Effect of Protein and Various Sources of Fat on the Metabolic Response to Refeeding following Severe Dietary Restriction in the Rat

by

Frances Irene King

A thesis submitted to the
Faculty of Graduate Studies in partial
fulfillment of the requirements for the
degree of Master of Science

Department of Foods and Nutrition
Faculty of Home Economics
University of Manitoba
Winnipeg April 1973

Suggested short title:

Effect of fat source and protein on refeeding

ABSTRACT

The object of the present study was to investigate the effect of isocaloric substitution of dietary carbohydrate by protein and various sources of fat on G-6-PDH and malic enzyme activities in liver and epididymal adipose tissue, and on hepatic fatty acid composition of restricted-refed rats. The effect of diet composition on blood glucose, serum cholesterol, and serum free fatty acids also was examined. Adult male rats (225-300g) were restricted to 5g daily for 8 days of a high carbohydrate, fat-free (FF) diet. The restricted rats were then refed 20 g daily of the FF diet, or a diet with 12% coconut oil (CO), lard (LA), rapeseed oil (RSO), or safflower oil (SO), or 27% casein (PRO) substituted isocalorically for glucose for periods of 3, 5, or 7 days. Hepatic dehydrogenase activities increased 9- to 10- fold in rats refed the FF diet, 6- to 7- fold in rats refed the CO diet, and 12- to 15- fold in rats refed the PRO diet for 3 to 7 days. Enzyme induction was suppressed to a 2- to 4- fold increase in rats refed the LA, RSO, and SO diets. Although a similar pattern prevailed in adipose tissue the effect of diet was less pronounced. Malic enzyme activity increased 3- to 8- fold in rats refed the FF

diet, 3- to 5- fold in rats refed the CO, LA, RSO, and SO diets, and 8- to 10- fold in rats refed the PRO diet. G-6-PDH activity increased only 2- to 5- fold irrespective of diet refed. Composition of the diet also had an appreciable effect on the fatty acid composition of liver lipid.Eicosatrienoic acid was observed in liver lipid of rats refed the PRO diet for 3, 5, and 7 days. Eicosatrienoic acid also was present in the liver lipid of rats refed the FF, CO, and LA diets for 7 days. A marked reduction was observed in linoleic and arachidonic acid levels in rats refed the FF, CO, and PRO diets when these rats were compared to restricted rats. No eicosatrienoic acid was observed in the liver lipid of rats refed RSO or SO diets for 7 days. Furthermore, linoleic and arachidonic acid levels increased slightly in the liver lipid of rats refed SO diets. Neither dietary restriction nor refeeding was found to have an effect on blood glucose, serum cholesterol, or serum free fatty acid levels. These results suggest that diet composition has a marked effect on the metablic response to refeeding in restricted-refed rats, especially on the activities of NADP-linked dehydrogenases in liver and adipose tissue, and on the fatty acid composition of liver lipid.

ACKNOWLEDGMENTS

The author wishes to thank Dr. B.E. McDonald, Professor in the Faculty of Home Economics at the University of Manitoba, for his technical assistance, cooperation, and guidance in the direction of this research and in the preparation of the manuscript.

The author also wishes to extend her appreciation to Dr. G. Atkinson of the Department of Statistics for his assistance with the statistical analyses.

Special thanks are extended to the writer's mother, Mrs. I. Quinn, for the typing of this manuscript and for the patience and understanding she demonstrated in carrying out this task.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

G-6-PDH	-	glucose-6-phosphate dehydrogenase
ME	-	malic enzyme
NADP	-	nicotinamide-adenine dinucleotide phosphate
NADPH	-	reduced form of NADP
RNA	-	ribonucleic acid
CoA	-	coenzyme A

LIST OF TABLES

LIST OF FIGURES

I. GENERAL INTRODUCTION

Starvation and subsequent refeeding of a high carbohydrate diet results in a marked hyperlipogenesis and an associated "overshoot" of NADP-linked dehydrogenases in the liver and adipose tissue of ad libitum fed rats. Recent evidence suggests that during the refeeding of a high-carbohydrate diet after starvation, new RNA is formed and this RNA persists so that during subsequent starvation refeeding episodes an even greater enzyme induction results than would occur after one starvation-refeeding cycle (Szepesi and Moser, 1971).

The composition of the refeeding diet may have a profound effect on the enzymatic response to refeeding following starvation. Dietary fat has long been known to prevent enzyme induction. In addition, it may be important in the prevention of the "metabolic memory of starvation" responsible for the increased enzyme induction with repeated starvation-refeeding cycles (Szepesi et al., 1972).

In the case of man, where liver is believed to be the major site of fatty acid production, increased inducibility of G-6-PDH and malic enzyme due to meal-skipping or periods of severe caloric restriction could lead

to an increased ability to synthesize fatty acids and thereby possibly lead to obesity. Evidence of such a response in man would be of importance in contributing to increased knowledge of etiology of major health problems such as obesity and cardiovascular disease.

The present study was undertaken to further clarify the effect of diet on the metabolic response to refeeding following severe dietary restriction.

II. STATUS OF THE PROBLEM

The response to a starvation-refeeding regimen appears to be an interesting example of the complicated regulatory mechanisms that occur in mammalian systems. The suppression of lipogenesis caused by fasting and the subsequent increase upon refeeding has been correlated with concomitant changes in the activities of various enzymes related to fatty acid synthesis, notably G-6-PDH and malic enzyme.

The nature of the refeeding diet markedly influences the response to refeeding following starvation. A high fat diet has consistently been shown to suppress the hyperlipogenic response in both liver and adipose tissue (Young et al., 1964; Leveille, 1967a and 1967b). The effect of dietary fat source on lipogenesis is not as well documented. The results of some studies suggest that the effect of the fat on lipogenesis is not related to the source of dietary fat (Dupont, 1966; Bottino et al., 1965), whereas other studies would suggest that unsaturated fatty acids especially linoleic, as well as arachidonic and α-linolenic, exert a special regulatory effect on lipogenesis and enzyme induction (Allmann, 1965; Chu et al., 1969). Furthermore, dietary fat may affect lipogenesis in a dif-

ferent manner than dehydrogenase activity depending upon fat source. Hepatic lipogenesis was found to be as low or lower in animals fed saturated fat when compared to animals fed unsaturated fat whereas NADP-linked dehydrogenase activity was higher in animals fed saturated fat (Tepperman and Tepperman, 1965).

In addition, much controversy surrounds the effect of dietary protein on enzyme activity and lipogenesis. Adequate dietary protein is necessary for G-6-PDH induction but not hyperlipogenesis in rat liver (McDonald and Johnson, 1965). Malic enzyme, however, was found to be only partially suppressed when a protein-free diet was refed following starvation (Tepperman et al., 1968). Nevertheless, dietary protein is essential for malic enzyme induction in adipose tissue (Jomain and Hanson, 1969). Thus there appears to be a dissociation in the responses of the two dehydrogenase enzymes in the two tissues considered primarily responsible for the biosynthesis of fatty acids.

Although many of the control mechanisms involved in the synthesis of fatty acids are known and understood, there are many gaps in the present understanding of this process. The object of the present study was to attempt to contribute to the understanding of lipogenesis and the relationship of the dietary factors involved in this process.

III. REVIEW OF LITERATURE

A. BIOSYNTHESIS OF FATTY ACIDS

1. Relationship of Enzyme Activity and Lipogenesis.

The fact that fatty acid biosynthesis depends upon a variety of nutritional and hormonal factors is well established. Because of the dependence of fatty acid biosynthesis on the reducing power derived from NADPH and the recognition of the pentose pathway as an important source of this reducing power, the two NADPH generating enzymes of the pentose pathway have received considerable attention. Of these enzymes, the activity of glucose -6- phosphate dehydrogenase (G-6-PDH) has been shown to be most critical (Leveille, 1966). It has also been demonstrated, however, that the pentose pathway could not generate more than about 50% of the NADPH required for high rates of lipogenesis in rat adipose tissue (Flatt and Ball, 1964). This led to a search for an alternative system of generating the necessary reducing power to support lipogenesis. Young et al. (1964), Wise and Ball (1964), and Pande et al. (1964) presented evidence suggesting that oxaloacetate derived from citrate cleavage could be converted to malate then to pyruvate as a result of the combined action of malic dehydrogenase and malic enzyme, thereby effecting a transfer of hydrogen

from NADH to NADP+ as shown by the following reactions:

OXALOACETATE + NADH $\longrightarrow$ MALATE + NAD+

MALATE + NADP+ $\longrightarrow$ PYRUVATE + NADPH $+CO_2$.

The subsequent demonstration that pyruvate carboxylase was present in the cytoplasm of rat adipose tissue (Ballard and Hanson, 1967) led to the proposal of a "transhydrogenation cycle" in which hydrogen would be transferred from NADH to NADP+ with ATP supplying the necessary energy. These reactions are described elsewhere in more detail (Leveille, 1970 and Leveille, 1972). Thus, because of their relationship to the biosynthesis of fatty acids, G-6-PDH and malic enzyme (NADP-malate dehydrogenase) are of particular interest to this review.

2. The Effect of Starvation and Refeeding on Lipogenesis and Enzyme Activity

The rate of lipogenesis is significantly reduced in the fasted and diabetic states, and is markedly increased by refeeding following a fast (Tepperman and Tepperman, 1958; Wise and Ball, 1964; Freedland, 1967; Leveille, 1969). Refeeding rats a balanced diet after a prolonged period of starvation causes an increase in lipogenesis in both adipose tissue and liver (Masoro *et al.*, 1962; Allmann *et al.*, 1965; Jansen *et al.*, 1967).

The consequences of fasting and refeeding follow-

ing a fast on the activity of those enzymes associated with lipogenesis have also been investigated. In the rat, the activities of hexose monophosphate shunt dehydrogenases, malic enzyme, and citrate cleavage enzyme are reduced by fasting and return to above normal levels upon refeeding (Tepperman and Tepperman, 1958; Tepperman and Tepperman, 1964; Kornacker and Lowenstein, 1965; McDonald and Johnson, 1965).

The enzymatic adaptations that occur during hyperlipogenesis are due to increased activity in the glycolytic pathway and citric acid cycle. As a result, citrate synthesis increases such that citrate diffuses into the extramitochondrial compartment where increased citrate cleavage enzyme activity cleaves citrate to oxaloacetate and acetyl CoA, thus providing for a rapid transfer of mitochondrially generated acetyl CoA into the cell cytoplasm where fatty acid synthesis occurs. The details of these reactions, as well as the specific roles of liver and adipose tissue have been summarized by Tepperman and Tepperman (1970), and Watt (1970). The role of G-6-PDH and malic enzyme in this series of reactions is to provide for de novo synthesis of fatty acids by supplying NADPH.

Increased activity of the NADP-linked enzymes is not believed to be the factor responsible for the hyperlipogenic response observed during refeeding following

starvation. Rather, present evidence suggests that an increased rate of glucose conversion to fatty acids is the stimulus causing increased activity of malic enzyme and G-6-PDH (Leveille, 1966; Foster and Srere, 1968; Yeh and Leveille, 1970).

III. B. FACTORS RELATED TO ENZYME INDUCTION

1. De Novo Enzyme Synthesis

Recently, it has been shown that treatment of starved rats with 8-azaguanine prevents the "overshoot" in G-6-PDH and malic enzyme during refeeding, but not the return of these enzymes to ad libitum values (Szepesi and Freedland, 1969). 8-Azaguanine appears to be incorporated into messenger RNA and ribosomal RNA (Kwan and Webb, 1967). Only the translation of messenger RNA formed during antibiotic administration would, therefore, be affected. Thus it has been suggested that recovery of ad libitum - fed values following starvation is a process which is independent of de novo RNA synthesis, whereas increased de novo RNA synthesis is required for the "overshoot".

Furthermore, it has been shown that a second cycle of starvation - refeeding can lead to an even greater "overshoot" in enzyme activity than a single episode of starvation and refeeding (Szepesi and Berdanier, 1970). In addition, Szepesi et al. (1971b) have demonstrated maximum activity of G-6-PDH and malic enzyme during the third starvation-refeeding cycle with no further increases during a fourth cycle. Because there is evidence to suggest that the specific RNA components responsible for the maintenance of hepatic G-6-PDH and malic enzyme are stable during starvation

(Szepesi et al., 1971a), there is support for the suggestion that successive episodes of starvation and refeeding lead to decreased dependence on transcriptional factors in the development of the enzyme "overshoot". Consequently the control of enzyme induction shifts from transcriptional to translational control (Szepesi et al., 1971b). It appears than that de novo RNA synthesis is a more critical factor in initiating enzyme induction, especially during the first three starvation-refeeding cycles, than it is in maintaining enzyme induction once it has been established.

2. Dietary Carbohydrate

Data presented by Szepesi et al.(1971 a) not only suggest that the specific RNA components responsible for the maintenance of hepatic G-6-PDH and malic enzyme levels are stable during starvation, but that the expression of RNA in terms of enzyme activity is influenced by the diet fed. There is considerable evidence to suggest that enzymatic "overshoot" is strictly dependent on dietary carbohydrate and that if rats are fed a high protein, low fat, carbohydrate-free diet the "overshoot" in G-6-PDH and malic enzyme activities does not occur (Potter and Ono, 1961; Szepesi and Berdanier, 1971; Szepesi and Moser, 1971).

Because glucose derived from dietary carbohydrate is known to increase insulin secretion (Curry, 1970; Pallotta and Kennedy, 1968), the possibility that increased insulin release upon refeeding a high carbohydrate diet may be the signal for subsequent hyperlipogenesis has been studied (Fabry et al., 1970; Szepesi and Berdanier, 1971; Szepesi et al., 1972).

Fabry et al. (1970) suggest that insulin is not the factor responsible for induced hyperlipogenesis. These authors also postulate that the high rate of lipogenesis associated with refeeding high levels of dietary carbohydrate can be attributed to the greater demands on the adipocyte during the refeeding of a high carbohydrate diet. In this instance the adipocyte must synthesize new enzyme proteins related to the synthesis of triglycerides from carbohydrate precursors as well as synthesize those proteins required for cellular hypertrophy associated with the storage of increased amount of triglyceride.

More recently, however, Szepesi and Berdanier (1971) suggested that the control of malic enzyme synthesis may be regulated by insulin and that the requirement for dietary carbohydrate is separate from the requirement for insulin. They also concluded that the control of G-6-PDH appeared to require factors other than or in

addition to, insulin. In subsequent investigation of this hypothesis, however, Szepesi et al. (1972) concluded that insulin either is not the signal for enzyme overshoot or that its effect can be completely overriden by a high-fat diet. This conclusion is supported by the observations of Fabry et al. (1970). It appears then, that not only de novo RNA synthesis and dietary carbohydrate may affect enzyme induction but that the composition of the refeeding diet may also be of importance in modifying the physiological responses observed.

III. C. EFFECTS OF DIET COMPOSITION

1. Carbohydrate

The absolute requirement for dietary carbohydrate for the induction of G-6-PDH and malic enzyme during refeeding following starvation has been outlined above (Sec.III.B.2). It appears that dietary carbohydrate is essential for enzyme induction apart from any effect it may have on insulin secretion. In fact, Leveille (1970) has suggested that the observed depression in enzyme activity associated with feeding dietary fat or protein is due to a reduction of glucose rather than an increase of fat or protein in the diet. More recently, however, it has been demonstrated that when the level of dietary carbohydrate was maintained constant and levels of fat and protein were varied, a low-protein-high-fat diet resulted in a greater decrease in lipogenesis than an isocaloric high-protein-low-fat diet (Allee et al., 1971). These results suggest that dietary protein and dietary fat have specific effects on lipogenesis unrelated to the amount of carbohydrate with which they are fed. In other words, the depressing effect of dietary fat or protein is not simply due to the removal of glucose from the diet.

2. Fat

Feeding a high fat diet has been shown to depress fatty acid synthesis in rat liver and adipose tissue (Hill et al., 1958; Hill et al., 1960; Leveille, 1967 a and b). Jansen et al. (1966) found, however, that hepatic fatty acid synthesis in mice was depressed to a much greater extent by dietary fat than was synthesis outside the liver. Furthermore the same authors reported that elevation in liver lipogenesis during refeeding was greatest on a diet containing 1% of corn oil, whereas in extrahepatic tissues the increase in lipogenesis was greater when the mice were refed a diet containing 20% of corn oil (Jansen et al., 1968). From these results, it appears that the control of induced hyperlipogenesis during refeeding may be different in liver than that in extrahepatic tissues.

The effect of source of dietary fat on fatty acid synthesis is, however, less clear. There is some evidence to suggest that all long chain fatty acids are equally effective in depressing lipogenesis in the rat and pig (Hill et al., 1958; Hill et al., 1960; Bottino et al., 1965; Dupont, 1966; Allee et al., 1972). Allmann et al. (1965) found that refeeding starved rats a fat-free diet resulted in increased activity of liver fatty acid synthetase and G-6-PDH. There was also a decrease in the amount of linoleic acid relative to other liver fatty acids. These

changes are essentially the same as those observed in early linoleic acid deficiency. By using fasting and refeeding as a method of decreasing hepatic linoleic acid concentration, Inkpen et al. (1969) were able to demonstrate that the capability for 9 - desaturation of long chain fatty acids by microsomes is suppressed by dietary linoleate. On the basis of this observation these authors suggest that hepatic linoleate is a key factor in the cellular control mechanism for lipogenesis. In addition, Chu et al. (1969) found that linoleic acid, arachidonic acid, and α -linolenic acids had a regulatory effect on fatty acid synthetase and G-6-PDH activity. These authors suggested that hepatic linoleate concentration per se is not a critical factor in the regulation of these enzyme activities because the effect of arachidonate and α -linolenate was observed without an increase in liver linoleate.

Moreover, research conducted by Tepperman and Tepperman (1965) has added another dimension to the effect of fat source on enzyme activity and hepatic lipogenesis. They found the aggregate hexosemonophosphate dehydrogenase activity was higher in the livers of rats fed a diet containing saturated fat (hydrogenated coconut oil) than it was in livers from animals fed a corn oil diet. Lipogenesis as measured by the incorporation of acetate -1- C^{14} into fatty acids by liver slices, was as low or lower in animals

fed saturated fat as it was in those fed a corn oil diet. It was suggested that the increased hexosemonophosphate shunt dehydrogenase activity is due to the desaturation and chain lengthening that occurs when animals are fed a saturated fat diet.

The effects of dietary fat and in particular the source of dietary fat on lipogenesis and the activity of enzymes associated with this process require further research before they can be clearly explained.

3. Protein

The effects of dietary protein on the activity of NADP-linked dehydrogenases and lipogenesis have not been clearly documented. There are many contradictory reports in the literature concerning the role of dietary protein in the hyperlipogenic response.

Many investigators have found that dietary protein is essential for the "overshoot" of G-6-PDH (Potter and Ono, 1961; Vaughan and Winders, 1964; McDonald and Johnson, 1965; Tepperman et al., 1968; Jomain and Hanson, 1969). In fact, Vaughan and Winders (1964) postulated that dietary protein was the limiting factor in the rapid regeneration of hexosemonophosphate shunt dehydrogenase. Potter and Ono (1961) found that induction of G-6-PDH

in starved-refed animals did not occur unless the diet contained adequate amounts of both carbohydrate and protein. No response in G-6-PDH activity was obtained with either 2% protein: 89% glucose or with 91% protein: 0% glucose. Although the optimum response occurred with 30% protein: 61% glucose, the "overshoot" in G-6-PDH activity was not significantly lowered unless dietary protein levels fell below or exceeded 18% and 60% respectively. The decrease in dehydrogenase activity observed on low protein diets was not associated with a decrease in hepatic lipogenesis (McDonald and Johnson, 1965; Tepperman et al., 1968), but was associated with low rates of fatty acid synthesis in adipose tissue (Jomain and Hanson, 1969).

In addition the response of malic enzyme to protein deficient diets has been found to differ from that of G-6-PDH. Tepperman et al. (1968) found a partial suppression of malic enzyme activity when a zero protein diet was refed. Oliver and McDonald (1967) found no depression in hepatic malic enzyme activity with a diet deficient in protein. Furthermore when diets containing 75% protein and 17% alanine, 90% protein, 75% protein and 17% glutamic acid, and zero protein high-carbohydrate were fed to rats following fasting, hepatic malic enzyme was stimulated only by the zero protein high-carbohydrate diet (Vaughan and Winders, 1964). Dietary protein appears

to be essential for malic enzyme induction in adipose tissue, however, since refeeding starved rats a protein deficient diet does not produce the elevation of malic enzyme that is characteristic of animals refed a balanced diet (Jomain and Hanson, 1969).

The effect of increased dietary protein on the metabolic response to meal-feeding also has been investigated. Leveille (1967a) found that increasing the level of dietary protein decreased fatty acid synthesis in adipose tissue in both nibbling and meal-fed rats. As the protein level of the diet was increased, there was also a decrease in the activity of malic enzyme. Reynolds et al. (1971) also found that G-6-PDH activity decreased when rats were shifted from a 12% protein diet to a 60% protein diet. In addition, it was found that increasing dietary protein from 12% to 24% significantly reduced in vitro lipogenesis in pig adipose tissue (Allee et al., 1971). Malic enzyme activity also was reduced in the adipose tissue of these pigs. There was, however, no significant reduction in G-6-PDH activity. In a second experiment dietary protein had no effect on any of these parameters. The different responses of meal-fed or ad libitum-fed animals to increased levels of dietary protein as compared to starved-refed animals may be a result of the greater physiological stress imposed upon the metabolic system of a starved-refed animal. Meal-feed-

ing and starvation-refeeding are known to cause many adaptations in enzyme activity which are similar. Beyond this, however, these two situations may not be directly comparable. More research is required before the effect of dietary protein on lipogenesis and enzyme activity can be defined.

III. D. FATTY ACID COMPOSITION OF LIVER AS AFFECTED BY STARVATION AND REFEEDING

In the rat, as in other mammalian species, changes in fatty acid metabolism begin to occur within twenty-four hours of fasting. Among other things, starvation-refeeding has been found to affect the total fatty acid composition of rat liver (Allmann *et al.*, 1965; Williams *et al.*, 1967).

Allmann *et al.* (1965) found a small decrease in the proportion of linoleic acid following starvation for forty-eight hours in rats that were fed laboratory chow prior to starvation. Refeeding a fat-free diet resulted in the characteristic "overshoot" in enzymes catalyzing fatty acid biosynthesis. Coincident with the rise in the activity of these enzymes was an even greater fall in the proportions of hepatic linoleic and arachidonic acids. It was suggested that this may have been attributable to the increased accumulation of saturated and monounsaturated fatty acids (palmitoleic, stearic, and oleic) rather than an actual decrease in linoleate and arachidonate *per se*.

That the latter possibility in fact was the case was established by Williams *et al.* (1967) who determined the liver fatty acid composition in rats fed a purified diet before fasting. They found that fasting increased the relative proportions of linoleate and arachidonate in total

liver fatty acids but that the absolute amounts remained constant. Refeeding lowered the relative proportions of linoleate and arachidonate to one-third of fasting values. Again this change was due more to relative than absolute changes in liver fatty acid composition. Generally, the effect of refeeding on liver fatty acid composition was similar to that observed by Allmann et al.(1965). It appears, however, that the pre-starvation diet may affect the response of liver fatty acids to starvation.

Recently, Rogers (1971) suggested that the significance of the results of Allmann et al. (1965) may be in doubt in view of the low values reported for arachidonic acid and the apparent absence of C22 polyunsaturates. This may explain the apparent differences in proportions of liver fatty acids reported by Allmann et al. (1965) and Williams et al. (1967) as a result of fasting.

The changes in liver fatty acid concentration brought about by refeeding a fat-free diet are essentially analogous to those observed in early linoleic acid deficiency (Allmann et al. 1965). Due to the very brief periods of linoleic acid restriction, however, Allmann et al. (1965) were barely able to detect eicosatrienoic acid formation. Further research is required to demonstrate the presence of an essential fatty acid deficiency in rats refed a fat-free diet.

Rogers (1971) found that changes in the fatty acid patterns of phospholipids in response to fasting and re-feeding differed from those discussed above which represent changes in the fatty acid composition of the total liver lipid. These observations by Rogers (1971) suggest that the fatty acid composition of liver triglycerides differ markedly from those of liver phospholipids. Fasting resulted in a significant decrease in the relative proportions of linoleic acid and a corresponding increase in arachidonic acid in total liver phospholipid. Refeeding for forty-eight hours had no effect on the relative proportions of these fatty acids. Fasting can, therefore, affect the proportions of major fatty acids in the phospholipid as well as in the triglyceride fraction. The significance of such changes in fatty acid proportions in the different lipid fractions, however, is unknown.

III. E. EFFECTS OF STARVATION AND REFEEDING ON BLOOD GLUCOSE AND SERUM LIPIDS

1. Blood Glucose

Insulin is thought to play a role in the maintenance of the physiological activities of three key glycolytic enzymes in the liver; glucokinase, phosphofructokinase, and pyruvate kinase. Insulin supposedly acts as an inducer in the biosynthesis of these enzymes (Sillero et al., 1969). These enzymes would, therefore, be expected to decrease in activity in states involving hypoinsulinism such as diabetes or starvation, and return to normal in conditions of hyperinsulinism. Since glucokinase is responsible for the first step in the metabolism of glucose by liver, the response of this enzyme to diet has been a special interest to many workers.

Liver glucokinase activity has been found to decrease during starvation and return to normal with refeeding (Weber et al. 1966; Blumenthal et al., 1964). In addition, the type of diet refed fasted rats had a pronounced effect on liver glucokinase activity (Blumenthal et al., 1964).

Coupled with decreased glycolytic activity during starvation is an increase in gluconeogenesis. This

is promoted by the decreased insulin supply during starvation because insulin acts as a suppressor of the biosynthesis of gluconeogenic enzymes (Weber *et al.*, 1965).

Blood glucose levels, therefore, should tend to remain within normal limits during starvation because glycolysis is suppressed and gluconeogenesis induced. In fact, Braun *et al.* (1967) found that in meal-fed rats the overall effect of insulin on carbohydrate metabolism was enhanced by meal-feeding. This enhanced action of insulin would tend to maintain normal blood glucose levels in the periods between meals.

Mammalian systems maintain homeostasis by their ability to adapt to alterations in the external or internal environment by decreasing or increasing the rate of production of various rate-limiting metabolites. One of the key biochemical parameters which the organism must maintain at all costs is an adequate blood glucose level. The significance of this is shown by the fact that low blood glucose of a few minutes duration leads to loss of consciousness and, if hypoglycemia continues, to convulsions, coma, and death (Weber *et al.*, 1965).

Although considerable research has been done on key glycolytic and gluconeogenic enzymes little has been done on level of blood glucose *per se* as influenced by

diet or starvation and refeeding. Glucose tolerance is known to be decreased in diabetes, a situation often compared to starvation. As well, glucose tolerance may be decreased in obese humans (Morse et al., 1960). Since the hyperlipogenic pattern associated with refeeding may predispose to obesity, it would be of interest to determine blood glucose levels as affected by starvation and refeeding.

2. Serum Lipids

The effect of diet, particularly dietary fat, on serum lipid patterns has been studied extensively because of the implication of cholesterol in cardiovascular disease in man. It is generally accepted that dietary fats having a large proportion of saturated fatty acids will elevate serum cholesterol levels while dietary fats high in poly-unsaturated fatty acids will depress serum cholesterol levels. Nevertheless, Dupont (1966) found that there was very little effect of age, sex, or diet on the overall pattern of serum cholesterol when weanling rats were fed diets containing 42% corn oil or 40% beef tallow and 2% corn oil although there was a trend toward increased serum cholesterol in rats fed beef tallow for both sexes.

Although a tremendous volume of data has been accumulated on the relationship of diet to serum lipid patterns in the fed animal, very little information has been reported on the effects of fasting and refeeding following fasting on blood lipid patterns. Kerpel et al. (1971) found that fasting caused a decrease in the level of cholesterol ester. The level of total cholesterol, however, remained unchanged. Refeeding chow for one day restored serum lipid patterns to normal whereas refeeding 20% w/w glucose in water for one day resulted in a further marked decrease in serum cholesterol, especially esterified cholesterol. By the fourth day of glucose refeeding, however, serum lipid levels had risen to pre-refeeding

values. Kerpel et al. (1971) explained these observations by postulating that as fasting progresses the supply of free fatty acid from adipose tissue is diminished so that the liver cannot maintain normal levels of esterified serum lipids. Hepatic lipogenesis which accompanies refeeding of a glucose solution appears to be inadequate to compensate for the reduced rate of fatty acid mobilization. Serum cholesterol levels might be expected to be maintained during fasting but not after glucose refeeding because adipose tissue is a storage depot for cholesterol, which also is mobilized during free fatty acid release, although more slowly than fatty acids.

This hypothesis may apply, however, only when fasted animals are refed a glucose solution. The glucose refed animals did not have higher rates of lipogenesis than chow-refed animals. If glucose were refed in a balanced diet hepatic lipogenesis might be rapid enough to allow serum cholesterol levels to return to normal more quickly.

The effect of diet composition and nutrient balance has not been extensively studied either in cardiovascular research or in the fasted-refed animals. Kritchevsky and Tepper (1971) recently reported studies wherein rats were fed diets in which 50% of the total calories were either carbohydrate, protein, or fat (corn oil). The high protein diet resulted in the highest serum cholesterol levels. When 0.5% cholesterol was added to the diet, however, the high carbo-

hydrate diet was as cholesteremic as the protein diet. These findings suggest the need for further investigation on the effect of carbohydrate-protein-fat interactions, including the composition of dietary fat on serum cholesterol levels.

Kerpel et al. (1971) also investigated the effects of fasting and refeeding upon serum free fatty acids. Serum free fatty acids increased upon fasting, but after seven days of starvation their concentration dropped to that observed in the fed rats. When animals were fasted for three days, refeeding with glucose for one day decreased the free fatty acid level to less than 50% of the fasted level. On the other hand, refeeding with chow for one day decreased the free fatty acid level to only one-third of the pre-fasting level, and free fatty acid levels did not return to normal until the fourth day. The increased level of free fatty acids that occurs upon starvation is a result of fatty acid mobilization from adipose tissue depot sites. As starvation progresses the supply of fat is decreased thus serum free fatty acids return to normal. These findings are supported by the observations of Yeh and Leveille (1970) who found plasma free fatty acids in chicks increased with time of fasting when the fasting period was relatively short, and that these returned to normal upon refeeding.

The varying effects of fat, carbohydrate, and protein upon lipid metabolism have been studied by a number of investigators. The effects of diet upon serum lipid levels and serum glucose levels following starvation and refeeding have received relatively little attention. Further research is required before a clear understanding of the nature of changes in blood components is possible.

IV. OBJECTIVES OF RESEARCH

The present study was undertaken to determine the effect of composition of the refeeding diet on the metabolic response to realimentation following severe dietary restriction. An attempt was made to clarify the effect of dietary fat source on the metabolic response to refeeding as well as the effect of isocaloric substitution of protein for glucose in the refed diet.

Malic enzyme and G-6-PDH activity were measured at three time intervals in both liver and adipose tissue in order to determine the effect of time on the activity of these enzymes in both tissues.

Finally, since factors pre-disposing to human obesity are believed to promote high serum cholesterol levels and a decreased glucose tolerance, serum cholesterol, blood glucose, and free fatty acids were measured in the serum of rats that had been subjected to a starvation-refeeding regimen.

V. EXPERIMENTAL PROCEDURE

A. DESIGN OF EXPERIMENT

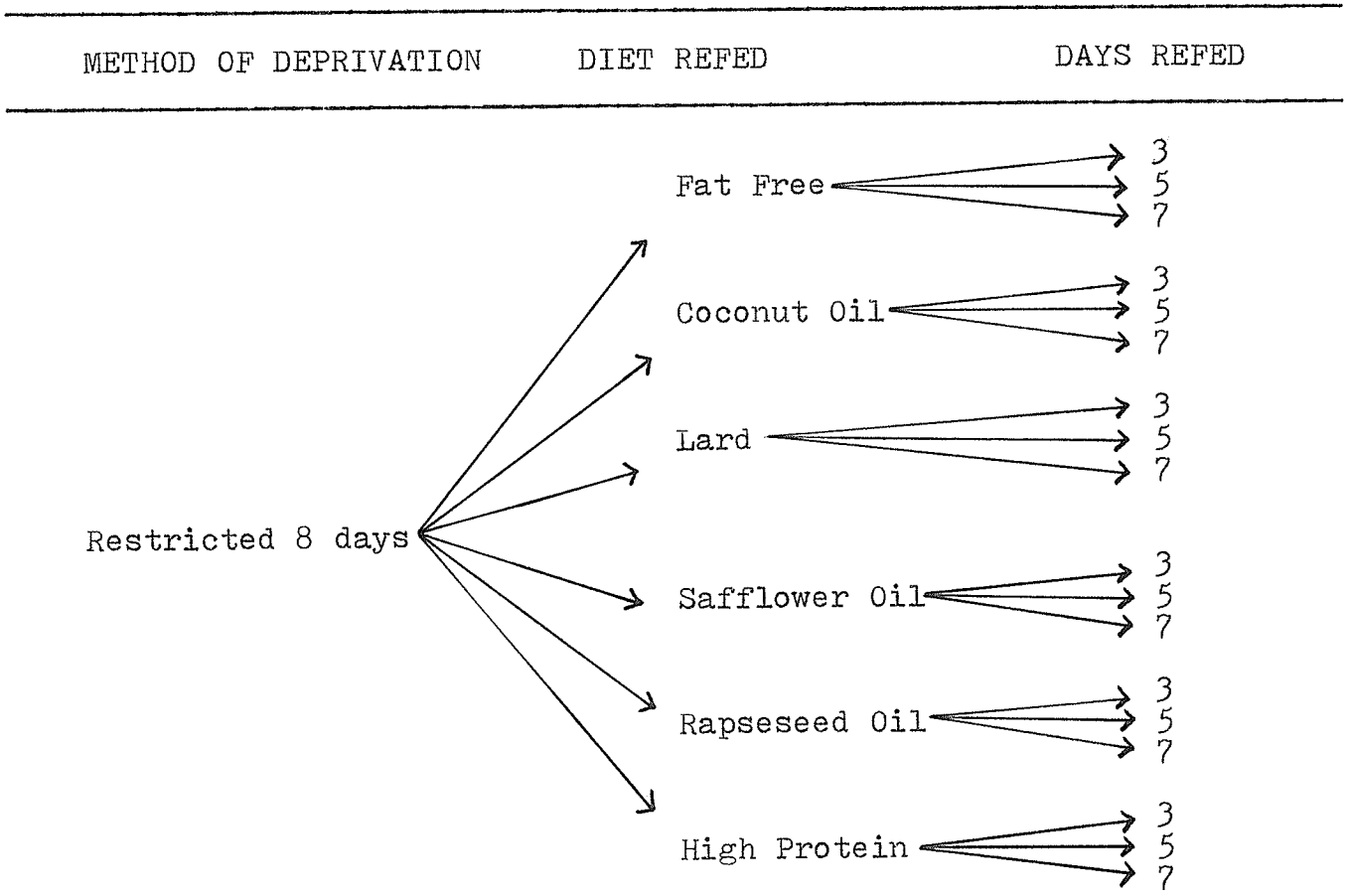
Adult male Sprague-Dawley rats (Dept. of Oral Biology, Faculty of Dentistry, Univ. of Man.) weighing 225-300 g were used in the present experiment. Three groups of thirty rats each were purchased at two week intervals and assigned at random to the various treatment groups. Controls included eight groups of four animals each. One Group was fed lab chow[1] ad libitum for 8 days, six groups were fed 20 g daily of one of the six experimental diets; fat free (FF), coconut oil (CO), lard (LA), safflower oil (SO), rapeseed oil (RSO), or high protein (PRO), for 8 days. The remaining control group was restricted to 5 g daily of the FF diet for 8 days. Following restriction the rats were assigned to groups of 3 rats each and fed 20 g daily of one of the six experimental diets for 3, 5 or 7 days. The rats were slaughtered at the end of the respective refeeding periods.

Eight animals were slaughtered each day. Rats from the same treatment were slaughtered on different days in order to distribute the variation within treatments over several days.

---

1 Rat Lab Chow: Ralston Purina Co., St Louis, Missouri, U.S.A.

DESIGN OF EXPERIMENT



CONTROLS

Restricted 8 days

Fed lab chow ad lib.

Fed experimental diet - 20 g daily for 8 days

Figure 1A.

V. B. DIETS

The diets (table 1) were similar to those previously used in the Department of Foods and Nutrition, University of Manitoba (Watt, 1970). Four fat sources were used (CO, LA, SO, RSO) in an attempt to determine the effect of fat source on the metabolic response to refeeding. Substitution was made on the basis of 4 Cal/g for protein, 9 Cal/g for fat, and 0 Cal/g for cellulose. The diets were mixed in a Hobart[1] mixer and stored in a refrigerator at 5°C.

---

1 The Hobart Manufacturing Co., Ltd., Ontario, Canada

TABLE I

COMPOSITION OF RAT DIETS

INGREDIENT	Amount (%)		
	0 Fat	12% Fat	High Protein
Soybean Protein	18.0	18.0	18.0
Casein (Vitamin Free)	--	--	27.0
DL-Methionine	0.6	0.6	0.6
Vitamin mix[1]	0.6	0.6	0.6
Glucose	73.0	46.0	46.0
Alphacel	3.8	18.8	3.8
Mineral mix[2]	4.0	4.0	4.0
Fat source[3]	--	12.0	--

1 Composition of vitamin mix: vitamin A (250,000 IU/g), 3.6 g; vitamin D (850,000 IU/g), 0.056g; alpha-tocopherol succinate (890 IU/g), 5.62 g; menadione, 2.25 g; inositol, 5.00 g; choline chloride, 75.0 g; niacin, 4.5g; riboflavin, 1.00 g; pyridoxine·HC1, 1.00 g; thiamin·HC1, 1.00 g; Ca pantothenate, 3.00 g; D-biotin, 20 mg; folic acid, 90 mg; vitamin B_{12} 1.35 mg, and dextrose, 898g.

2 Composition of mineral mix: NaCL, 108 g; $K_3C_6H_5O_7.H_2O$, 236.25 g; K_2HPO_4, 77.25 g; $CaHPO_4.2H_2O$, 355 g; $CaCO_3$, 168.4g; mg CO_3, 40.75 g; $FeSO_4.7H_2O$, 14.88 g; $CuSO4.5H_2o$, 178 mg; $MnSO_4.H_2O$, 1.38 g; KI, 4.5 mg; $ZnSO_4.7H_2O$, 1.82 g.

3 Fat sources included; coconut oil, safflower oil, lard and rapeseed oil.

V.C. FEEDING AND MANAGEMENT OF EXPERIMENTAL ANIMALS

Rats were allowed to adjust to their new environment before being assigned to the respective treatments. During the pre-experimental period the rats were housed 4 to 6 per cage in wire-floored cages measuring 24x12 inches. The rats were fed lab chow[1] ad libitum and tap water was available at all times from water bottles located at the front of each cage. Room temperature fluctuated between 20-21°C. Lighting was controlled so that it was dark from 7:00 P.M. until 7:00 A.M.

During the experimental trials rats were housed individually in wire-floor cages measuring 8x12 inches. All dietary regimens were initiated at 8:30 A.M. Restricted animals were fed 5.0 g daily; 2.5 g at 8:30 A.M. and 2.5 g at 4:30 P.M. During refeeding the rats were fed 20 g of diet daily; 10 g at 8:30 A.M. and 10 g at 4:30 P.M.

Following the specified treatments, rats were anesthetized by an injection of Nembutal[2] (0.8 ml/kg), and blood was removed by heart puncture. Immediately following bleeding the rats were decapitated using a guillotine[3] and the tissue to be assayed rapidly excised.

---

1 Rat Lab Chow: Ralston Purina Co., St. Louis, Missouri, U.S.A.

2 Sodium Pentobarbital, 60 mg/ml, Abbott Laboratories, Ltd., Montreal, Canada.

3. Harvard Apparatus Inc., Dover, Mass. U.S.A.

V.D. PREPARATION OF TISSUE FOR CHEMICAL ANALYSES

The blood obtained by heart puncture was collected in a heparinized 10 ml Vacutainer[1] tube, and the tubes stored in ice until they were centrifuged[2] at 1000xG for 10 minutes at 0°C. The plasma was removed with a syringe and stored at -20°C for subsequent analyses.

The livers and epididymal fat pads were quickly excised following decapitation of the rats. The livers were rinsed with cold tap water, blotted with filter paper, and weighed. Epididymal fat pads were carefully removed from blood vessels and connective tissue and weighed.

Approximately 2.0 g of liver (a composite of a piece from each lobe) was minced with scissors and homogenized in buffer, 1:9 W/V, (McDonald and Johnson, 1965) using a Potter-Elvehjem tissue homogenizer.[3] Similarly the epididymal fat pads were weighed and homogenized in 0.15 molar KCl solution, 1:9 W/V, using a Virtis No. 23 tissue homogenizer.[4]

---

1 #3200 KA Becton, Dickinson and Co., Canada Ltd., Clarkson, Ontario

2 Model B-20 Centrifuge, International Equipment Co., Needham Hts., Mass., U.S.A.

3 Arthur H. Thomas Company, Philadelphia, Pa.

4 Virtis Research Co., Model 23, Gardiner, New York, U.S.A.

Both homogenates were centrifuged at 0°C in a refrigerated centrifuge.[1] Centrifugation was carried out for 10 minutes at 10,800 x G and 20 minutes at 45,900 x G. The supernatant fraction was removed with a syringe and used for enzyme analyses. A portion of the supernatant was stored at - 20°C for later protein determination.

The remainder of each liver was stored at -20°C in three ounce, screw-cap glass jars for later lipid extraction.

---

1 Model B-20, International Equipment Co., Needham Hts., Mass., U.S.A.

V.E. CHEMICAL ANALYSES

1. Protein Assay

Protein content of the liver and adipose tissue supernatants was measured by the method of Lowry et al. (1951) using commercial Folin Ciocalteau Reagent.[1] The procedures used were identical to those outlined by Stitchell (1971).

2. Enzyme Assays

The G-6-PDH activity was assayed by the method of Lohr and Waller (1963) and the malic enzyme activity by the method of Ochoa (1955). The procedures used were identical to those outlined by Stitchell (1971) and involved a measurement of the rate of formation of NADPH at 340 mμ in a Unicam SP800 A UV spectrophotometer,[2] equipped with a SP20 Series recorder.

3. Lipid Analyses

a. Total hepatic lipid extraction

Frozen livers were thawed at room temperature and the total lipid extracted following the procedure of Bligh and Dyer (1959). The procedures used were identical to those outlined by Watt (1970).

---

1 Fisher Scientific Co., Toronto, Ontario.

2 Pye Unicam Ltd., Cambridge, England

b. Determination of fatty acid patterns in liver lipids

Fatty acid methyl esters were prepared using boron trifluoride as catalyst, according to the method of Metcalfe et al. (1966). The methyl esters were dissolved in petroleum ether and fatty acids resolved with 2.7 m by 2 mm i.d. stainless-steel columns packed with 10% EGSS-Y organosilicone polyester on 100/120 mesh Gas Chrom Q[1], using a Varian Aerograph[2] model 1740-1 gas chromatograph fitted with dual columns, hydrogen flame detectors, a Varian Aerograph model 477 electronic digital integrator, and a Varian Aerograph model 20 strip chart recorder. Helium flow rates were 30-35 ml/min. Oven temperature was isothermal at 200°C with injection port and detectors at 280° and 240°C, respectively. Identification of the fatty acid methyl esters was made by comparison with known standards.[3]

4. Blood Analyses

a. Blood glucose concentration

The glucose concentration of deproteinized plasma was determined by a modification of the procedure

---

1 Applied Science Laboratories, Inc. State College, Pa.

2 Varian Aerograph, 6358 Viscount Road Malton, Ontario

3 The Hormel Institute Austin, Minn.

of Raabo and Terkildsen (1960) as outlined in Sigma[1] Tech. Bulletin 510-5-69. Duplicate determinations were made for each sample. Colorimetric measurements were made in a Coleman Junior Spectrophotometer[2], model 6A, at a wavelength of 450 mμ.

b. Serum total cholesterol

Total serum cholesterol was determined by the method of Pearson et al. (1952). Duplicate determinations were made for each sample. Colorimetric measurements were made in a Coleman Junior Spectrophotometer[2], model 6A, at a wavelength of 550 mμ.

c. Free fatty acids

Free fatty acid determination was carried out according to the method of Mosinger (1965). Plasma volume varied from 0.2-0.5 ml, depending on the amount of sample available, whereas the original method called for 1.0 ml of plasma. Four ml of heptane was used in the extraction instead of 3.0 ml recommended in the method. Colorimetric measurements were made in a Coleman Junior Spectrophotometer[2], model 6A, at a wavelength of 550 mμ.

---

1 Sigma Chemical Company, St. Louis, Mo.

2 Coleman Instruments, Inc., Maywood, Illinois, U.S.A.

V.F. STATISTICAL ANYALYSES

Statistical analyses were carried out on the data for G-6-PDH and malic enzyme in liver and adipose tissue. A log transformation was performed on all original data and the transformed data was subjected to analysis of variance and Duncan's Multiple Range Test according to the method described by Snedecor and Cochran (1967). Significance was determined by the F-test at $P<0.05$ (Appendix Tables I, II, III and IV).

VI RESULTS AND DISCUSSION

A. GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND MALIC ENZYME ACTIVITIES

Restricting food intake to 5 g daily of a fat-free diet had no effect on malic enzyme activity or G-6-PDH activity when enzyme activities in the liver and epididymal adipose tissue of restricted rats were compared to those of rats fed laboratory chow ad libitum (restricted vs. chow-fed controls, Tables II to V). However, enzyme activities of restricted rats tended to be considerably lower than those of non-restricted rats fed 20 g daily of the FF or PRO diets for 8 days (restricted vs. fat-free and high protein controls, Tables II to V; Figures 1 and 2). The extent of elevation in response to 20 g daily of these two fat-free diets varied with the experimental diet fed; in general, enzyme activities were higher for rats fed the PRO diet. On the other hand, feeding non-restricted rats 20g daily of similar diets containing 12% added fat did not result in an elevation in enzyme activities except for rats fed the diet containing coconut oil. These observations are not surprising because dietary fat has been found to suppress the activities of these two enzymes. The laboratory chow[1] contained a minimum of 4.5% crude fat which may

---

1 Rat Lab Chow: Ralston Purina Co., St. Louis Missouri, U.S.A.

TABLE II

Liver malic enzyme activity in restricted-refed rats[1]

Dietary Regimen	Malic Enzyme Activity (n moles NADPH produced/mg protein /min) Controls[2]	Restricted-Refed[3] 3	5	7
Chow	17.6± 7.5			
Restricted	18.4± 4.8			
Fat-free	63.5±21.7	173.9±16.2	163.5±28.5	192.6±37.8
Coconut oil	45.6±17.0	100.8± 6.9	126.0±43.8	129.1±26.6
Lard	26.1± 2.8	86.4± 4.9	45.8±18.2	54.0±11.0
Safflower oil	22.6± 6.4	61.8±34.3	30.0± 5.5	41.9±10.4
Rapeseed oil	24.4± 6.3	59.1± 7.5	63.5±10.9	48.8±10.9
High protein	58.1±13.4	228.2±67.4	248.8±29.6	202.8±29.1

1 Analysis of variance for this data is shown in Appendix Table I.

2 Each value represents the mean ± S.D. of 4 rats fed lab chow *ad libitum* for 8 days, restricted to 5 g daily of fat-free diet for 8 days, or fed 20 g daily of experimental diet for 8 days.

3 Each value represents the mean ± S.D. of 3 rats restricted to 5 g daily of fat-free diet for 8 days and refed experimental diet, 20g daily, for 3, 5 or 7 days.

TABLE III

Liver G-6-PDH activity in restricted-refed rats[1]

Dietary Regimen	G-6-PDH Activity (n moles NADPH produced /mg protein /min)			
		Restricted-Refed[3]		
	Controls[2]	3	5	7
Chow	32.0±10.8			
Restricted	30.3± 5.8			
Fat-free	79.5±26.0	313.1± 8.0	285.9± 72.5	294.6±36.1
Coconut oil	48.9±20.9	211.5± 7.6	231.3± 66.8	206.0±48.1
Lard	38.9± 9.0	138.4± 4.3	89.6± 32.4	92.4±41.0
Safflower oil	29.8± 7.8	79.1± 25.9	45.2± 19.5	70.3±18.5
Rapeseed oil	35.5±13.3	120.8± 28.0	109.2± 18.5	83.3±14.5
High protein	139.8±34.7	433.2±100.9	443.1±128.3	397.4±35.1

[1] Analysis of variance for this data is shown in Appendix Table II.

[2,3] See footnotes 2 and 3, Table II.

TABLE IV

Malic enzyme activity in epididymal adipose tissue of restricted-refed rats[1]

Dietary Regimen	Malic Enzyme Activity (n moles NADPH produced /mg protein /min)			
		Restricted-Refed[3]		
	Controls[2]	3	5	7
Chow	185.2± 97.7			
Restricted	137.8±170.8			
Fat-free	462.0±192.2	539.8±232.2	707.5±147.7	1065.2±164.4
Coconut oil	243.0± 40.0	655.0± 61.0	668.9±267.2	708.2± 74,3
Lard	155.4± 44.0	421.1±102.1	574.6±252.8	638.5±356.6
Safflower oil	173.1± 81.6	307.4± 58.4	467.4±131.7	652.9±192.8
Rapeseed oil	168.1± 54.5	348.5± 42.9	282.5± 39.6	726.7±120.3
High protein	777.1±159.4	1060.2±437.6	1190.8±199.1	1413.2±148.7

1 Analysis of variance for this data is shown in Appendix Table III.

2,3 See footnotes 2 and 3, Table II.

TABLE V

G-6-PDH activity in epididymal adipose tissue of restricted-refed rats[1]

Dietary Regimen	G-6-PDH Activity (n moles NADPH produced /mg protein /min)			
		Restricted-Refed[3]		
	Controls[2]	3	5	7
Chow	117.0±33.1			
Restricted	112.1±127.5			
Fat-free	184.8± 20.0	280.6±136.2	362.6± 67.7	400.1±134.0
Coconut oil	140.9± 15.9	265.6± 36.9	259.1±108.5	256.0±105.0
Lard	106.9± 14.7	211.5± 41.1	193.5± 53.9	250.1± 55.0
Safflower oil	94.5± 10.1	211.9± 74.4	198.9± 84.4	231.3± 41.7
Rapeseed oil	99.7± 18.8	154.4± 10.73	193.6± 76.3	294.9± 23.1
High protein	245.8± 47.1	329.5±145.1	369.9± 31.2	576.7± 87.9

[1] Analysis of variance for this data is shown in Appendix Table IV.

[2,3] See footnotes 2 and 3, Table II.

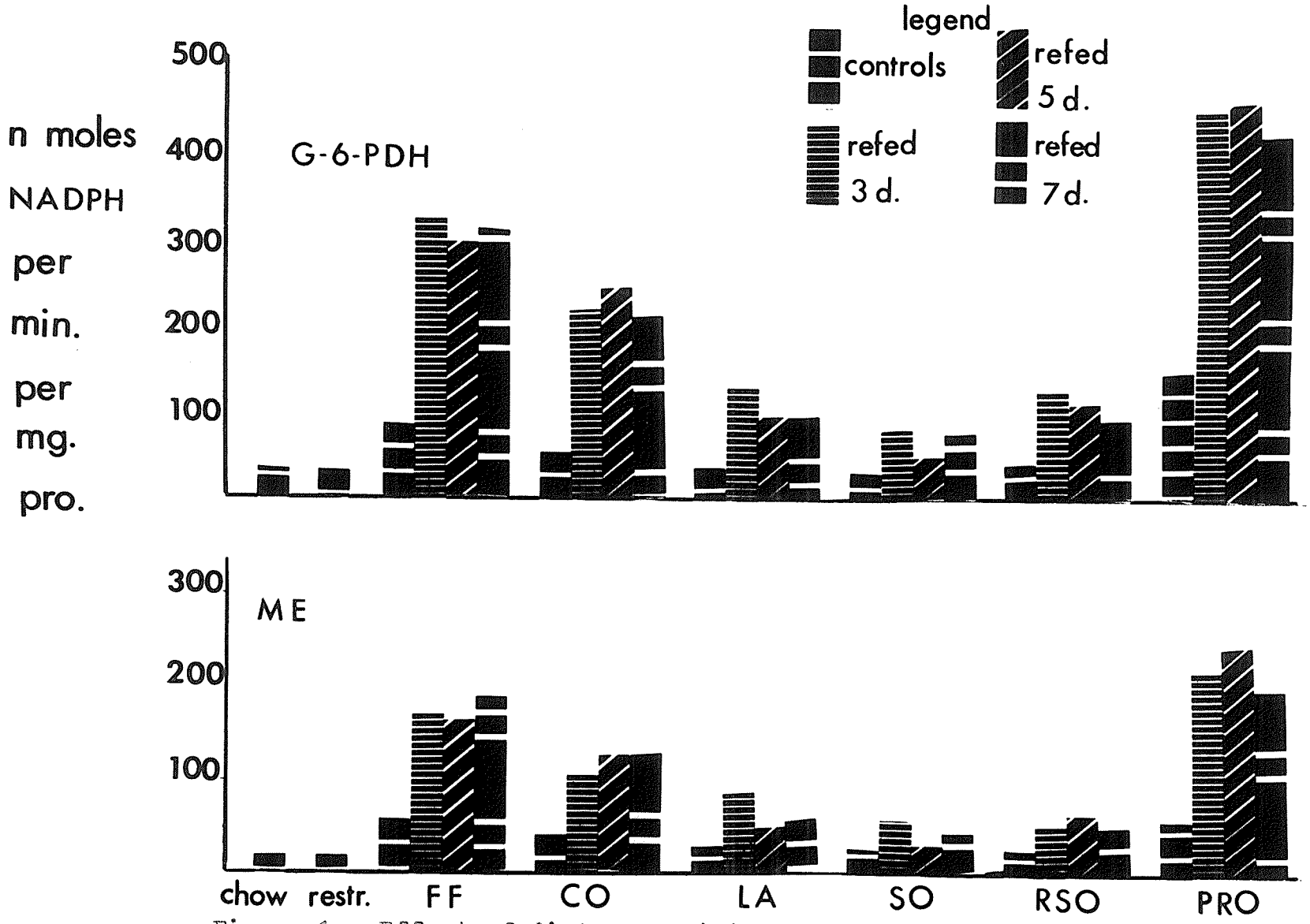


Figure 1. Effect of diet composition and duration of refeeding on enzyme activities in liver.

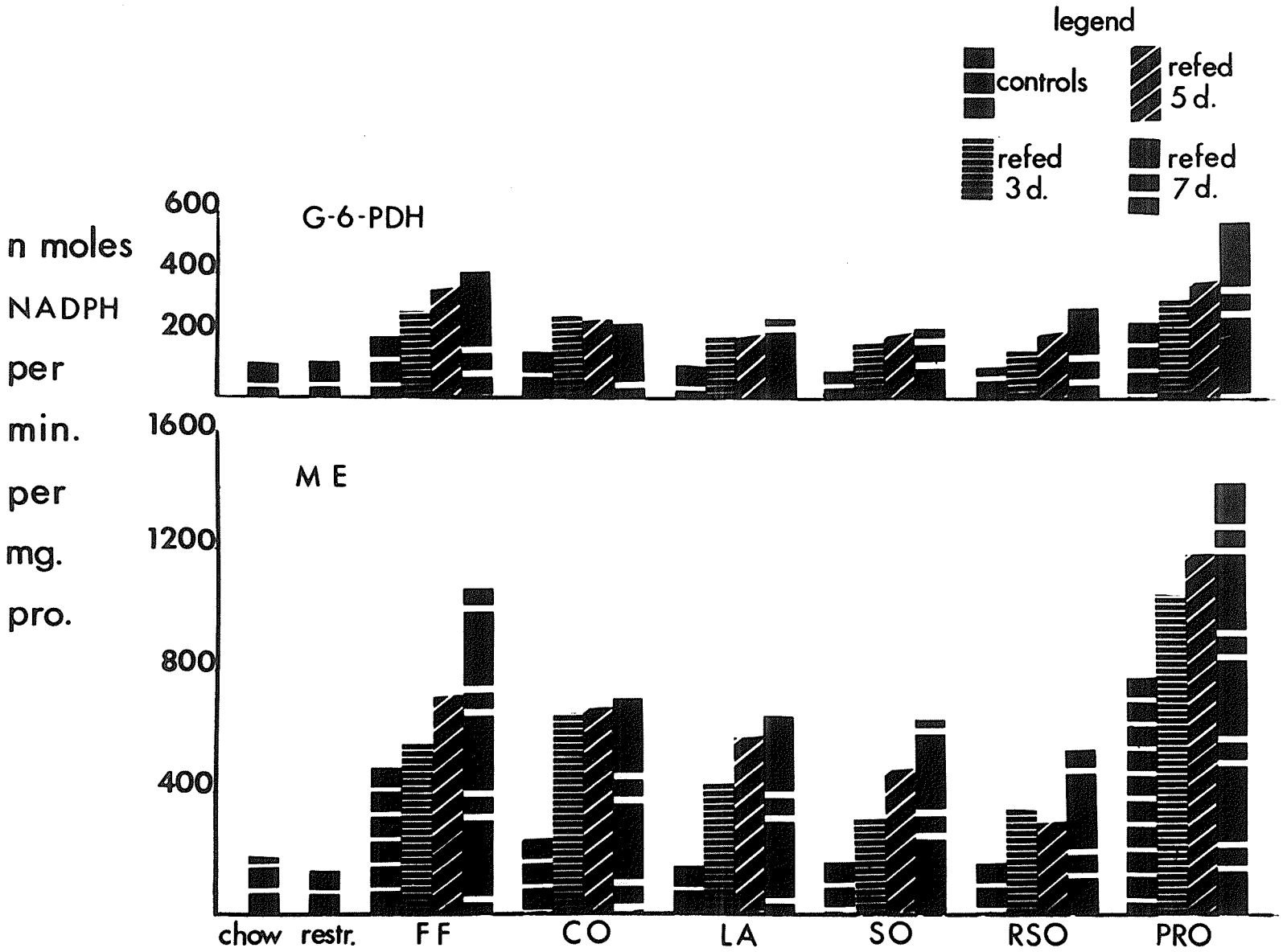


Figure 2. Effect of diet composition and duration of refeeding on enzyme activities in epididymal adipose tissue.

partially account for the fact that controls fed laboratory chow ad libitum had the same enzyme activities as those restricted to 5 g of FF diet daily. Another factor that may contribute to the relatively low enzyme activities observed for the chow-fed controls is that these animals were fed ad libitum. All rats receiving the experimental diets were fed twice daily, resulting in a situation analogous to meal-feeding which has been found to increase the activity of G-6-PDH and malic enzyme in rat adipose tissue (Leveille and Hanson, 1966). The meal-feeding effect was especially evident in restricted animals who tended to eat the entire 2.5 g allotment immediately upon being fed. Thus enzyme activities in the liver and adipose tissue of rats fed laboratory chow ad libitum may not be directly comparable to enzyme activities in liver and adipose tissue of restricted controls.

If restricted animals are compared to non-restricted controls fed 20 g of the FF diet daily, then restriction may be considered to have had a suppressing effect on the activities of G-6-PDH and malic enzyme in both liver and epididymal adipose tissue. Watt (1970) also found that dietary restriction depressed G-6-PDH and malic enzyme activities in liver and adipose tissue when the composition of the diet was identical for restricted

animals and ad libitum fed controls. However, the results of the present study differ from those of Watt (1970) for the non-restricted control rats fed diets containing 12% lard, safflower oil, and rapeseed oil. Watt (1970) found that dietary restriction to 5 g daily of both a FF diet and diet containing 12% corn oil depressed G-6-PDH and malic enzyme activities in liver and adipose tissue even when enzyme activities were compared to control animals fed the 12% corn oil diet ad libitum for 10 days. The results of the present study are, however, in agreement with those of Jomain and Hanson (1969) who observed that fasting did not suppress G-6-PDH or malic enzyme activities in adipose tissue when enzyme activities of fasted rats were compared to those of rats fed a balanced diet containing 9% corn oil. It appears, therefore, that dietary restriction may have a depressing effect on G-6-PDH and malic enzyme activities in liver and adipose tissue depending on the dietary regimen of fed controls.

Refeeding following diet restriction resulted in the characteristic "overshoot" of G-6-PDH and malic enzyme activities in both liver and epididymal adipose tissue (Tables II to V; Figures 1 and 2). The results of the present study suggest that the composition of the diet

has an effect on the enzymatic response to refeeding following dietary restriction. The magnitude of the response to refeeding, however, depends on how the comparisons are made. When malic enzyme and G-6-PDH activities in the liver and fat pads of animals refed for 3 days following dietary restriction are compared to those of control animals fed 20 g daily of an identical diet for 8 days the extent of enzymatic "overshoot" is less pronounced than when dehydrogenase activities in liver and adipose tissue of the restricted-refed rats are compared to those of the restricted-control rats fed 5 g of the FF diet daily.

For example, the "overshoot" in hepatic malic enzyme ranged from a low of approximately 2.2-fold in rats refed the CO diet to a high of approximately 3.9-fold in rats refed the PRO diet when the response of the refed rats is compared to that of non-restricted controls fed 20 g of the same diet for 8 days. Similarly the increased activity of G-6-PDH in adipose tissue ranged from a low of 1.3-fold in rats refed the PRO diet to a high of 2.5-fold in rats refed the RSO diet. However, when the response to refeeding is measured by comparing enzyme activities of restricted-refed rats with those of rats restricted to 5 g of FF diet for 8 days, a marked increase was observed

for G-6-PDH and malic enzyme activities of rats refed the FF, CO, and PRO diets. The increase in hepatic enzyme activities of refed rats over those of restricted rats was 9- to 10- fold in rats refed the FF diet, 6- to 7- fold in rats refed the CO diet, and 12- to 15- fold in rats refed the PRO diet. This marked "overshoot" in enzyme activities was suppressed to a 2- to 4- fold increase in rats refed the LA, RSO, and SO diets. Refeeding also induced an appreciable response in malic enzyme activity in the adipose tissue. Malic enzyme activity in the epididymal fat pads increased 3- to 8- fold in rats refed the FF diet for 3 to 7 days, 4- to 5- fold in rats refed the CO diet, 3- to 5- fold in rats refed the LA, RSO, and SO diets, and 8- to 10- fold in rats refed the PRO diet. The response of G-6-PDH to refeeding was less pronounced in fat pads, however, with the increase in activity over that of restricted rats being only 2- to 3- fold, irrespective of diet, at 3 days refeeding. At 7 days fat pad G-6-PDH activity had increased 3.5- fold for rats refed the FF diet and 5- fold for rats refed the PRO diet, but only slightly more than 2- fold for rats refed the fat containing diets.

The marked "overshoot" in enzyme activity observed when restricted animals were refed a fat-free

diet has been reported by several other investigators (Tepperman and Tepperman, 1958; Potter and Ono, 1961; Leveille and Hanson, 1966; Fabry et al., 1970; and Szepesi and Berdanier, 1971). In addition, the results of the present study support those of Allee et al. (1971) who demonstrated that the hyperlipogenic response to refeeding following dietary restriction was not due to the presence of glucose per se in the refeeding diet. In other respects, however, the results of the present study do not support those of Allee et al. (1971). They found that the addition of protein to the refeeding diet suppressed the hyperlipogenic response to refeeding. This suppression was not as great as that observed when an isocaloric amount of fat was substituted for glucose in the refeeding diet and, in a second experiment, substitution of protein for glucose did not suppress the hyperlipogenic response to refeeding. The results of the effect of dietary protein on the hyperlipogenic response in their study are therefore inconclusive.

In the present study, both G-6-PDH and malic enzyme activities in the liver and adipose tissue of rats refed the PRO diet were significantly higher ($P<0.05$) than enzyme activities in rats refed LA, RSO, or SO diets (Appendix Tables V to VIII). Activities of both dehydrogenases also were significantly higher ($P<0.05$) in the fat pads of rats refed the PRO diet than in rats refed the

CO diet. Similarly, hepatic G-6-PDH activity of rats refed the PRO diet was significantly higher ($P<0.05$) than that of rats refed the CO diet and, although the differences in hepatic malic enzyme activity between the PRO- and CO- refed rats were not significant ($P>0.05$), malic enzyme activity in the PRO-refed rats tended to be higher than in the CO- refed rats. In fact, activities of the dehydrogenases in liver and fat pads were higher for rats refed the PRO diet than for those refed any of the other diets although differences between the PRO and FF diets were not significant. These results also disagree with those of Leveille (1967a) and Reynolds et al. (1971) who found a decrease in the activities of malic enzyme and G-6-PDH, respectively, when the level of dietary protein was increased.

On the other hand, the results of the present study support those of Potter and Ono (1961) and McDonald and Johnson (1965) who found that adequate amounts of both carbohydrate and protein were required for induction of G-6-PDH activity in the livers of starved-refed rats. Potter and Ono (1961) found that 18% dietary protein was the minimum required for enzyme "overshoot" in rats that had been starved for 2 days and that the level of protein required for maximum enzyme induction was 30% although no significant decrease in enzyme activity occurred until

dietary protein exceeded 60%. Thus the trend for higher enzyme activities when dietary protein was increased from 18% to 45% in the refeeding diet in the present study coincides with the observations by Potter and Ono (1961) since restriction for 8 days has been found comparable to starvation for 4 days (Watt, 1970). In fact it is possible that the 45% level of dietary protein used in the present study was very near the optimum required for maximum enzyme induction since it is reasonable to assume that a slightly higher level of dietary protein may be required for tissue replacement after the longer and probably more severe degree of starvation in the present study than that of Potter and Ono (1961). Even if this postulate were not true the level of 45% dietary protein was still within the range where Potter and Ono (1961) found that dietary protein did not significantly reduce the enzyme activity observed at the optimum level of 30% dietary protein.

The apparent discrepancy among experiments in the effect of dietary protein on enzyme induction may possibly be explained by differences in experimental procedures. The observations of Leveille (1967a) and Reynolds et al. (1971) were made on non-starved animals that were meal-fed. In the present study and that of Potter and Ono (1961) the response to dietary protein was determined with animals that had been severely restricted

or starved. Thus, although many of the enzymatic adaptations that occur with starvation-refeeding regimens are similar to those that occur in meal-fed animals, these two physiological states may result in very different responses to diet composition. The enzymatic adaptations that occur in meal-fed animals, for example, are indicative of an increase in the maximal capacity for lipogenesis and represent the increased efficiency with which meal-fed animals store energy in the form of fat (Leveille, 1972). An emaciated animal, on the other hand, is not only required to handle the food presented to him in an efficient manner but must replace almost completely its fat stores as well as synthesize new protein required for enzymes and body tissues which were depleted during starvation. The fact that other body tissues as well as fat stores must be replenished is illustrated by the data in Table VI which shows that restriction to 5 g daily of the FF diet for 8 days resulted in a considerable loss in body weight (chow vs. restricted controls). Restricted animals had smaller epididymal fat pads and livers than non-restricted controls (Tables VII to IX). The fact that the proportional decrease in liver size (g liver /100g body weight) was less pronounced than the actual decrease in liver weight (liver wt.; chow vs. restricted controls,

TABLE VI

Body weights of restricted-refed rats

Dietary Regimen	Total Body Weight (g)			
	Controls[1]	Restricted-Refed[2]		
		3	5	7
Chow	391±37			
Restricted	178±18			
Fat-free	252±15	202±10	215±21	222±20
Coconut oil	257±12	196±15	201±17	217±4
Lard	258±14	192±20	223± 7	222± 6
Safflower oil	249± 4	193±20	216±28	231±19
Rapeseed oil	189±14	185±22	219±22	209±15
High protein	269±27	192±26	232±17	238±15

[1,2] See footnotes 2 and 3, Table II.

TABLE VII

Weight of epididymal adipose tissue relative to body weight in restricted-refed rats

Dietary Regimen	Relative Fat Pad Size (g fat pad /100g body weight)			
		Restricted-Refed[2]		
	Controls[1]	3	5	7
Chow	0.74±0.21			
Restricted	0.14±0.10			
Fat-free	0.64±0.16	0.35±0.12	0.40±0.08	0.71±0.23
Coconut oil	0.71±0.26	0.35±0.05	0.50±0.06	0.71±0.07
Lard	0.69±0.14	0.42±0.06	0.44±0.04	0.63±0.21
Safflower oil	0.67±0.10	0.29±0.04	0.47±0.09	0.73±0.12
Rapeseed oil	0.66±0.04	0.37±0.10	0.57±0.04	0.57±0.09
High protein	0.73±0.17	0.25±0.10	0.45±0.07	0.65±0.32

[1,2] See footnotes 2 and 3, Table II.

TABLE VIII

Weight of liver relative to body weight in restricted-refed rats

Dietary Regimen	Relative Liver Size (g liver /100 g body weight)			
	Controls[1]	Restricted-Refed[2] 3	5	7
Chow	3.94±0.37			
Restricted	2.83±0.16			
Fat-free	3.58±0.51	4.78±0.51	4.32±0.38	3.89±0.30
Coconut oil	3.49±0.31	4.00±0.24	3.92±0.10	3.45±0.13
Lard	3.38±0.34	3.73±0.23	3.45±0.30	3.46±0.27
Safflower oil	3.40±0.35	3.94±0.17	3.55±0.52	3.42±0.19
Rapeseed oil	3.64±0.34	3.72±0.56	3.76±0.38	3.37±0.14
High protein	3.77±0.21	4.79±0.13	4.15±0.52	3.98±0.40

[1,2] See footnotes 2 and 3, Table II.

TABLE IX

Liver weights of restricted-refed rats

Dietary Regimen	Total Liver Weight (g)			
		Restricted-Refed[2]		
	Controls[1]	3	5	7
Chow	15.37±1.70			
Restricted	5.01±0.24			
Fat-free	8.94±0.86	9.61±0.66	9.22±0.43	8.62±0.62
Coconut oil	8.93±0.58	8.03±0.37	7.86±0.62	7.40±0.24
Lard	8.70±0.96	7.15±0.37	7.67±0.53	7.70±0.78
Safflower oil	8.47±1.02	7.59±0.49	7.58±0.81	7.90±0.87
Rapeseed oil	9.18±0.79	7.13±0.47	8.16±0.07	7.04±0.38
High protein	10.14±1.14	10.07±1.53	9.57±0.58	9.43±0.45

[1,2] See footnotes 2 and 3, Table II.

Tables VIII and IX) suggests that, although liver proteins contributed to a large extent to gluconeogenesis during restriction, protein stores in other parts of the body also contributed to this process.

Thus, the very high dehydrogenase activities that occur upon refeeding, especially in the liver, also may occur in other parts of the body; especially the increase in G-6-PDH, the activity of which increases when new protein is synthesized (Mahler and Cordes, 1971). Increased malic enzyme activity may be more directly related to hepatic lipogenesis because the latter is more dependent on malic enzyme activity in periods of refeeding following restriction particularly if it is assumed that the NADPH generated by the increased activity of G-6-PDH is used for other biosynthetic processes.

The greater activity of malic enzyme in adipose tissue also may be explained in a similar manner. As outlined by Fabry et al. (1970), when a high carbohydrate diet is refed the adipocyte must synthesize new enzyme proteins related to the synthesis of triglycerides from carbohydrate precursors as well as synthesize those proteins required for cellular hypertrophy associated with the storage of increased amount of triglycerides. This would explain why G-6-PDH activity is higher in the adipose tissue of animals refed FF and PRO diets when compared to

those refed the fat-containing diets. In addition, the fact that less protein biosynthesis would probably occur in the adipocyte than in the hepatocyte during refeeding may explain why G-6-PDH activity is lower than malic enzyme activity in adipose tissue whereas in liver the reverse was observed. The results of the present study are similar to those reported by Young et al. (1964), Pande et al. (1964) and Watt (1970), who also found malic enzyme activity higher in adipose tissue than liver, and support the suggestion that adipose tissue could play a more important role than liver in lipogenesis in the rat, especially during refeeding.

As previously noted, malic enzyme activity in epididymal adipose tissue showed a more pronounced response to refeeding following dietary restriction, as well as greater variation in response to composition of the refeeding diet, than did G-6-PDH activity. In other words, malic enzyme activity increased to a greater extent relative to restricted controls than did G-6-PDH activity regardless of the composition of the refeeding diet.

Both hepatic enzymes increased to the same extent relative to restricted controls. These observations support the suggestion by Watt (1970) that different mechanisms may exist for the control of enzyme activity in liver and adipose tissue since the magnitude and pattern of response to the refeeding diet was different in the two tissues.

Although there tended to be specific trends in the pattern of metabolic response with time, the results of the present study suggest that the type of diet refed was more important in suppressing the enzymatic response which occurred in liver and adipose tissue during refeeding than the length of time the diet was refed. The effect of diet on G-6-PDH and malic enzyme activities in liver and adipose tissue was statistically significant ($P<0.05$) whereas the effect of time was not (Appendix Tables I to IV). Watt (1970) reported similar results in liver but found that the effects of both time and type of diet refed were statistically significant ($P<0.05$) for malic enzyme activity in adipose tissue. The apparent discrepancy between these two studies may be partially accounted for by the fact that Watt (1970) measured enzyme activities at 48, 72, 96, and 120 hours (2, 3, 4, and 5 days) whereas enzyme activities in the present study were measured at 3, 5, and 7 days. Szepesi

and Berdanier (1971) observed maximum values for G-6-PDH and malic enzyme activities 3 days after refeeding following a 48 hour fast. Watt (1970) may have measured enzyme activities during the time period when the greatest change in enzyme activities was taking place whereas activities in the present study had probably reached maximum values and were tending to plateau. Although the length of time rats were refed following restriction was not an important factor in the present study, hepatic dehydrogenase activities had begun to decline at 7 days refeeding whereas there was a trend toward increased dehydrogenase activies in epididymal adipose tissue at this time (Tables II to V; Figures 1 and 2). These findings support the suggestion by Jansen et al. (1968) that the mechanism involved in the hyperlipogenic response in liver during refeeding may not be the same as the mechanisms in extrahepatic tissues. In fact, the trend toward decreased hepatic enzyme activities in the present study suggests that much of the initial activity in liver following refeeding may be associated with the synthesis of new hepatic tissue other than lipid. This postulate is substantiated by the observation that dietary restriction resulted in a considerable decrease in relative liver size (liver wt. /100g body wt.) (Table VIII). In general, liver weight increased more rapidly than body weight during the initial

periods of refeeding. Although differences were small, the relative liver size at 3 and 5 days refeeding was greater than at 7 days refeeding. Thus changes in relative liver size tended to parallel changes in hepatic enzyme activities.

The fact that the decrease in hepatic enzyme activities with time was not statistically significant may be explained by the observation (Jansen et al., 1968) that the length of time required for hepatic lipogenesis to return to normal was directly proportional to the length of starvation. Since restriction of rats to 5g of diet daily for 8 days, as used in the present study, has been found equivalent to 4 days starvation (Watt, 1970), enzyme activity would be expected to require a longer time to return to normal than following 2 days starvation. In fact, according to Szepesi and Berdanier (1971), approximately 7 days were required before enzyme activities returned to normal in rats starved for 48 hours.

The trend towards increased activities of the dehydrogenases in epididymal adipose tissue is not as easily explained. Jansen et al. (1968) found that lipogenesis in epididymal adipose tissue returned to normal before that in hepatic tissue. This observation by Jansen et al. (1968) is in contrast with that of Watt (1970) and

the present study where a gradual increase in enzyme activities occurred between 3 and 7 days refeeding. No explanation can be offered for this apparent discrepancy among investigations. Thus further research is required to determine the significance of this observation.

There is some disagreement, as outlined in Section III.C.2., regarding the effect of the source of dietary fat on enzyme induction and lipogenesis although it is generally accepted that dietary fat will suppress enzyme induction during refeeding following starvation. For this reason, the effect of four different fat sources on enzyme activities was investigated in the present study. The fats included were: coconut oil, which contains a high level of medium chain saturated fatty acids; lard, a saturated animal fat; safflower oil, a vegetable oil containing a high level of polyunsaturated fatty acids; and rapeseed oil, which contains a very low level of saturated fatty acids and a high proportion of long chain monounsaturated fatty acids. The fatty acid composition of these fats is provided in Table X.

Comparison of dehydrogenase activities of rats refed diets containing 12% fat with the activities of rats refed the fat-free diets (FF and PRO diets) showed G-6-PDH in both liver and adipose tissue, and hepatic malic enzyme activities to be significantly lower ($P<0.05$) in rats refed the LA, SO, and RSO diets than in rats refed the FF and

TABLE X

Fatty acid composition of dietary fats

Fatty Acid %	Fat Source				
	Coconut Oil	Lard	Safflower Oil	Rapeseed Oil	Chow
8:0	6.44				0.45
10:0	6.63				0.68
12:0	34.42				0.41
14:0	16.69	8.59	0.16		2.40
14:1					0.27
16:0	11.51	22.09	7.03	3.42	23.66
16:1		4.11		0.20	
16:2					0.73
18:0	4.67	13.19	2.78	1.56	7.94
18:1	10.22	35.23	13.66	22.61	26.48
18:2	4.17	11.03	76.32	16.64	29.22
18:3				7.45	
20:0			0.63		
20:1			0.29	12.79	
20:5					2.45
22:1				34.72	

PRO diets. Malic enzyme activity in epididymal adipose tissue of rats refed the LA, SO, and RSO diets also was significantly lower than in rats refed the PRO diet. However, malic enzyme activity was not significantly lower ($P>0.05$) in rats refed the LA, SO and RSO diets than in rats refed the FF diet (Appendix Tables V to VIII). Activities of both dehydrogenases also were significantly lower ($P<0.05$) in the fat pads of rats refed the CO diet than in rats refed the PRO diet. Similarly, hepatic G-6-PDH activity in rats refed the CO diet was significantly lower ($P<0.05$) than that in rats refed the PRO diet and, although malic enzyme activities in the livers of the PRO- and CO- refed rats did not differ significantly ($P>0.05$), activities in the CO-refed rats tended to be lower than in the PRO- refed rats. No differences were observed between FF- and CO- refed rats in the activities of either dehydrogenase in liver or in adipose tissue.

The observations in adipose tissue give support to the suggestion that all long chain dietary fatty acids are effective in suppressing lipogenesis in the rat (Hill et al., 1958; Hill et al. 1960; Bottino et al., 1965; Dupont, 1966). There is some evidence, however, to support the suggestions of Inkpen et al. (1969) that linoleic acid, and Chu et al. (1969) that arachidonic and α- linolenic acids as well as linoleic acid, play a

regulatory role in hepatic G-6-PDH activity.

The results of the present study suggest that source of dietary fat may appreciably influence the pattern of metabolic response to refeeding in the liver. Fats rich in polyunsaturated fatty acids appear particularly effective in suppressing induction of dehydrogenase enzymes and perhaps hyperlipogenesis in response to refeeding. On the other hand, the medium chain saturated fatty acids of coconut oil appear to be less effective in suppressing enzyme induction during refeeding following dietary restriction. Furthermore, although dietary fat containing a large proportion of linoleic acid appeared to be somewhat more effective in suppressing hepatic dehydrogenase activity, especially G-6-PDH, all dietary fats appeared similar in their ability to suppress enzyme induction in adipose tissue.

B. FATTY ACID COMPOSITION OF LIVER LIPIDS

The fatty acid composition of lipid from the livers of rats fed the various diets is given in Tables XI to XIV. Dietary restriction had little effect on the fatty acid composition of total liver lipid (Table XI). There was, however, a slight decrease in the proportion of linoleic acid as a result of dietary restriction. A

TABLE XI

Effect of diet on major fatty acids in total lipid fraction of rat liver

Dietary Regimen	Fatty Acid %[1]								
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	20:3	20:4
Fat-free			24.5 ±1.5	3.9 ±0.4	17.1 ±1.0	17.7 ±1.1	13.3 ±1.1	1.0 ±0.0	18.7 ±1.2
Coconut oil		2.7 ±0.7	24.6 ±2.8	2.9 ±0.9	16.5 ±1.4	19.6 ±1.8	16.3 ±5.2		12.5 ±2.6
Lard			22.0 ±1.1	2.3 ±1.5	16.1 ±2.7	24.4 ±3.2	15.1 ±2.0		15.5 ±1.5
Safflower oil			17.7 ±0.8	0.7 ±0.2	17.1 ±2.6	8.9 ±1.2	31.0 ±5.9		20.0 ±4.1
Rapeseed oil			16.5 ±1.6	1.1 ±0.3	14.2 ±1.1	16.8 ±10.5	25.3 ±10.4		16.7 ±2.8
High protein			23.8 ±4.3	4.6 ±0.6	19.7 ±5.7	18.2 ±2.5	11.5 ±2.5	3.3[2]	10.4 ±6.5
Chow			20.2 ±2.2	1.2 ±0.5	21.2 ±2.8	12.1 ±1.0	18.6 ±0.7		18.5 ±1.3
Restricted			19.1 ±0.9	1.8 ±1.2	18.7 ±2.1	12.9 ±1.8	15.8 ±1.9		24.2 ±1.9

[1] Each value represents the mean ± S.D. for 3 rats fed lab chow ad libitum for 8 days, restricted to 5g daily of fat-free diet for 8 days, or fed 20g daily of experimental diet for 8 days.

[2] Present in 1 of 3 rats.

TABLE XII

Effect of refeeding for 3 days following diet restriction on major fatty acids in total lipid fraction of rat liver

Dietary Regimen	Fatty Acid %[1]								
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	20:3	20:4
Fat-free			25.0 ±9.0	18.2 ±5.6	14.1 ±4.5	22.8 ±7.2	5.4 ±1.7		5.6 ±2.3
Coconut oil	3.6 ±3.3	5.9 ±2.3	36.8 ±0.5	10.6 ±1.9	6.9 ±1.7	26.3 ±1.5	3.3 ±1.4		5.5 ±2.5
Lard		1.8 ±0.6	31.2 ±6.7	7.8 ±3.0	11.1 ±5.4	31.6 ±8.2	5.2 ±3.7		10.5 ±6.0
Safflower oil			26.5 ±2.9	3.9 ±2.5	9.9 ±1.8	15.7 ±2.2	26.5 ±7.2		11.1 ±3.7
Rapeseed oil			25.6 ±5.3	4.1 ±1.9	12.3 ±5.4	26.9 ±5.9	12.0 ±3.1		9.5 ±5.1
High protein			32.2 ±3.0	10.0 ±2.8	7.3 ±2.7	35.7 ±5.1	3.3 ±1.1	2.3[2] ±0.9	2.7 ±1.7

Controls: See Table XI.

1 Each value represents the mean ± S.D. of 3 rats restricted to 5g daily of fat-free diet for 8 days, and refed 20g daily of experimental diet.

2 Present in 2 of 3 rats.

TABLE XIII

Effect of refeeding for 5 days following diet restriction on major fatty acids in total lipid fraction of rat liver

Dietary Regimen	Fatty Acid %[1]								
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	20:3	20:4
Fat-free		2.4 ±1.5	36.6 ±7.3	13.0 ±2.0	9.9 ±6.2	30.0 ±9.9	2.1 ±1.8		2.7 ±2.2
Coconut oil	2.5 ±1.8	4.4 ±1.4	31.0 ±6.1	8.3 ±1.2	11.5 ±3.1	24.4 ±4.2	6.0 ±3.3		7.4 ±4.5
Lard			23.3 ±1.7	4.1 ±0.4	16.6 ±0.8	23.9 ±3.4	10.0 ±1.4		15.9 ±1.3
Safflower oil		1.3 ±0.2	19.5 ±0.2	1.5 ±0.6	17.5 ±2.4	7.9 ±0.9	26.2 ±2.6		22.4 ±0.6
Rapeseed oil			27.3 ±6.3	4.5 ±1.3	10.1 ±3.5	28.1 ±6.1	9.9 ±2.8		8.9 ±4.4
High protein			27.3 ±7.5	7.3 ±2.2	14.8 ±5.0	29.1 ±7.9	4.9 ±3.1	5.1 ±3.1	7.6 ±4.6

Controls: See Table XI.

[1] See footnote 1, Table XII.

TABLE XIV

Effect of refeeding for 7 days following diet restriction on major fatty acids in total lipid fraction of rat liver

Dietary Regimen	Fatty Acid %								
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	20:3	20:4
Fat-free			33.7 ±0.5	10.2 ±0.7	9.7 ±0.9	31.4 ±3.0	3.6 ±1.0	3.0 ±0.8	5.7 ±1.3
Coconut oil	2.3 ±0.7	3.9 ±1.6	27.3 ±2.2	7.0 ±1.9	15.4 ±5.6	20.3 ±3.4	7.7 ±1.6	1.2 ±0.3	10.6 ±4.0
Lard			22.7 ±3.2	3.6 ±1.2	16.4 ±2.7	23.1 ±4.7	9.7 ±0.5	1.3 ±0.2	16.6 ±3.9
Safflower oil			20.2 ±4.7	1.3 ±1.0	17.7 ±3.1	8.3 ±2.8	23.6 ±5.9		22.4 ±3.6
Rapeseed oil			21.0 ±1.5	2.5 ±1.0	15.4 ±3.8	22.5 ±2.7	12.9 ±1.8		14.9 ±2.4
High protein			22.8 ±4.1	7.5 ±0.6	17.3 ±2.5	25.1 ±5.6	5.9 ±1.7	7.4 ±2.4	8.4 ±3.1

Controls: See Table XI.

[1] See footnote 1, Table XII.

similar observation was reported by Allmann et al. (1965) in rats as a result of a forty-eight hour fast.

Refeeding the fat-free diets following dietary restriction effected a marked change in liver fatty acid composition (Tables XII to XIV). An appreciable decrease in the proportions of linoleic and arachidonic acids occurred in rats refed the FF and PRO diets. Furthermore, eicosatrienoic acid was found in the livers of rats refed the FF and PRO diets. The levels of eicosatrienoic acid in liver appear to be directly related to the length of time the fat-free diets were refed. Furthermore, the levels of eicosatrienoic acid also appear to be related to the magnitude of dehydrogenase response which, in turn, appears to be related to the type of diet refed. Rats refed the PRO diet, which was found to produce the highest G-6-PDH and malic enzyme activities, also showed the largest accumulation of eicosatrienoic acid in liver lipid. In addition, refeeding a fat-free diet resulted in a large increase in palmitoleic and oleic acids. Eicosatrienoic acid also was found in hepatic lipid of all rats refed the CO and LA diets for 7 days. In fact, the fatty acid composition of liver lipid from rats refed the CO diet tended to coincide with the higher G-6-PDH activity observed for rats refed this diet compared to rats refed the SO diet. Hepatic malic enzyme activity also was significantly higher

($P<0.05$) for rats refed the CO diet than for rats refed the SO or RSO diets. Tepperman and Tepperman (1965) also found that hepatic dehydrogenase activities were higher in rats refed a CO diet than in rats refed a corn oil diet. However, hepatic lipogenesis in animals refed the CO diet was not elevated over that in animals refed the corn oil diet. On the other hand, the results of the present study suggest that both hepatic lipogenesis (on the basis of liver lipid concentrations - Table XV) and dehydrogenase activities tended to be elevated in rats refed the CO diet.

There were no appreciable decreases in linoleic and arachidonic acids in hepatic lipid of rats refed SO and RSO diets. In fact, linoleic and arachidonic acid levels in the liver lipid of rats refed the SO diet actually increased over the levels found in the liver lipid of chow-fed controls. These high levels of linoleic and arachidonic acids may account, in part, for the lower activities of hepatic dehydrogenases in rats refed the SO diet. In rats refed the RSO diet, the proportions of linoleic and arachidonic acids were only slightly lower than in restricted or chow-fed controls. Moreover, eicosatrienoic acid was not present in the livers of animals refed the SO or RSO diets.

The results of this study appear to confirm and

TABLE XV

Liver lipid concentration in restricted-refed rats

Dietary Regimen	Liver Lipid Concentration (mg lipid /g liver)			
		Restricted - Refed[2]		
	Controls[1]	3	5	7
Chow	23.8±3.2			
Restricted	28.7±0.0			
Fat-free	41.2±3.2	125.8±25.7	115.7±24.1	60.4±11.4
Coconut oil	39.0±8.9	87.7±27.9	51.7±13.4	46.1± 9.5
Lard	36.2±5.5	54.9±30.5	34.4± 7.1	37.2±0.0
Safflower oil	49.6±8.4	51.8± 6.3	28.7± 0.0	40.8± 1.0
Rapeseed oil	45.9±3.2	55.8±31.8	54.3±22.6	53.6± 8.4
High protein	23.1±6.3	77.7±24.3	29.7±11.9	28.5± 7.7

1,2 See footnotes 2 and 3, Table II.

extend those of Allmann et al. (1965). The appearance of eicosatrienoic acid in the livers of rats refed fat-free diets supports the suggestion that the pattern of changes observed in early linoleic acid deficiency appear to be the same as those accompanying fat-free refeeding. Furthermore, the results of the present study are not subject to the criticism that has been directed at the results of Allmann et al. (1965) (Rogers, 1971). In the present study C22 polyunsaturates, as well as fairly high levels of arachidonic acid, were found in the livers of control animals. The very long chain polyunsaturated fatty acids, such as C22 polyunsaturates, are reported for control animals only in the present study. These fatty acids were present in the liver lipids of rats fed the other diets but they were not measured due to the length of time required to resolve these fatty acids because isothermal conditions were used in the GC analyses carried out in this experiment. In addition, unless the sample injected was reasonably large these peaks were rather badly spread and difficult to quantitate. Thus most chromatograms were terminated with the appearance of arachidonic acid. All fatty acid composition tables were calculated assuming that 100% of the fatty acids had been resolved with the appearance of arachidonic acid so that the fatty acid composition of control rats would be

comparable to that of experimental rats.

As noted earlier, the results of the present study also give some support to the suggestion of Chu et al. (1969) that hepatic linoleate and arachidonate concentrations may have a special regulatory effect on hepatic G-6-PDH activity. Hepatic dehydrogenase activities were lowest in rats refed the SO diet. In addition, the highest levels of linoleic and arachidonic acids occurred in the liver lipid of these rats. It appears, therefore, that higher linoleate and arachidonate concentrations may be important in suppressing hepatic dehydrogenase activity.

C. BLOOD GLUCOSE AND SERUM LIPID LEVELS

There was no evidence in the present study to suggest that glucose tolerance was affected by dietary restriction followed by refeeding. Dietary restriction appeared to have a slight depressing effect on blood glucose levels (Table XVI) but upon refeeding, blood glucose levels generally returned to normal. The type of diet refed did not appear to have any effect on blood glucose levels. These results would be expected when the importance of maintaining adequate blood glucose levels is considered.

TABLE XVI

Effect of refeeding following diet restriction on blood glucose levels

Diet Refed	Blood Glucose mg% Days Refed 3	5
Fat-free	137.8±14.8[1]	181.0±27.1[2]
Coconut oil	132.3±23.5	150.5±19.0[3]
Lard	129.3±19.4	153.8±11.1[3]
Safflower oil	150.0±15.8	159.5±18.6[2]
Rapeseed oil	137.6±12.4	164.2±33.8[3]
High protein	117.4±17.6	152.7±11.1[3]

Controls[1]	
Fed chow, ad libitum, 8 days	174.5±49.3
Restricted, 5 g FF/day, 8 days	118.4±4.8

1 Mean ± S.D. of 4 rats.

2 Mean ± S.D. of 2 rats.

3 Mean ± S.D. of 3 rats.

Dietary restriction followed by refeeding also had little effect on serum lipids (Tables XVII and XVIII). Serum cholesterol levels appear somewhat lower in restricted animals but because of the marked variation among rats within treatment groups no differences were found among treatments.

Dietary restriction appeared to have a depressing effect on serum free fatty acids. Short periods of starvation have been found to increase serum free fatty acids (Kerpel et al., 1971; Yeh and Leveille, 1970). Furthermore Kerpel et al. (1971) found that after 7 days starvation serum free fatty acids had returned to normal. Thus the very low levels observed for serum free fatty acids in restricted animals in the present study cannot be adequately explained. The method used for the determination of serum free fatty acids in the present study was not completely satisfactory as there was considerable variation between duplicate assays. Because of the small sample of blood available it was not possible to use another method. Thus further research is required to determine the effect of diet restriction followed by refeeding on serum free fatty acids.

Unfortunately not only were there limitations in the methods used for the determination of serum lipids but because of the difficulty in obtaining blood by means

TABLE XVII

Effect of refeeding following diet restriction on serum cholesterol levels

Diet Refed	Serum Cholesterol mg/100 ml serum	
	Days refed	
	3	5
Fat-free	90.0±17.6[1]	84.3± 3.0[2]
Coconut oil	88.5± 3.8	97.8±14.9[3]
Lard	95.5± 5.6	93.6± 5.1[3]
Safflower oil	108.6±12.0	87.0±35.6[2]
Rapeseed oil	110.0±16.4	85.0± 9.2[3]
High protein	90.3± 5.3	90.0±12.3[3]
Controls[1]		
Fed chow, ad libitum, 8 days		76.6±8.7
Restricted, 5 g FF/day, 8 days		61.5±15.6

1 Mean ±S.D. of 4 rats.
2 Mean ±S.D. of 2 rats.
3 Mean ±S.D. of 3 rats.

TABLE XVIII

Effect of refeeding following diet restriction on serum free fatty acids

Diet Refed	Free Fatty Acids mg/100 ml serum	
	Days Refed	
	3	5
Fat-free	48.0±27.8[1]	27.0±15.0[2]
Coconut oil	25.2± 4.6[1]	32.2±24.2[3]
Lard	97.3±10.6[2]	88.0± 8.7[3]
Safflower oil	88.0± 8.8[3]	65.7±26.6[2]
Rapeseed oil	57.4±22.5[1]	42.5± 6.8[3]
High protein	71.0± 8.8[1]	66.4± 6.2[3]
Controls[3]		
Fed chow, ad libitum, 8 days		74.9±26.3
Restricted, 5g FF/day, 8 days		8.8± 6.6

[1] Mean ± S.D. of 4 rats.
[2] Mean ± S.D. of 2 rats.
[3] Mean ± S.D. of 3 rats.

of a heart puncture, blood samples were missing in some treatment groups. Nevertheless, the results of the present study suggest that the type of diet refed appeared to have little effect on blood glucose, serum cholesterol, or serum free fatty acid levels in restricted-refed rats.

VII. SUMMARY

The present study investigated the effect of composition of the refeeding diet on the metabolic response to refeeding following severe dietary restriction. Male rats weighing 225-300 g when placed on experiment were restricted to 5g daily of a high carbohydrate, fat-free diet. The effect of the composition of the refeeding diet on the response of two NADP-linked dehydrogenases in liver and epididymal adipose tissue, and on changes in the fatty acid composition of liver was determined. Induction of the two NADP-linked dehydrogenases, G-6-PDH and malic enzyme was greatest in both liver and adipose tissue of rats refed fat-free diets irrespective of whether the diets were high in carbohydrate (FF diet) or protein (PRO diet). In fact, the "overshoot" of these dehydrogenases was more marked in the PRO-refed rats which suggests that the response of these enzymes to the refeeding of a fat-free diet was not due to high levels of glucose per se in the refeeding diet.

The response of both enzymes to refeeding was more pronounced in liver than in adipose tissue. In addition, G-6-PDH activity in the liver was consistently higher than malic enzyme activity whereas, in adipose

tissue, the opposite prevailed. There was also a trend for enzyme activities to decrease by the 7th day of refeeding in hepatic tissue, but to continue to increase to this time in adipose tissue. These results suggest that different mechanisms may exist for the control of enzyme activity in liver and adipose tissue.

Refeeding diets in which 12% coconut oil, lard, rapeseed oil, or safflower oil isocalorically replaced glucose suppressed the marked "overshoot" in G-6-PDH and malic enzyme activities observed in response to the fat-free diets. Again pattern of response varied between the liver and adipose tissue. All dietary fats appeared similar in their ability to suppress the induction of these enzymes in adipose tissue whereas coconut oil did not suppress the induction of these enzymes in the liver. In addition, activities of these enzymes were lowest in the livers of rats refed the safflower oil diet. These results suggest that source of dietary fat may appreciably influence the pattern of metabolic response to refeeding in the liver.

Refeeding the fat-free diets effected a marked change in liver fatty acid composition. Eicosatrienoic acid was observed in the liver lipid of rats refed all diets except those containing rapseseed oil or safflower

oil as the source of dietary fat. The amount of eicosatrienoic acid in liver lipid appeared to be related to the "overshoot" in dehydrogenase activity suggesting that the pattern of change observed in early linoleic acid deficiency may be the same as that accompanying refeeding of diets low in essential fatty acids. These results also suggest that higher linoleate and arachidonate concentrations in liver lipid may be important in suppressing the characteristic "overshoot" observed in G-6-PDH and malic enzyme in rats refed fat-free diets.

There was no evidence on the present study to suggest that blood glucose, serum cholesterol, or serum free fatty acids were affected by dietary restriction or refeeding. The significance of these observations is, however, unclear at the present time.

The results of the present study suggest that diet markedly affects the metabolic response to realimentation following severe dietary restriction. Further research is required, to elucidate the manner in which diet composition affects the metabolic response herein investigated.

VIII. APPENDIX TABLES

APPENDIX TABLE I

Analysis of variance for malic enzyme activity in liver following log transformation of original data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance P<0.05
Total within treatment	85	10.5054	0.1235		
Between treatment					
1. Chow and restricted vs. experimental	1	2.5832	2.5832	20.9165	S[1]
2. Chow vs restricted	1	0.0039	0.0039	0.0315	NS[2]
3. Not restricted vs. restricted	1	2.8153	2.8153	22.7959	S
4. Diets	5	4.6203	0.9240	7.4817	S
5. Time	2	0.0552	0.0276	0.2234	NS
6. Interactions					
Diet x not restricted vs. restricted	5	0.1661	0.0332	0.2688	NS
Diet x time	10	0.2614	0.0261	0.2113	NS

[1] significant

[2] not significant

APPENDIX TABLE II

Analysis of variance for G-6-PDH activity in liver following log transformation of original data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance P<0.05
Total within treatment	85	12.1960	0.1434		
Between treatment					
1. Chow and restricted vs. experimental	1	2.3917	2.3917	16.6785	S[1]
2. Chow vs. restricted	1	0.0004	0.0004	0.0027	NS[2]
3. Not restricted vs restricted	1	3.6575	3.6575	25.5055	
4. Diets	5	5.6344	1.1268	7.8577	S
5. Time	2	0.0914	0.0457	0.3186	NS
6. Interactions					
Diet x not restricted vs. restricted	5	0.2375	0.0475	0.3312	NS
Diet x time	10	0.1831	0.0183	0.1276	NS

[1] significant

[2] not significant

APPENDIX TABLE III

Analysis of variance for enzyme activity in epididymal adipose tissue following log transformation of original data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance P<0.05
Total within treatment	85	8.9704	0.1055		
Between treatment					
1. Chow and restricted vs. experimental	1	2.8840	2.8840	27.3364	S[1]
2. Chow vs. restricted	1	0.2753	0.2753	2.6094	NS[2]
3. Not restricted vs restricted	1	2.4196	2.4196	22.9345	S
4. Diets	5	2.5300	0.5060	4.7962	S
5. Time	2	0.3173	0.1586	1.5033	NS
6. Interactions					
Diet x not restricted vs. restricted	5	0.2409	0.0481	0.4559	NS
Diet x time	10	0.3033	0.0303	0.2872	NS

[1] significant

[2] not significant

APPENDIX TABLE IV

Analysis of variance for G-6-PDH in epididymal adipose tissue following log transformation of original data

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Significance P<0.05
Total within treatment	85	3.8928	0.0457		
Between treatment					
1. Chow and restricted vs. experimental	1	1.0143	1.0143	22.1947	S[1]
2. Chow vs. restricted	1	0.0781	0.0781	1.7089	NS[2]
3. Not restricted vs restricted	1	1.3854	1.3854	30.3150	S
4. Diets	5	1.1071	0.2214	4.9037	S
5. Time	2	0.1315	0.0657	1.4376	NS
6. Interactions					
Diet x not restricted vs. restricted	5	0.0467	0.0093	0.2035	NS
Diet x time	10	0.1297	0.0129	0.2822	NS

[1] significant

[2] not significant

APPENDIX TABLE V

Duncan's Multiple Range Test for liver malic enzyme activity following log transformation of original data[1]

High protein	Fat-free	Coconut oil	Lard	Rapeseed oil	Safflower oil
2.1653	2.1027	1.9307	1.6581	1.6342	1.5286

SE = 0.0974

APPENDIX TABLE VI

Duncan's Multiple Range Test for liver G-6-PDH activity following log transformation of original data[1]

High protein	Fat-free	Coconut oil	Rapeseed oil	Lard	Safflower oil
2.4849	2.2889	2.0972	1.9038	1.8737	1.6857

SE = 0.1048

[1] All values underscored by the same line are not significantly different.

P	2	3	4	5	6
rp(5%)	2.82	2.97	3.07	3.13	3.19
Rp(ME)	0.2746	0.2892	0.2990	0.3048	0.3107
Rp(G-6-PDH)	0.2955	0.3112	0.3217	0.3280	0.3343

APPENDIX TABLE VII

Duncan's Multiple Range Test for malic enzyme activity in epididymal adipose tissue following log transformation of original data[1]

High protein	Fat-free	Coconut oil	Lard	Rapeseed oil	Safflower oil
3.0114	2.7868	2.6792	2.5363	2.5255	2.5097

SE = 0.0900

APPENDIX TABLE VIII

Duncan's Multiple Range Test for G-6-PDH activity in epididymal adipose tissue following log transformation of original data[1]

High protein	Fat-free	Coconut oil	Rapeseed oil	Lard	Safflower oil
2.5371	2.4399	2.3188	2.2470	2.2359	2.2099

SE = 0.0591

[1] All values underscored by the same line are not significantly different.

P	2	3	4	5	6
rp(5%)	2.82	2.97	3.07	3.13	3.19
Rp(ME)	0.2538	0.2673	0.2763	0.2817	0.2871
Rp(G-6-PDH)	0.1666	0.1755	0.1814	0.1849	0.1885

IX. BIBLIOGRAPHY

Allee, G.L., O'Hea, E.K., Leveille, G.A. and Baker D.H. 1971. Influence of dietary protein and fat on lipogenesis and enzyme activity in pig adipose tissue. J. Nutr. 101: 869-878.

Allee, G.L., Romsos, D.R., Leveille, G.A., and Baker, G.H. 1972. Lipogenesis and enzymatic activity in pig adipose tissue as influenced by source of dietary fat. J. Anim. Sci. 35: 41-46.

Allmann, D.W., Hubbard, D.D. and Gibson, D.M. 1965 Fatty acid synthesis during fat-free refeeding of starved rats. J. Lipid Res. 6: 63.74.

Bligh, E.G., and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J Biochem. Physiol. 37: 911-917.

Blumenthal, M.D., Abraham, S., and Chaikoff, I.L. 1964. Dietary control of liver glucokinase activity in the normal rat. Arch. Biochem. Biophys. 104: 215-224.

Bottino, N.R., Anderson, R.E., and Reiser, R. 1965. Dietary fatty acids: their metabolic fate and influence on fatty acid biosynthesis. J. Amer. Oil Chem. Soc. 42: 1124-1129.

Braun, T., Vrana, A., and Fabry, P. 1967. Enhanced hypoglycemic effect of exogenous insulin associated with an increased response of adipose tissue and a diminished response of the diaphragm in meal-fed rats. Experientia 23: 468-470.

Chu, L.C., McIntosh, D.J., Hincenbergs, I., and Williams, M.A. 1969. Dietary unsaturated fatty acids and liver fatty acid synthetase in rats. Biochim. Biophys. Acta 187: 573-575.

Curry, D.L., 1970. Factors affecting insulin secretion in vitro. Amer. J. Clin. Nutr. 23: 305-310.

Dupont, J. 1966. Synthesis of cholesterol and total lipid by male and female rats fed beef tallow or corn oil. Lipids 1: 409-414.

Fabry, P., Kleinfield, R., Tepperman, H.M. and Tepperman, J. 1970. Effect of diet and insulin on the morphology and TPNH generating enzyme activities of rat adipose tissue (34521). Proc. Soc. Exp. Biol. Med. 133: 577-581.

Flatt, J.P., and Ball, E.G. 1964. Studies on the metabolism of adipose tissue. XV. An evaluation of the major pathways of glucose catabolism as influenced by insulin and epinephrine. J. Biol. Chem. 239: 675-685.

Foster, D.W., and Srere, P.A. 1968. Citrate cleavage enzyme and fatty acid synthesis. J. Biol. Chem. 243: 1926-1930

Freedland, R.A. 1967. Effect of progressive starvation on rat liver enzyme activities. J. Nutr. 91: 489-495.

Hill, R., Linazasoro, J.M., Chevallier, F., and Chaikoff, I.L. 1958. Regulation of hepatic lipogenesis: The influence of dietary fats. J. Biol. Chem. 233: 305-310.

Hill, R., Webster, W.W. Linazasoro, J.M., and Chaikoff, I.L. 1960. Time of occurrence of changes in the liver's capacity to utilize acetate for fatty acid and cholesterol synthesis after fat feeding. J. Lipid. Res. 1: 150-153.

Inkpen, C.A., Harris, R.A. and Quackenbush, F.W. 1969. Differential responses to fasting and subsequent feeding by microsomal systems of rat liver: 6- and 9- desaturation of fatty acids. J. Lipid Res. 10: 277-282.

Jansen, G.R., Hutchison, C.F., and Zanetti, M.E. 1966. Studies on lipogenesis in vivo. Effect of dietary fat or starvation on conversion of ^{14}C glucose into fat and turnover of newly synthesized fat. Biochem. J. 99: 323-332.

Jansen, G.R., Zanetti, M.E., and Hutchison, C.F. 1967. Studies on lipogenesis in vivo. Comparison of cholesterol and fatty acid synthesis in rats and mice. Biochem. J. 102: 864-869.

Jansen, G.R., Zanetti, M.E., and Hutchison, C.F. 1968. Studies on lipogenesis in vivo. Lipogenesis during extended periods of refeeding after starvation. Biochem. J. 106: 345-353.

Jomain, M., and Hanson, R.W. 1969. Dietary protein and the control of fatty acid synthesis in rat adipose tissue. J. Lipid Res. 10: 674-680.

Kerpel, S., Rubenstein, B., and Rubinstein, D. 1971. The effect of prolonged fasting and of glucose refeeding on rat serum and liver lipid levels. Lipids 6: 332-340.

Kornacker, M.S., and Lowenstein, J.M. 1965. Citrate and the conversion of carbohydrate into fat. The activity of citrate-cleavage enzyme and acetate thiokinase in livers of starved and refed rats. Biochem. J. 94: 209-215.

Kritchevsky, D. and Tepper, S.A. 1971. Influence of isocaloric, isogravic diets on serum and liver lipids in rats. Nutrition Reports International 3: 283-289.

Kwan, S.W., and Webb, T.E. 1967. A study of the mechanism of polyribosome breakdown induced in regenerating liver by 8- azaguanine. J. Biol. Chem. 242: 5542-5548.

Leveille, G.A. 1966. Glycogen metabolism in meal-fed rats and chicks and the time of lipogenic and enzymatic adaptive changes. J. Nutr. 90: 449-460.

Leveille, G.A. 1967 a. Influence of dietary fat and protein on metabolic and enzymatic activities in adipose tissue of meal-fed rats. J. Nutr. 91: 25-34.

Leveille, G.A. 1967 b. Influence of dietary fat level on the enzymatic and lipogenic adaptations in adipose tissue of meal-fed rats. J. Nutr. 91: 267-274.

Leveille, G.A. 1969. In vivo fatty acid and cholesterol synthesis in fasted and fasted - refed chicks. J. Nutr. 98: 367-372.

Leveille, G.A. 1970. Adipose tissue metabolism : influence of periodicity of eating and diet composition. Fed. Proc. 29: 1294-1301.

Leveille, G.A. 1972. Lipogenic adaptations related to pattern of food intake. Nutr. Rev. 30: 151-155.

Lohr, G.W., and Waller, H.D. 1963. Methods in Enzymatic Analyses. H.W. Bergmeyer, Editor. Acad. Press, N.Y. p. 744-751.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Mahler, H.R., and Cordes, E.H. 1971. Biological Chemistry. Harper and Row, New York. p. 535.

Masoro, E.J., Korchak, H.M., and Porter, E. 1962. A study of the lipogenic inhibitory mechaniasm induced by fasting. Biochim. Biophys. Acta 58: 407-416.

McDonald, B.E., and Johnson, B.C. 1965. Metabolic response to realimentation following chronic starvation in the adult male rat. J. Nutr. 87: 161-167.

Metcalfe, L.D., Schmitz, A.A., and Pelka, J.R. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Analytical Chem. 38: 514-515.

Morse, W.J., Sidorov, J.J., Soeldner, S., and Dickson, R.C. 1960. Observations on carbohydrate metabolism in obesity. Metabolism 9: 666-679.

Mosinger, F. 1965. Photometric adaptation of Dole's microdetermination of free fatty acids. J. Lipid Res. 6: 157-159.

Ochoa, S. 1955. Malic enzyme from pigeon liver and wheat germ. In Methods in Enzymology. S.P. Colowick and N.O. Kaplan (eds.). Acad. Press, N.Y., Vol. 1, p.739-748.

Oliver, A.K., and McDonald, B.E. 1967. Effect of diet composition on hepatic NADP-linked dehydrogenase and fatty acid synthesis. Fed. Proc. 26: 637(abstr.)

Pallotta, J.A., and Kennedy, P.J. 1968. Response of plasma insulin and growth hormone to carbohydrate and protein feeding. Metabolism 17: 901-908.

Pande, S.V., Khan, R.P., and Venkitasubramanian, T.A. 1964. Nicotinamide adenine dinucleotide phosphate - specific dehydrogenases in relation to lipogenesis. Biochim. Biophys. Acta. 84: 239-250.

Pearson, S., Stern, S., and McGavack, T.H. 1952. A rapid procedure for the determination of total cholesterol in serum. J. Clin. Endochrinol. and Metab. 12: 1245-1246.

Potter, V.R., and Ono, T. 1961. Enzyme patterns in the rat liver and morris hepatoma 5123 during metabolic transitions. Cold Spring Harbor Symposia Quantitative Biology 26: 355-362.

Raabo, E., and Terkildsen, T.C. 1960. On the enzymatic determination of blood glucose. Scand. J. Clin. Lab. Invest. 12: 402-407.

Reynolds, R.D., Potter, V.R., and Pitot, H.C. 1971. Response of several hepatic adaptive enzymes to a shift from low to high protein diet in intact and adrenalectomized rats. J. Nutr. 101: 797-802.

Rogers, C.G. 1971. Fatty acid composition of liver mitochondria and microsomes in fed and fasted rats. Nutrition Reports International 4: 351-362.

Sillero, A., Sillero, M.A.G., and Sols, A. 1969. Regulation of the level of key enzymes of glycolysis and gluconeogenesis in liver. European J. Biochem. 10: 351-354.

Snedecor, G.W., and Cochran, W.G. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa.

Stitchell, B. 1971. Effect of exogenous insulin and its relationship to carbohydrate intake in the metabolic response of starved-refed rats. (M. Sc. Thesis, University of Manitoba).

Szepesi, B., and Freedland, R.A. 1969. Differential requirement for de novo RNA synthesis in the starved-refed rat; inhibition of the overshoot by 8-azaquanine after refeeding. J. Nutr. 99: 449-458.

Szepesi, B., and Berdanier, C.D. 1970. Insulin and enzyme responses in the starved-refed rat. Fed. Proc. 29: 256 (Abstr.).

Szepesi, B., and Berdanier C.D. 1971. Time course of the starve-refeed response in rats; the possible role of insulin. J. Nutr . 101: 1563-1574.

Szepesi, B., and Moser, P. 1971. Persistence of increased inducibility of NADP-linked dehydrogenases in rat liver. Proc. Soc. Exp. Biol. Med. 136: 200-202.

Szepesi, B., Berdanier, C.D., Diachenko, S.K., and Moser, P.B. 1971a. Effect of length of starvation, refeeding, and 8- azaquanine on serum insulin and NADP-linked dehydrogenases of rat liver. J. Nutr. 101:1147-1152.

Szepesi, B., Berdanier, C.D., and Egawa, M. 1971b. Evidence for increased constitutiveness of NADP-linked dehydrogenases in the starved-refed rat. J. Nutr. 101: 863-868.

Szepesi, B., Vegors, R., and De Mouy, J.M. 1972. On the possible role of insulin in the starve-refeed response. Nutrition Reports International 5: 281-286.

Tepperman, H.M., and Tepperman, J. 1958. The hexose-monophosphate shunt and adaptive hyperlipogenesis. Diabetes 7: 478-485.

Tepperman, H.M. and Tepperman, J. 1964. Patterns of dietary and hormonal induction of certain NADP-linked liver enzymes. Amer. J. Physiol. 206: 357-361.

Tepperman, H.M. and Tepperman, J. 1965. Effect of saturated fat diets on rat liver NADP-linked enzymes. Am. J. Physiol. 209: 773-780.

Tepperman, H.M., De La Garza, S.A., and Tepperman, J. 1968. Effects of dehydroepiandrosterone and diet protein on liver enzymes and lipogenesis. Am. J. Physiol. 214: 1126-1132.

Tepperman, J., and Tepperman, H.M. 1970. Gluconeogenesis, lipogenesis, and the Sherringtonian metaphor. Fed. Proc. 29: 1284-1293.

Vaughan, D.A., and Winders, R.L. 1964. Effects of diet on HMP dehydrogenase and malic (TPN) dehydrogenase in the rat. Am. J. Physiol. 206: 1081-1084.

Watt, M. 1970. Effect of dietary fat and diet restriction versus inanition on the metabolic response of rats during refeeding. (M. Sc. Thesis. University of Manitoba).

Weber, G., Singhal, R.L., and Srivastava, S.K. 1965. Effect of nutritional state on hormonal regulation of liver enzymes. Can. J. Biochem. 43: 1549-1563.

Weber, G., Singhal, R.L., Stamm, N.B., Les, M.A., and Fisher, E.A. 1966. Synchronous behaviour pattern of key glycolytic enzymes: glucokinase, phosphofructokinase, and pyruvate kinase. In Advan. Enzyme Reg. G. Weber (ed.). Pergamon Press, Oxford, England. Vol 4, p. 49-81.

Williams, M.A., Tamai, K.T. McIntosh, D.J. 1967. Effects of fasting on liver lipids in rats fed a purified diet. Biochim. Biophys. Acta 137: 187-189.

Wise, E.M. and Ball, E.G. 1964. Malic enzyme and lipogenesis. Proc. Nat. Acad. Sci. U.S.A. 52: 1255-1263.

Yeh, Y.Y., and Leveille, G.A. 1970. Hepatic fatty acid synthesis and plasma free fatty acid levels in chicks subjected to short periods of food restriction and refeeding. J. Nutr. 100: 1389-1398.

Young, J.W. Shrago, E., and Lardy, H.A. 1964. Metabolic control of enzymes involved in lipogenesis and gluconeogenesis. Biochemistry 3: 1687-1692.