of triglycerides ($p<0.01$) and lipids ($p<0.05$) in the body than did those on the 15% fat ration. The difference was observable at both feeding frequencies and at both levels of dietary PUFA but was significant ($p<0.05$) for the MF animals and for rats receiving the 11% PUFA diets. This finding is consistent with the report of Wood and Reid (1975) that fat deposition was enhanced by fat feeding although the capacity for de novo synthesis was depressed. This would suggest that dietary fat was deposited in adipose tissue through the action of LPL on TG of the circulating lipoproteins. Although not statistically significant, there was a consistently higher LPL activity and a greater ratio of LPL:HSL in adipose tissue of rats given the 30% fat diet.

In the AD rats, the LPL:HSL ratio was positively correlated with serum triglycerides ($p<0.004$) as well as body fat (Table 4). The finding is not consistent with the concept of LPL as the lipid-clearing enzyme and HSL as the mobilizing enzyme. Persson (1973) reported that serum TG were negatively correlated with heparin-releasable activity in human subjects. Huttunen et al. (1976) also demonstrated a negative correlation between serum TG and LPL activity

Table 5. Effects of dietary fat levels on enzyme activities and tissue lipids.
(means and standard deviations)

Dietary Fat	Adipose			Serum	Body	
	LPL	HSL	LPL:HSL	TG	Lipid	TG
	mU/mg	mU/mg		mg/dl	%	%
15% fat (AD)	11.98 ± 2.93	6.76 ± 1.74	1.83 ± 0.53	67.01 ± 45.59	13.85 ± 3.25	9.58 ± 3.13
30% fat (AD)	14.20 ± 2.65	6.87 ± 1.48	2.13 ± 0.47	76.26 ± 55.17	15.80 ± 2.14	11.20 ± 2.06
15% fat (MF)	19.30 ± 6.30	9.01 ± 1.51	2.15 ± 0.63	73.35 ± 35.10	13.11 ± 3.44	9.06 ± 2.70
30% fat (MF)	19.54 ± 2.91	8.60 ± 2.61	2.50 ± 0.97	107.74[b] ± 48.91	15.65[b] ± 3.39	11.33[b] ± 3.58
15% fat 2.5% PUFA	17.18 ± 6.99	7.86 ± 1.87	2.19 ± 0.66	88.31 ± 46.84	13.91 ± 3.62	9.93 ± 3.39
30% fat 2.5% PUFA	17.93 ± 2.74	8.13 ± 1.59	2.24 ± 0.30	103.29 ± 46.86	15.80 ± 3.72	11.58 ± 3.79
15% fat 11% PUFA	14.42 ± 5.08	8.05 ± 2.11	1.82 ± 0.48	53.38 ± 22.71	13.06 ± 3.07	8.73 ± 2.25
30% fat 11% PUFA	16.05 ± 4.57	7.41 ± 2.79	2.40[b] ± 1.06	83.28 ± 59.41	15.64[b] ± 1.69	10.97[b] ± 1.79
15% fat All	15.76 ± 6.14	7.96 ± 1.96	2.00 ± 0.60	70.28 ± 39.95	13.47 ± 3.32	9.31 ± 2.88
30% fat groups	16.96 ± 3.85	7.76 ± 2.28	2.32 ± 0.78	93.63 ± 53.29	15.72[b] ± 2.81	11.26[a] ± 2.90

[a] 30% fat significantly greater than 15% fat ($p<0.01$)

[b] 30% fat significantly greater than 15% fat ($p<0.05$)

of post-heparin plasma. But Krauss et al. (1974) found no correlation between serum TG concentrations and extrahepatic LPL of post-heparin plasma. Nilsson-Ehle (1974) also reported that LPL activity in post-heparin plasma was not correlated with LPL activity in adipose tissue of normal subjects. Possibly the divergent results stem from different assay methods and from the use of different amounts of heparin; heparin was not used in the present study. The failure of serum TG to decline, despite a high LPL activity, may also be a function of the nutritional state of the animals. In this study, the rats were fasted 11 hours prior to sacrifice, Cryer et al. (1976) noted that LPL in adipose was highly correlated with TGFA uptake from the circulation when insulin levels were high following carbohydrate feeding.

The positive correlation ($p<0.003$) between serum TG and body fat in the AD rats (Table 4) suggests that when serum triglycerides are elevated there is a responsive rise in adipose LPL activity which, in turn, promotes fat deposition. The significantly ($p<0.05$) higher serum triglycerides and greater body fat of the MF animals consuming the 30% fat diet, coupled with their high ratio of LPL:HSL, would support this thesis.

Responses to dietary PUFA:

The effects of PUFA, as percent of dietary calories, appear in Table 6. Once again, there was a relationship between adipose LPL activity and the serum triglyceride level. With the 11% PUFA diet,

Table 6. Effects of dietary polyunsaturated fat levels on enzyme activities and tissue lipids. (means and standard deviations)

Dietary PUFA	Adipose			Serum	Body	
	LPL	HSL	LPL:HSL	TG	Lipid	TG
	mU/mg	mU/mg		mg/dl	%	%
2.5% PUFA (AD)	14.49 ± 2.96	6.80 ± 1.17	2.17 ± 0.49	98.58[a] ± 56.15	15.46 ± 3.30	11.34 ± 3.15
11% PUFA (AD)	11.87 ± 2.45	6.83 ± 1.94	1.82 ± 0.49	44.03 ± 18.39	14.27 ± 2.42	9.55 ± 2.06
2.5% PUFA (MF)	20.24 ± 5.37	9.04 ± 1.41	2.25 ± 0.53	93.37 ± 38.24	14.33 ± 4.10	10.24 ± 4.04
11% PUFA (MF)	18.60 ± 4.24	8.56 ± 2.66	2.40 ± 1.06	87.73 ± 52.69	14.43 ± 3.16	10.14 ± 2.56
2.5% PUFA 15% fat	17.18 ± 6.99	7.86 ± 1.87	2.19 ± 0.66	88.31[b] ± 46.84	13.91 ± 3.62	9.93 ± 3.39
11% PUFA 15% fat	14.42 ± 5.08	8.05 ± 2.11	1.82 ± 0.48	53.38 ± 22.71	13.06 ± 3.07	8.73 ± 2.25
2.5% PUFA 30% fat	17.93 ± 2.74	8.13 ± 1.59	2.24 ± 0.30	103.29 ± 46.86	15.80 ± 3.72	11.58 ± 3.79
11% PUFA 30% fat	16.05 ± 4.57	7.41 ± 2.79	2.40 ± 1.06	83.28 ± 59.41	15.64 ± 1.69	10.97 ± 1.79
2.5% PUFA All	17.56[b] ± 5.23	8.00 ± 1.71	2.22 ± 0.51	95.80[b] ± 46.66	14.86 ± 3.73	10.75 ± 3.63
11% PUFA Groups	15.23 ± 4.83	7.72 ± 2.46	2.12 ± 0.87	67.33 ± 45.60	14.35 ± 2.77	9.85 ± 2.30

[a] 2.5% PUFA significantly greater than 11% PUFA ($p<0.01$)

[b] 2.5% PUFA significantly greater than 11% PUFA ($p<0.05$)

both the LPL activity and the serum TG were significantly ($p<0.05$) lower than with the 2.5% PUFA diet. The lowering of serum TG with the high PUFA diet was significant for the AD animals ($p<0.01$) and for rats receiving the 15% fat ration ($p<0.05$), and the trend toward lower LPL and serum TG was apparent in all other subgroups.

Thus, feeding high PUFA diets to rats resulted in a lower concentration of TG in serum and lower fat deposition compared with that by low PUFA diets. However, the total level of dietary fat also influenced the fat deposition in the body.

The LPL response was unexpected in view of the report of Pawar and Tidwell (1968b) that the more unsaturated fat diet induced LPL activity in adipose tissue. However, the lowered serum TG agrees with the reports of Bagdade et al. (1970) and Engelberg (1966) who noted lower serum TG when polyunsaturated fats were fed. Engelberg proposed that the high PUFA diet, by increasing the unsaturation of lipids in the circulating lipoproteins, could increase their solubility and enhance the substrate-enzyme contact. Thus, in the present experiment, the ingestion of the high PUFA diet may have facilitated the interaction of the lipoprotein TG with LPL, resulting in a more rapid removal of the TG from circulation. The lower level of LPL in adipose tissue may then be secondary to the reduction in serum TG.

In contrast to the report of Pawar and Tidwell (1968a) that adipose tissue of PUFA-fed rats showed a decreased in vitro release of fatty acids and glycerol, the HSL activity in the present study was relatively stable under all dietary conditions. If differences

did occur as a result of the dietary treatment, they may have been obliterated by the 11-hour fast that was imposed prior to sacrifice. In a preliminary experiment, it was found that protamine-resistant lipase activity increased with prolonged fasting. This is in agreement with the results of Rizack (1961) who observed a higher lipolytic activity in extracts of adipose tissue of starved rats than in that of fed rats.

VI. SUMMARY AND CONCLUSIONS

This experiment was designed to compare the effects of meal-feeding versus ad libitum-feeding on body fat accumulation and enzyme activity in adipose tissue of rats fed diets supplying two levels of dietary fat and two levels of polyunsaturated fat.

The experimental diets provided total fat as either 15% or 30% of calories. At each level of total fat, polyunsaturated fatty acids (PUFA) supplied either 2.5% or 11% of calories. Two groups of rats were assigned to each of the four diets. The ad libitum-fed (AD) animals were allowed unlimited access to food. The meal-fed (MF) animals were allowed access to food for only 3-1/2 hours daily.

At the end of five weeks, the animals were fasted for 11 hours and then sacrificed. Lipoprotein lipase (LPL) of adipose tissue was measured as the NaCl-inactivated lipase activity of the epididymal fat pad. Hormone-sensitive lipase (HSL) of adipose tissue was measured as the protamine-resistant lipase activity. The total lipids and triglycerides of the carcass were measured and expressed as percent of body weight and the serum triglyceride (TG) concentration was determined.

The MF rats consumed less food than the AD animals and lost body weight initially. Thereafter, they gained weight at a rate similar to that of the control rats although their caloric intake was only 75% that of the controls. Weight gain per 100 Kcal ingested was greater in MF rats but there was no difference in percentage of

body fat.

The two enzymes, functioning in fat deposition (LPL) and fat mobilization (HSL), were positively correlated under all conditions. Higher activities of both enzymes were observed in the MF rats than in the AD animals. This suggested a greater turnover of body fat.

Total lipids and triglycerides, as percentage of body weight, were positively correlated at both feeding frequencies. For the AD animals, body fat deposition was correlated positively with LPL activity of adipose tissue, negatively with HSL activity, and positively with the LPL:HSL ratio. The LPL:HSL ratio in adipose tissue of the MF rats was higher than that of the AD rats. It is postulated that body fat deposition in the MF rats would have exceeded that of the AD rats, had their caloric intake been equivalent.

Deposition of body fat was significantly greater in the rats receiving 30% of calories as fat, as contrasted with those on the 15% fat diet. This finding agreed with the higher LPL activity and higher ratio of LPL:HSL in adipose tissue of rats given the 30% fat diet.

For the AD rats, positive correlations were observed between serum TG and body fat, and between these parameters and the LPL:HSL ratio. It is proposed that when serum TG are elevated there is a responsive rise in adipose LPL activity and an increase in the LPL:HSL ratio which would promote fat deposition in the body. This concept is supported by the finding of significantly higher serum TG, greater body fat deposition, and higher LPL:HSL ratios in the MF rats

consuming the 30% fat diets.

Lower serum TG were observed in the AD rats fed the high PUFA diets, regardless of the level of total dietary fat. This was associated with lower activity of adipose LPL, a lower ratio of LPL:HSL, and less deposition of body fat.

The following conclusions are drawn:

1. Meal feeding induces an increased turnover of body fat by increasing the activities of LPL and HSL in adipose tissue.
2. Body fat deposition is a function of the ratio of LPL:HSL in adipose tissue.
3. LPL activity increases in response to a rise in serum TG.
4. The percentage of fat in the body and ratio of LPL:HSL are greater at the higher intake of fat calories.
5. The percentage of fat in the body, serum TG, and LPL activity are reduced at the higher intake of calories from PUFA.

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APPENDICES

Table i.

Hubbell-Mendel-Wakeman salt mixture:

Aluminum Potassium Sulfate	0.017 %
Calcium Carbonate	54.300 %
Copper Sulfate.$5H_2O$	0.090 %
Ferric Phosphate	2.050 %
Magnesium Carbonate	2.500 %
Magnesium Sulfate.$7H_2O$	1.600 %
Manganese Sulfate.H_2O	0.035 %
Potassium Chloride	11.200 %
Potassium Iodide	0.008 %
Potassium Monophosphate	21.200 %
Sodium Chloride	6.900 %
Sodium Fluoride	0.010 %

Vitamin mixture:

Thiamine-HCl	0.04 %
Riboflavin	0.08 %
Pyridoxine-HCl	0.05 %
Ca-Pantothenate	0.40 %
Inositol	2.00 %
Menadione	0.04 %
Folic Acid	0.04 %
Niacin	0.40 %
Choline Dihydrogen Citrate	42.38 %
Biotin premix[a]	0.30 %
B-12 premix[b]	1.00 %
Corn Starch	53.27 %

[a]Biotin premix: 1.0 gm biotin mixed with 99 gm corn starch.

[b]Vitamin B-12 premix: 1 gm vitamin B-12 mixed with 500 gm corn starch.

Table ii. Average caloric intake per week.

Feeding Frequency	Fat Level	PUFA Level		
		2.5%	11%	2.5% & 11%
		gm	gm	gm
AD	15%	551 ± 132	487 ± 170	517 ± 156
AD	30%	570 ± 121	509 ± 144	538 ± 136
AD	15% & 30%	561 ± 126	498 ± 157	[a]527 ± 146
MF	15%	356 ± 111	313 ± 136	334 ± 125
MF	30%	373 ± 148	337 ± 133	355 ± 140
MF	15% & 30%	364 ± 130	325 ± 134	[a]345 ± 133
AD & MF	15%	447 ± 155	400 ± 176	423 ± 167
AD & MF	30%	465 ± 168	423 ± 162	444 ± 166
AD & MF	15% & 30%	[b]456 ± 161	[b]411 ± 169	433 ± 166

[a]AD significantly greater than MF ($p<0.001$)

[b]2.5% PUFA significantly greater than 11% PUFA ($p<0.004$)

Table iii. Average weight gain over four-week period.

Feeding Frequency	Fat Level	PUFA Level		
		2.5%	11%	2.5% & 11%
		gm	gm	gm
AD	15%	194 ± 22	192 ± 17	193 ± 19
AD	30%	187 ± 28	190 ± 19	189 ± 23
AD	15% & 30%	190 ± 24	191 ± 18	[a]191 ± 21
MF	15%	110 ± 19	106 ± 31	108 ± 25
MF	30%	121 ± 17	118 ± 30	120 ± 24
MF	15% & 30%	115 ± 18	112 ± 30	[a]114 ± 25
AD & MF	15%	149 ± 48	149 ± 51	149 ± 48
AD & MF	30%	152 ± 41	154 ± 44	153 ± 42
AD & MF	15% & 30%	150 ± 44	152 ± 47	151 ± 45

[a]AD significantly greater than MF ($p<0.001$)

Table iv. Average weekly weight gain per 100 kcal consumed.

Feeding Frequency	Fat Level	PUFA Level		
		2.5%	11%	2.5% & 11%
		gm	gm	gm
AD	15%	8.65	9.00	8.83
AD	30%	8.04	8.85	8.44
AD	15% & 30%	8.34	8.92	8.64
MF	15%	6.88	6.95	6.92
MF	30%	7.05	7.62	7.34
MF	15% & 30%	6.97	7.29	8.52
AD & MF	15%	7.77	7.98	7.88
AD & MF	30%	7.55	8.24	7.89
AD & MF	15% & 30%	7.66	8.11	7.88