ABSTRACT

THE INFLUENCE OF DIETARY POLYUNSATURATED AND SATURATED FATTY ACIDS ON HEPATIC AND ADIPOSE TISSUE FATTY ACID SYNTHESIS IN THE MEAL-FED RAT

By

Steven Donald Clarke

The primary objective of this research was to evaluate the contention that low dietary levels of polyunsaturated fatty acids specifically depress the rates of liver and adipose tissue fatty acid synthesis and the activities of glucose-6-phosphate dehydrogenase (EC1.1.1.49), malic enzyme (EC1.1.1.40), citrate cleavage enzyme (EC4.1.3.8), acetyl-CoA carboxylase (EC6.4.1.2) and fatty acid synthetase in rats trained to consume their daily food during a three hour period each day. Pure methyl esters of palmitate ($C_{16:0}$), stearate ($C_{18:0}$), oleate ($C_{18:1}$), linoleate ($C_{18:2}$) and linolenate ($C_{18:3}$) were employed as supplements to either a fat-free, high carbohydrate diet or a low fat, essential fatty acid adequate diet. Constant intakes of basal diet were generally maintained among all treatments. $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ were determined to have apparent digestibilities of 88, 87 and 89%, respectively, and these were supplemented to the basal diet as 3% of the amount of food allotted daily to each rat. However the poor digestibilities

of $C_{16:0}$ and $C_{18:0}$ (40 and 35%) required that the level of supplementation be raised to 7 and 8%, respectively.

The first part of this investigation measured the response of hepatic and adipose fatty acid synthesis and the activities of lipogenic enzymes to the supplementation for 7-10 days of various fatty acids to either fat-free, or low fat, high carbohydrate diets. Dietary $C_{18:2}$ and/or $C_{18:3}$ resulted in a consistent and significant decline in the activities of hepatic glucose-6-phosphate dehydrogenase, malic enzyme, citrate cleavage enzyme, acetyl-CoA carboxylase and fatty acid synthetase. In contrast the additon of $C_{16:0}$, $C_{18:0}$, or $C_{18:1}$ to the basal diets had little suppressive action on the activities of these enzymes. Dietary polyunsaturated fatty acids were also associated with a 25% decline in the activities of hepatic pyruvate kinase (EC2.7.1.40) and glucokinase (EC2.7.1.2). Unlike the liver, adipose tissue glucose-6-phosphate dehydrogenase, malic enzyme and fatty acid synthetase activities remained unaffected by the addition of either saturated or unsaturated fatty acids to the diets. The in vivo incorporation of ^3H_2O into liver fatty acids was significantly reduced by nearly 40% among all experiments involving dietary $C_{18:2}$ and $C_{18:3}$. Like the enzyme activities, adipose tissue fatty acid synthesis was not depressed by dietary fatty acids of any type. These effects of $C_{18:2}$ and $C_{18:3}$ on hepatic lipogenesis after 7-10 days were not due to differences among treatments in carbohydrate intake or variations in absorbed fat, and were independent of essential fatty acid status.

The second phase of research was directed at quantitating the concentration and composition of plasma free fatty acids, the concentration of hepatic long chain acyl-CoA esters and the hepatic ratio of lactate and pyruvate in rats fed the fat-free diet for five to seven days with and without 3% $C_{18:2}$ or $C_{18:3}$. The liver tissue samples for determination of metabolites were collected by rapid freezing in situ using clamps precooled in liquid nitrogen. The concentration of these known effectors of hepatic fatty acid synthesis were not altered by dietary $C_{18:2}$ or $C_{18:3}$. The composition of plasma free fatty acids showed a fourfold rise in unesterified linoleate after $C_{18:2}$ feeding. If long chain acyl-CoA esters or free fatty acids play a role in regulating hepatic fatty acid synthesis, the action must be more dependent on the composition than the total concentration of acyl esters.

Because an animal very likely has reached a new steady-state after supplementing $C_{18:2}$ or $C_{18:3}$ for 7-10 days, accurate detection of the initial point of inhibition exerted by $C_{18:2}$ or $C_{18:3}$ in rat liver may be difficult. Therefore the characterization of changes in activities of certain key hepatic lipogenic and glycolytic enzymes as well as quantitative comparison to the rate of in vivo fatty acid synthesis during the attainment of a new steady-state was of particular importance.

The in vivo rate of C_2-unit incorporation into hepatic fatty acids, calculated from ^3H_2O incorporation, revealed that six to eight

meals of 3% $C_{18:2}$ supplementation led to maximal depression in fatty acid synthesis. Furthermore within a treatment group the in vitro activity of fatty acid synthetase and acetyl-CoA carboxylase was nearly identical to the in vivo rate of C_2-unit incorporation into hepatic fatty acids. Time sequence studies demonstrated that a minimum of three meals containing $C_{18:2}$ or $C_{18:3}$ or about a 48 hour time span was essential before a significant decline in the rate of hepatic fatty acid synthesis was detectable. The consumption of four $C_{18:2}$ containing meals resulted in a degree of depression in fatty acid synthesis that was very similar to that found after six meals of $C_{18:2}$. However the activity of fatty acid synthetase in vitro was not significantly reduced by three or four meals of $C_{18:2}$ addition, and it was well above the in vivo rate of C_2-unit incorporation into fatty acids. Glucokinase activity also remained high during the transitional period and generally its activity was not reduced by $C_{18:2}$.

Low dietary levels of polyunsaturated fatty acids very effectively reduce the rate of rat liver fatty acid synthesis as well as activities of many lipogenic enzymes. The initial point of inhibition of hepatic fatty acid synthesis exerted by polyunsaturated fats would appear to be after glucose phosphorylation and prior to malonyl-CoA utilization.

THE INFLUENCE OF DIETARY POLUNSATURATED AND SATURATED FATTY ACIDS ON HEPATIC AND ADIPOSE TISSUE FATTY ACID SYNTHESIS IN THE MEAL-FED RAT

BY

STEVEN DONALD CLARKE

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science
and Human Nutrition

1976

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Dale Romsos, Associate Professor, for his guidance and support in the development of the research topic. In addition the suggestions and advice of Dr. G. A. Leveille, Chairman of the Department of Food Science, Dr. M. R. Bennink, Assistant Professor, and Dr. W. G. Bergen, Associate Professor, during the preparation of this manuscript, were greatly appreciated.

My wife, Karen, and daughter, Kimberly, deserve special thanks for their unending patience, help and understanding during my graduate program.

TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd.)

TABLE OF CONTENTS (cont'd.)

TABLE OF CONTENTS (cont'd.)

LIST OF TABLES

LIST OF TABLES (cont'd.)

LIST OF TABLES (cont'd.)

Table Page

PART V
CHANGE IN HEPATIC FATTY ACID SYNTHESIS IN MEAL-FED RATS DURING SEVEN DAYS OF FEEDING POLYUNSATURATED FATTY ACIDS

LIST OF ABBREVIATIONS

AcCBX	acetyl-CoA carboxylase
ADP	adenine diphosphate
AMP	adenine monophosphate
ATP	adenine triphosphate
BHT	butylated hydroxytoluene
$C_{16:0}$	palmitate
$C_{16:1}$	palmitoleate
$C_{18:0}$	stearate
$C_{18:1}$	oleate
$C_{18:2}$	linoleate
$C_{18:3}$	linolenate
$C_{20:3}$	eicosatrienoate
$C_{20:4}$	arachidonate
cAMP	cyclic adenine monophosphate
CCE	citrate cleavage enzyme
CO_2	carbon dioxide
CoA	coenzyme A
CV	coefficient of variation
FAS	fatty acid synthetase
FF	fat-free
F6P	fructose-6-phosphate

LIST OF ABBREVIATIONS (cont'd.)

FDP	fructose-1,6-diphosphate
G6PD	glucose-6-phosphate dehydrogenase
HCO_3	bicarbonate
^3H_2O	tritiated water
IP	intraperitoneal
kg	kilogram
ME	malic enzyme
mg	milligram
Mg^{++}	magnesium
min	minute
mM	millimolar
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide, reduced
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate, reduced
PDH	pyruvate dehydrogenase
PFK	phosphofructokinase
PG	prostaglandins
PK	pyruvate kinase
µg	micrograms
µM	micromolar

INTRODUCTION

An elevation in blood triglycerides and cholesterol in humans has received wide acceptance as a warning sign for cardiovascular problems. One approach to controlling hypertriglyceridemia has been the dietary manipulation of reducing the proportion of saturated fat (e.g. butter, beef tallow, lard) and increasing the amount of polyunsaturated fat (e.g. corn oil, safflower oil) (1). The validity of this approach has not been universally accepted and the data are not all supportive (2-5). For example an isolcaloric exchange of safflower oil or corn oil for butter fat in diets of human subjects resulted in a significant drop in plasma cholesterol and triglycerides (3). On the other hand, subjects that ate a low fat diet displayed no reduction in blood low density lipoproteins when fed additional ethyl linoleate or stearate (6).

Attempts have been made at attributing a mechanism of action to polyunsaturated fats by utilizing rats and mice as experimental models (7-10). Initial observations with high fat diets indicated polyunsaturated fats suppressed liver and adipose tissue fatty acid synthesis with greater efficacy than did saturated fats. Such an effect in man, whose major site of de novo fatty acid synthesis is the liver, would have significant implications. A reduction in the amount of de novo fat synthesis in liver would potentially lead to less

transport of triglyceride via blood to peripheral tissues and hence a lowered blood triglyceride concentration.

Because of high dietary fat levels and because of variations in fatty acid composition of dietary fats, earlier studies were unable to explain the mechanism by which polyunsaturated fats affected rates of lipogenesis. One method adopted to avoid these difficulties was to supplement a fat-free diet of mice and rats with low levels of pure esters of individual fatty acids (7-10). However specific differences in their action on liver and adipose lipogenesis attributed to individual fatty acids were overshadowed by the following oversights: a) variation among experiments and treatments in the type and amount of carbohydrate eaten by animals, b) differences among methyl esters in digestibility, c) the assumption that lipogenic enzyme activities reflected rates of fatty acid synthesis, and d) failure to adequately examine the influence of dietary fatty acids on adipose tissue lipogenesis.

Therefore the primary objective of this research was to reexamine the contention that low levels of dietary polyunsaturated fatty acids specifically inhibit liver and adipose tissue fatty acid synthesis and associated enzymes in the meal-eating rat. Particular attention was given to possible differences in digestibility among the fatty acid methyl esters investigated and attention was also directed at correlating in vivo rates of fatty acid synthesis to in vitro lipogenic enzyme activities. The second phase of experimentation investigated parameters which could potentially explain the inhibition of

fatty acid synthesis by dietary fatty acids, specifically linoleate and linolenate.

PART 1

REVIEW OF LITERATURE

DE NOVO FATTY ACID SYNTHESIS

De novo fatty acid synthesis involves a series of cytosolic reactions which unite eight acetate units to form the long chain fatty acid palmitate. The following equations depict the reactions:

(1) citrate + ATP + CoA → acetyl-CoA + oxaloacetate + ADP + P_i

(2) HCO_3^- + ATP + acetyl-CoA $\rightleftharpoons$ malonyl-CoA + ADP + P_i

(3) acetyl-CoA + ACP - SH $\rightleftharpoons$ acetyl-S-ACP + CoA

(4) acetyl-S-ACP + 7 malonyl-CoA + 14NADPH + 14 H^+ → palmitoyl-CoA + 7 CO_2 + 7 H_2O + 14NADP

The following discussion pertains to nonruminant animals. Citrate conversion to acetyl-CoA plus oxaloacetate is catalyzed by citrate cleavage enzyme. Carboxylation of acetyl-CoA is carried out by the biotin containing enzyme, acetyl-CoA carboxylase. ACP refers to acyl carrier protein which is a fundamental constituent of fatty acid synthetase enzyme complex (11). Fatty acid synthetase is the enzyme complex responsible for joining carbon-carbon bonds of acetate units originating from malonyl-CoA, and in so doing oxidizes NADPH. The liberation of CO_2 in these reactions ensures irreversibility (11, 12).

NADPH is essential for fatty acid synthesis and is generated in most animals by flow of glucose-6-phosphate through the enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of the hexose monophosphate shunt. In addition to hexose shunt production of NADPH, reducing equivalents can result from oxaloacetate in

cytosol via reduction by NADH to malate and subsequent malate decarboxylation to pyruvate catalyzed by malic enzyme with the generation of NADPH. In chickens this is the primary source of NADPH (11, 13).

Citrate originates from the mitochondria via condensation of mitochondrial oxaloacetate and acetyl-CoA. Citrate efflux from the mitochondria is under carrier mediated control and could be a key point in regulating rates of fatty acid synthesis. In de novo fatty acid synthesis mitochondrial acetyl-CoA primarily is derived from pyruvate decarboxylation through the regulatory enzyme complex, pyruvate dehydrogenase (14).

The dietary precursor of pyruvate will depend on the type of carbohydrate being consumed by an individual or animal. The immediate control step of pyruvate production is at the level of the allosteric enzyme pyruvate kinase which catalyzes dephosphorylation of phosphoenolpyruvate to pyruvate (15). If the dietary carbohydrate is glucose then two steps in glycolysis (a) glucose phosphorylation by glucokinase and (b) fructose 1, 6-diphosphate synthesis by phosphofructokinase are potential points for regulating glucose degradation, glycolytic flow, and hence pyruvate production (15). Fructose and glycerol are sources of dietary carbohydrate which by-pass these two regulatory steps and result in different effects on fatty acid synthesis in liver and adipose tissue (15, 16).

Clearly several locations exist for modulation of glycolytic flux and de novo fatty acid synthesis. Control of these points is under hormonal and metabolite mediation and likely involves a

combination of both factors. In the following sections several steps of glycolysis and lipogenesis will be reviewed with particular reference to regulatory significance.

LONG-TERM AND SHORT-TERM REGULATION OF RATES OF DE NOVO FATTY ACID SYNTHESIS

Control of fatty acid synthesis can be via long-term regulation. Long-term control would be effectors or regulation which requires many hours or days for effectiveness, whereas short-term control is a response by the system in seconds or minutes to both positive and negative modulators.

Long-term control of fatty acid synthesis is best characterized by the time required to observe alterations in tissue content of catalytic enzyme machinery. Generally lipogenic enzymes are considered to be in ample concentrations and increase in amount in response to greater substrate flux (17, 18). However key enzymes, such as glucokinase, phosphofructokinase, acetyl-CoA carboxylase and fatty acid synthetase respond to dietary and hormonal manipulations in a way which suggests that the level of the enzyme could be a regulator of fatty acid synthesis. For example under conditions of high carbohydrate intakes by rats the maximal rate of fatty acid synthesis may be restricted to the level of fatty acid synthetase enzyme in liver (19).

Antibodies have been prepared against several lipogenic enzymes of rat, mouse, and chicken liver and/or adipose tissue (13, 20-24).

Quantitative precipitin analyses indicate that changes in tissue enzyme activity reflect shifts in enzyme protein content.

The fluctuation in enzyme protein content appears to be a product of differential rates of enzyme synthesis and not variation in catalytic efficiency or precursor protein transformation (20-24).

Protein synthesis can be controlled at transcription, translation or both. Those factors which have been proposed to precipitate modifications in rates of enzyme synthesis include variations in hormonal relationships, cAMP and metabolite concentrations (25, 26).

Based on the following data, Nepokroeff et al. (27) have proposed that variation in the activity of lipogenic enzymes which occurs with dietary manipulation is a product of coordinated regulation of synthesis of glucokinase, glucose-6-phosphate dehydrogenase, malic enzyme, citrate cleavage enzyme, acetyl-CoA carboxylase and fatty acid synthetase. Streptozotocin-diabetic rats fasted for 48 hours and refed a high carbohydrate diet displayed no elevation above fasting levels in lipogenic enzyme activities, whereas those refed animals administered insulin had linear increases for 48-60 hours in lipogenic enzyme activities (27). The mode of action of refeeding carbohydrate in normal rats was suggested to be via stimulation of insulin release (28). In comparison to insulin, injecting glucagon to normal, refed rats every 12 hours for 48 hours prevented the rise in lipogenic enzyme activity by 50% with the most notable effect on

glucokinase which was only 14% of normal (27). To attribute these effects of glucagon or insulin specifically to control of coordinated synthesis of the enzymes involved without considering substrate flux or metabolite changes may oversimplify the issue. Glucokinase may regulate the rate limiting step in hepatic glucose utilization. Thus effects on glucokinase activity, may be the only specific effect of insulin or glucagon. The resulting activity of other lipogenic enzymes then reflects reduced metabolite flow or concentrations. This is substantiated by the observation that fatty acid synthesis, acetyl-CoA carboxylase activity and fatty acid synthetase activity were maintained at normal rates in diabetic rats fed fructose (25).

From the foregoing discussion it is clear that the exact role the metabolite and hormonal factors play in enzyme protein synthesis regulation is undefined at this time and likely involves a combination of interrelated factors.

Short-term regulation of fatty acid synthesis involves altering the rate of fatty acid synthesis without changing the total content of liver and/or adipose tissue glycolytic and lipogenic machinery. Such control mechanisms permit the organism to maintain metabolic homeostasis during intermittent periods of energy consumption and deprivation. The nature of short-term regulators has been linked to changes in the concentration of several metabolites such as ATP, cAMP, long chain acyl-CoA esters, fructose-6-phosphate, NADH/NAD ratios (19, 29-32).

The quantitative importance of short-term regulators is typified in meal-eating rats. Rates of liver and adipose tissue fatty acid synthesis peak very rapidly after beginning a meal, begin to decline within six hours post-meal initiation, and are negligible just prior to a meal (33, 34). These fluctuations are also seen in malonyl-CoA concentration changes in meal-eating rats (19). In contrast to the diurnal variation in fatty acid synthesis, total in vitro activities of acetyl-CoA carboxylase, fatty acid synthetase, glucokinase etc., remain essentially constant throughout the 24 hour period (35).

A number of locations for short-term regulation have been proposed: 1) reduced uptake and/or phosphorylation of glucose, (2) less pyruvate production and/or decarboxylation, (3) suppressed citrate efflux from mitochondria and hence reduced substrate for fatty acid synthesis, and (4) less amount of active acetyl-CoA carboxylase. Although various metabolites may act as the immediate agent, fluctuations in their concentration frequently are hormonally mediated; and as with control of enzyme protein synthesis, short-term effectors probably involve a combination of hormone and metabolite changes.

REGULATION VIA FLUCTUATION IN ENZYME ACTIVITY

Glucokinase and hexokinase. The utilization of circulating glucose for the production of pyruvate (via glycolysis) and ultimately as a substrate for de novo fatty acid synthesis initially requires

phosphorylation to glucose-6-phosphate. This reaction is catalyzed by hexokinase which exists in four isozyme forms. Control of intracellular glucose utilization at this point is a key regulatory step (15, 37).

Three of the hexokinases (Types I to III) are low k_m enzymes located in most tissues including the liver. Type IV hexokinase called glucokinase is exclusively found in the liver and is the enzyme of major importance in phosphorylating intracellular hepatic glucose. Glucokinase has a high k_m for glucose (10mM vs. 0.1mM for Types I-III), is not inhibited by glucose-6-phosphate and varies in activity with changes in carbohydrate intake (36) in the rat, hamster, mouse, human and dog. Ruminants and chickens have very little glucokinase activity in the liver (37, 15). Since the liver is freely permeable to glucose, several investigators believe that the phosphorylation of glucose catalyzed by glucokinase is the rate limiting step in overall hepatic glucose utilization (37, 38).

After consuming a carbohydrate meal the concentration of glucose in portal blood increases to concentrations exceeding 10-12 mM (36). At this level of glucose the low k_m hexokinase would be saturated and has been estimated to have a maximum catalytic activity of 0.1 μmoles/min/g liver, 25^{o}. In contrast glucokinase will not be at near maximal velocity and has an estimated maximum catalytic activity of 1.5 μmoles/min/g liver, 25^{o}. In addition, a build up of glucose-6-phosphate in the liver would inhibit hexokinase but have no effect on glucokinase (39). Thus these two properties of the enzyme catalyzing the possible rate limiting step of glucose metabolism in liver

are compatible with the anatomical location and elevated rate of glycolysis, glycogen synthesis and lipogenesis following carbohydrate consumption (36).

The level of glucokinase in the liver is dependent on both the amount of dietary glucose as well as circulating insulin (36). Rats fed a carbohydrate-free high fat diet possessed very low glucokinase activities. The oral administration of a glucose solution to the animals caused a tremendous increase in glucokinase activity within three hours. However oral administration of glucose plus anti-insulin serum treatment prevented the rise in glucokinase activity (36). In addition, insulin administration alone to diabetic rats could not enhance glucokinase activity (40). The action of insulin appeared to be specific for glucokinase since normal levels of phosphofructokinase and pyruvate kinase could be maintained in diabetic rats by feeding a fructose-glycerol diet (37). Fasting also led to a rapid decline in glucokinase activity in rats, mice and hamsters but not guinea pigs (41). Upon refeeding of a high carbohydrate diet glucokinase activity increased, with the greatest effect resulting from dietary glucose (42).

From changes in enzyme activity during fasting, the half-life of glucokinase has been estimated at 32 hours. Evidence with protein synthesis and transcription inhibitors has indicated that an elevation in glucokinase activity is a product of enhanced enzyme synthesis (37). In addition immunological titrations revealed that the amount of

enzyme activity in vitro in rats of various nutritional states reflected the amount of active enzyme protein (37).

Glucokinase activity is not only altered by the level of dietary fat but also responds differently to the composition of dietary fat (43, 44). Replacing 15% of the glucose in a high carbohydrate diet of gerbels with safflower oil was associated with a significant decline in glucokinase activity. However, the addition of 15% coconut oil caused no change in glucokinase activity relative to the high carbohydrate-fed group. The reason for more efficient inhibition of glucokinase by safflower oil-containing diet was unknown but this observation was in accord with observation in mice and rats that polyunsaturated fats inhibit hepatic lipogenic enzymes with greater efficacy than saturated fats (7-10). Inhibition at the first step of glucose utilization in liver would be an efficient mechanism by which to inhibit lipogenesis resulting from dietary triglyceride. The reasons for differential response to saturated and unsaturated fatty acids at this time can only be speculative and require further elucidation.

Glucokinase activity in vitro has been shown to be inhibited by added free fatty acids, acetyl-CoA and phosphoenolpyruvate (45). The negative effect of free fatty acids could be prevented by the addition of glucose. These observations were interpreted as a control mechanism in vivo for glucokinase during the transition of the liver glycolysis to gluconeogenesis. However the inhibition of glucokinase by free fatty acids was time and dose dependent which supports the contention that free fatty acids and their CoA derivatives exert a

non-specific detergent effect on most enzymes except acetyl-CoA carboxylase (29). Similarly the inhibitory effect of acetyl-CoA and phosphoenolpyruvate was concentration dependent and non-competitive with glucose (45). More recent alternatives for the regulation of glucose uptake and release in liver during the transition from glycolysis to gluconeogenesis have been (a) substrate cycling catalyzed by glucokinase and glucose-6-phosphatase and (b) compartmentation of glucokinase and glucose-6-phosphatase (36). Unlike the liver, adipose tissue is not freely permeable to glucose (36). Therefore in order for adipose tissue to utilize glucose for de novo fatty acid synthesis, glucose must first pass through the adipocyte plasma membrane. The entry of glucose into fat cells is greatly facilitated by insulin and may be the rate limiting step in adipose tissue glucose utilization (36). Under such conditions adipose tissue hexokinase activity may play less of a key role in determining rates of glucose utilization than does glucokinase activity in the liver. Nevertheless adipose hexokinase is an adaptive enzyme, responsive to nutritional manipulation and may still serve an important function in regulating the rate of substrate flow through glycolysis and ultimately lipogenesis (46).

Hexokinase in rat adipose tissue is the low k_m isozyme and the variety which predominates appears to be age dependent (15). Fasting and alloxan-diabetes in the rat was associated with a decrease in hexokinase activity, while refeeding a high carbohydrate diet or injecting insulin resulted in an increase in hexokinase

activity (15). Human adipose tissue hexokinase activity responds to fasting and refeeding in a manner similar to rats, but diabetic humans did not display a reduction in hexokinase activity nor did the activity increase upon insulin injection (47).

From the use of protein synthesis inhibitors and ^{14}C-histidine incorporation into hexokinase, the elevation in activity appears to involve enzyme protein synthesis. The stimulus for synthesis includes both glucose and insulin (15, 48). A short-term regulatory mechanism for hexokinase activity may exist in the ratio of soluble vs. membrane bound enzyme (49, 51). A very large portion of hexokinase activity has been identified as associated with mitochondria (50). In chick brain evidence has indicated that conditions which lower ATP availability (e.g. anoxia) increased the amount of mitochondrially bound hexokinase which increased the k_i for glucose-6-phosphate five-fold and reduced the k_m for ATP seven-fold (51). In vitro bound hexokinase was solubilized from the mitochondria and reduced in activity by added glucose-6-phosphate (50). Binding of hexokinase to mitochondria purportedly stabilizes the enzyme and places it in closer proximity to ATP generating machinery (50, 52). Even though in rats fed a high carbohydrate diet the amount of bound hexokinase was greater than in fasted rats, the exact physiological role soluble and bound enzymes play in glucose metabolism of adipose tissue requires further elucidation (53).

Phosphofructokinase. Phosphofructokinase (PFK) exists in isozyme form among tissues with the L-type in liver, M-type in muscle,

and a M-L variety in adipose tissue. Purified PFK (generally M-type) is inhibited by ATP, citrate and creatine; and stimulated by fructose-1,6-diphosphate (FDP), AMP, cAMP and P_i (30, 54). In addition to these metabolite effectors of PFK, a phosphorylated form of PFK has been isolated from mouse skeletal muscle (55). The active form of PFK has been proposed to be a phosphorylated tetramer which dissociates to inactive protomers upon dephosphorylation by a magnesium dependent phosphatase (56). The reactivation rate by ATP of the inactive protomers in rat liver was greatly reduced by only six hours of fasting but the rate was restored to normal within six hours of refeeding following a 24 hour fast (56). The physiological significance of these modifiers varies with tissue and the effect of the modifiers is not always consistent with metabolite concentrations and physiological conditions. For example, injection of glucagon into portal vein of rats increased liver cAMP levels three fold but PFK activity was depressed by 50% within four minutes (30). Insulin on the other hand increased PFK activity 50% with no change in cAMP concentration (30). Three days of fasting reduced rates of glycolysis in rat liver while the cAMP concentration dramatically rose (57). These observations are not consistent with cAMP activation of purified PFK but are in accord with known effects of fasting on rates of glycolysis (57). If ATP inhibits PFK then a low ATP/ADP ratio would favor PFK activation and promote gylcolysis. However physiological states such as fasting and diabetes result in low rates of hepatic glycolysis and a low ATP/ADP ratio (31). Because the ratio of

ATP/ADP and the absolute content of ATP in adipose tissue changed very little in fed, fasted-refed or fasted rats, Ballard (32) has questioned the significance of the ATP/ADP ratio as a control mechanism of adipose tissue fatty acid synthesis. In support of this the concentration of adenine nucleotides per milligram wet tissue or per milligram DNA in rat epididymal fat pads incubated in vitro with albumin, insulin or epinephrine did not vary from pads of normal rats incubated with no additions (58).

Attributing control of glycolytic flux and in particular PFK activity to one ratio or metabolite may be an oversimplistic approach (54). The oscillatory behavior of glycolytic flux in rat muscle extracts indicated that PFK activity is not only dependent on ATP/ADP but greatly affected by FDP concentration plus the concentration of AMP and fructose-6-phosphate (F6P) (54). Several workers have suggested that a better indicator of glycolytic rate may be the ratio of F6P or FDP to ATP (38, 54, 59). This approach is supported by in vitro metabolite data in fat pads isolated from normal and diabetic rats (58). The addition of insulin to the media increased the uptake of glucose, the concentration of glucose-6-phosphate (G6P) and presumably F6P, and the level of glycerol-phosphate which collectively indicated an accelerated glycolytic flow. The inclusion of epinephrine with insulin reduced G6P content of the fat pads by 50% and glycerol-phosphate content by several fold. Under both conditions ATP/ADP ratios did not significantly change, although citrate was elevated (58). These parameter variations are in accord with

insulin's ability to promote lipogenesis and epinephrine's effects in fasting.

The role of citrate as an inhibitor of PFK and hence glycolysis is sometimes inconsistent with the part it plays in promoting lipogenesis in liver and adipose tissue (15). Little difference in the hepatic content of citrate existed between starved, diabetic, fasted-refed or insulin-injected diabetic rats (19, 38, 60). In adipose tissue both fasting and epinephrine treatment (in vitro) resulted in a significant increase in rat adipose tissue citrate levels (32, 58), which agrees with its inhibitory effect on glycolysis and PFK activity but disagrees with its stimulatory role for acetyl-CoA carboxylase. Similarly resting muscle preferentially utilizes circulating free fatty acids which leads to a build-up of muscle citrate and thus potentially could slow glucose utilization via glycolysis by PFK inhibition (61).

Recently Ramadoss et al.(62) demonstrated that free fatty acids inactivated rabbit muscle PFK. This effect could be lessened by prior incubation of the enzyme with F6P, AMP, FDP or cAMP. Albumin would partially protect the enzyme if added before the free fatty acids. However once the enzyme was inactivated by palmitate or oleate, albumin was unable to reverse the inhibition. Because of the high concentrations of palmitate and oleate utilized and the irreversible nature of the inhibition, the effect probably was a nonspecific detergent action of the free fatty acids rather than a specific regulatory mechanism (29).

In addition to modulation of PFK activity by several different metabolites the actual amount of enzyme can vary with nutritional conditions. PFK activity in rat liver and adipose tissue was depressed by fasting and alloxan diabetes (15, 63). Refeeding or insulin injection resulted in the activity of PFK returning to normal levels (15, 64). The effect of insulin may be secondary to its action on glucose uptake and phosphorylation because PFK activity could be maintained at high levels in the liver of diabetic rats fed a fructose-glycerol diet (37).

Pyruvate kinase. Pyruvate kinase (PK) catalyzes the formation of pyruvate from phosphoenolpyruvate with an equilibrium constant greatly in favor of pyruvate formation (36, 65). The in vitro activity of PK suggests it catalyzes a reaction at near equilibrium rates whereas the mass-action ratio indicates the reaction is not near equilibrium (36). The allosteric properties of PK offer an explanation for this discrepancy and contribute to the regulatory role PK plays in glucose metabolism via glycolysis (63). PK is sharply inhibited by ATP and alanine while the inhibition is rapidly reversed by FDP (66). During gluconeogenesis inhibition of PK activity is desireable to avoid futile dephosphorylation of phosphoenolpyruvate. Thus in light of alanine's major contribution as a gluconeogenic precursor, its inhibition of PK activity appears reasonable. Similarly activation of PK by FDP would facilitate flow of glucose through glycolysis and enhance pyruvate production (36, 15, 38). However the function of ATP is unclear since in rat

liver its concentration increases during high rates of lipogenesis and glycolysis which is inconsistent with ATP inhibition of phosphofructokinase and PK (38). As previously cited the ratio of FDP/ATP may be an important, fundamental factor in controlling PK activity (38).

Like phosphofructokinase, PK does appear to be adaptative in rat liver but does not respond to dietary manipulation in chick liver and only minimal changes occur in rat adipose (15). Fasted and alloxan diabetic rats displayed lowered hepatic PK activities while refeeding or insulin injection elevated PK activity above normal. The increase in hepatic PK was prevented by first administering actinomycin or ethionine which suggested the increase in enzyme activity under these conditions represented de novo enzyme synthesis (45). As with phosphofructokinase the effect of insulin may be secondary to its primary effect on glucose phosphorylation in liver because PK activity could be maintained above normal in diabetic rats fed fructose (37). Adipose tissue PK was not reduced in fasting rats or by treating them with alloxan (64). The enzyme did slightly increase in refed rats fed a high carbohydrate diet and in meal-trained rats (67, 68).

Pyruvate dehydrogenase. In rats, mice, chickens, pigs and presumably man the conversion of pyruvate to acetyl-CoA in liver and/or adipose tissue is fundamental to producing substrate for fatty acid synthesis. This reaction is catalyzed by pyruvate dehydrogenase

(PDH), an intramitochondrial enzyme, which is very important in modifying rates of pyruvate decarboxylation.

PDH is a nonadaptive enzyme whose activity is controlled by shifts between phosphorylated (inactive) and nonphosphorylated (active) forms of the enzyme (69, 14). Phosphorylation of PDH is governed by a kinase requiring Mg-ATP and which is independent of cAMP (14, 15). The inactivation of PDH in isolated mitochondria of rat heart was prevented by high concentrations of pyruvate (70). Presumably pyruvate inhibits kinase activity (71). Dietary regimens which are associated with high rates of glycolysis and lipogenesis would favor pyruvate production and enhance active amounts of PDH. Rats in a fed state have been shown to have one-sixth active PDH in liver and two-thirds dephosphorylated form in adipose tissue (72). A large proportion of active hepatic PDH has been found in rats refed a high carbohydrate diet, injected with insulin or perfused with fructose (73-75). Similarly adipose tissue PDH was activated by adding glucose, fructose or pyruvate to an in vitro incubation media and by insulin in the absence of substrate (76). The action of insulin in adipose has been proposed to be via an enhancement of PDH-phosphatase activity. Such an effect of insulin would potentially override the negative influence of elevated ATP/ADP ratio in liver and adipose tissue of rats during high rates of lipogenesis (58, 77).

Dephosphorylation of PDH is catalyzed by a Mg^{++} - dependent phosphatase (15). The phosphatase has a K_m for Mg^{++} which is well above the total cell Mg^{++} concentration. Therefore its activity is

sensitive to changes in Mg^{++} levels (15). An increase in intramitochondrial ATP concentration would lead to a greater proportion of Mg-ATP complex, reduce the availability of Mg^{++} and thereby lower phosphatase activity (15, 54). A reciprocal relationship between mitochondrial ATP levels and the proportion of active PDH has been demonstrated in rat liver mitochondria (14). Free fatty acids and/or their CoA esters have been proposed to control pyruvate utilization by regulating the proportion of active PDH (14, 73, 78, 79). Injection of oleate promoted the amount of phosphorylated rat liver PDH; addition of oleate or octanoate to an in vitro media depressed the proportion of rat adipose tissue active PDH (73, 78). The reduced quantity of active PDH in liver and adipose elicited by free fatty acids was suggested to be due to the inhibition of mitochondrial adenine nucleotide translocase activity by long chain acyl-CoA esters (14, 80) which led to an increase in mitochondrial ATP concentration.

In addition to ATP pyruvate dehydrogenase activity is affected by several metabolities: pyruvate, NADH, acetyl-CoA and ADP (79, 81-83). The effects of these metabolites are exerted on the inactivating kinase portion of the PDH-complex (79, 83). The PDH-kinase has been shown to be activated by NADH and acetyl-CoA and inhibited by pyruvate and ADP (79). Recent investigations with isolated rat liver (81) and heart (79) mitochondria incubated with octanoate or palmitoylcarnitine have demonstrated an increase in the amount of NADH and acetyl-CoA with a concomittant decrease in pyruvate decarboxylation and active PDH (79, 81). Because these changes were independent of changes in ATP

concentration or ATP/ADP, the inhibitory action of fatty acids on the activity of PDH may be more related to increases in NADH/NAD and acetyl-CoA/CoA ratios from β-oxidation than to elevated ATP/ADP ratio resulting from translocase inhibition (79). This is also consistent with a lower NADH/NAD ratio during high rates of hepatic lipogenesis even though ATP/ADP ratio increases (36).

Acetyl-CoA carboxylase. Dietary and hormonal manipulations which increase or decrease rates of liver and adipose tissue fatty acid synthesis are also associated with parallel changes in acetyl-CoA carboxylase (AcCBX) activity (13, 25, 84-87). Hepatic AcCBX activity is reduced in rats, mice and chicks by fasting, high fat diet and alloxan-diabetes (25, 84-86) and elevated in activity by refeeding high carbohydrate diet, feeding a fat-free diet, and feeding fructose (13, 25, 84-86). Quantitative antibody precipitin analyses have indicated that these changes reflect alterations in enzyme protein content which is accomplished by differential rates of enzyme synthesis without changes in rates of degradation of AcCBX (84).

Glucocorticoid administration to adult rats depressed rat adipose tissue AcCBX content without affecting rat liver AcCBX. Glucagon injection during refeeding of a high carbohydrate diet prevented the rise in rat liver AcCBX by 50% (87). Similarly insulin injection to diabetic rats elevated hepatic and adipose AcCBX (25). The effects of these hormones have been proposed to be prior to aldolase because fructose feeding to diabetic rats maintained normal rates of liver fatty acid synthesis and AcCBX (25).

Although the level of AcCBX probably has a governing effect on rates of fatty acid synthesis under prolonged nutritional situations, its half-life of 40-50 hours does not explain the normal diurnal short term changes which occur in rates of fatty acid synthesis.

The key role which AcCBX plays in short term or fine regulation of fatty acid synthesis can be appreciated from the allosteric properties of the enzyme (15). From electron microscopy and density-gradient centrifugation purified AcCBX has been shown to exist in an inactive protomer which can be converted to an active polymer (11). Polymer formation of purified AcCBX is enhanced by citrate while depolymerization is promoted by long chain fatty acyl-CoA derivatives (88, 89). This mechanism has been confirmed in rat liver and adipose tissue and avian liver (90, 91). The K_i of AcCBX for fatty acid CoA derivatives varies with the type of fatty acid and appears to be lowest for saturated fatty acyl-CoA esters (92).

The physiological significance of citrate's influence on AcCBX activity is open to question. The citrate content of liver (whole tissue analysis) in rats fed a high fat diet was two to three times higher than nibbling, meal-fed, or refed rats and yet the rate of fatty acid synthesis in the fat-fed rats was very low (19). Similarly fat pads of fasted rats contained more citrate per gram of wet tissue than tissue from fed or refed rats (32). These observations are consistent with citrate's negative effect

on glycolysis via inhibiting phosphofructokinase but inconsistent with its activator role with AcCBX. One difficulty with total tissue analysis is an inability to differentiate metabolite compartments which could alter the local effective concentration of a metabolite. Thus total tissue citrate may not change but the concentration in various micropools could fluctuate.

Several observations support a reversible, specific inactivation of AcCBX by long chain fatty acyl-CoA compounds and support the contention AcCBX is a key step in short-term regulation of fatty acid synthesis. Many enzymes are inhibited by CoA derivatives of fatty acids (93, 94) but these effects were shown to be nonspecific and irreversible, and likely the result of a detergent action of the fatty acids. Goodridge (29) clearly demonstrated that palmitoyl-CoA in the presence of high amounts of albumin inhibited both AcCBX and ^{14}C-citrate incorporation into fatty acids. This inhibition could be reversed by increasing the albumin concentration of the system. Following intubation of corn oil to rats previously fed a fat free diet, a two fold increase in hepatic fatty acyl-CoA concentration and a 100% reduction in hepatic in vitro fatty acid synthesis occured within two hours (95, 96). Since this time period was too short to attribute the change in fatty acid synthesis to a reduced enzyme protein concentration, the alternative explanation could be an inactivation of some enzyme in lipogenic pathway, presumably AcCBX (24, 95, 96).

Data for the effect of acyl-CoA esters in adipose tissue are more variable. The in vitro exposure of rat fat pads to insulin plus glucose doubled the amount of active AcCBX without influencing total AcCBX activity. Insulin alone had no effect (89). Conversely the inclusion of epinephrine with insulin and glucose caused a 50% drop in polymeric form of AcCBX (88). A negative correlation between AcCBX activity and adipose tissue fatty acyl-CoA concentrations was observed but this correlation was inconsistent (88). For example, the long chain fatty acyl-CoA level in adipose tissue of rats fasted for 36 hours was significantly below the concentration in fed animals, but during this time total adipose AcCBX changed very little (88). These results are in contrast to data derived from rat liver (97) where the long chain acyl-CoA content in liver of fasted rats (18-48 hours) was twice normal. The inconsistency in data between liver and adipose tissue may be related to the high triglyceride content of adipose tissue which must be extracted before analysis for CoA derivatives (88).

Fatty acid synthetase. For several years the reaction catalyzed by acetyl-CoA carboxylase was considered the rate limiting step in lipogenesis (98). However following improvements in acetyl-CoA carboxylase assay system, data now indicate that either carboxylase or fatty acid synthetase (FAS) catalyzed reactions may be rate limiting depending upon the nutritional state of the animal (19, 70).

Unlike acetyl-CoA carboxylase or pyruvate dehydrogenase, allosteric regulation of FAS probably is insignificant in the short term control of fatty acid synthesis (35). Early work with purified pigeon liver and rat liver FAS indicated inhibition of the enzyme by palmitoyl-CoA (99, 100). However Goodridge (29) clearly demonstrated that palmitoyl-CoA inhibition of enzymes other than acetyl-CoA carboxylase was due to an irreversible nonspecific detergent effect. Pigeon liver FAS was reported to be stimulated by phosphorylated sugars (e.g. FDP) and inhibited by malonyl-CoA (101). However the physiological significance of these observations remains to be defined because the concentrations of phosphorylated sugars used were well above the physiological level (102). Further research has been unable to confirm the earlier work of Wakil and associates (101, 102).

FAS activity varies with nutritional and hormonal conditions (86-88). Feeding a high carbohydrate fat-free diet to rats, mice and chicks resulted in a relatively high liver and/or adipose tissue FAS activity (7-10, 103). Addition of safflower oil, corn oil, linoleate or linolenate to the fat-free diet precipitated a rapid and marked decline in FAS activity in rat, mouse and chick liver (7-10, 103). Fasting for 24-48 hours also greatly reduced rat liver FAS activity while refeeding led to a large increase in FAS activity (20).

Alloxan-diabetic rats possessed very low liver and adipose tissue FAS activities but treatment with insulin or fructose feeding restored hepatic FAS activity to near normal (25). The injection

of glucagon to rats during a refeeding period greatly prevented the expected rise in hepatic FAS activity as well as several other lipogenic enzymes (27). However this preventative effect may well have been secondary to the near total lack of increase in glucokinase activity (27). Rat adipose tissue FAS activity was reduced by glucocorticoid administration but liver FAS was unaffected (87). Clearly FAS responds to different dietary and hormonal manipulations. Whether these effects are primary to the agent or secondary to some other change (e.g. glucokinase vs. FAS activity) remains to be ascertained.

Changes in FAS activity have been demonstrated not to be the product of alterations in catalytic efficiency but reflect actual variations in enzyme protein content (20, 25). Control of enzyme protein content occurs by altered rates of synthesis and/or degradation. Under most conditions the level of FAS activity in a tissue is varied by differential rates of synthesis (20, 26). The rate of degradation of FAS has been estimated in nonfasted rats and a half-life of 47-75 hours has been reported (20, 26).

In the absence of definitive data for allosteric effectors of FAS, Guynn et al. (19) have concluded that in rats fed a high carbohydrate diet the rate of hepatic fatty acid synthesis depends on the quantity of FAS. This conclusion was based on the observation that animals fed the carbohydrate diet has a build-up of liver malonyl-CoA content. Such control of hepatic fatty acid synthesis

would be long-term in nature and does not preclude short-term or fine control through allosteric changes in glycolytic enzymes, pyruvate dehydrogenase or acetyl-CoA carboxylase.

Citrate cleavage enzyme. In nonruminant animals citrate cleavage enzyme (CCE) catalysis of citrate hydrolysis to oxaloacetate and acetyl-CoA has been considered a major mechanism in the generation of cytosolic acetyl-CoA for de novo fatty acid synthesis. Like several other lipogenic enzymes CCE activity in liver and adipose tissue of rats appears to be adaptive. Because an increase in CCE activity was associated with greater rates of enzyme synthesis in rat liver, Gibson et al. (104) concluded that differential rates of enzyme synthesis govern the level of CCE activity. CCE activity was shown to be reduced in rat and chick liver, and rat and pig adipose tissue by fasting, fat-feeding and alloxan diabetes (105-109). Conversely feeding a high carbohydrate diet promoted CCE synthesis and increased its activity in rat liver and adipose (105, 106).

Current data have not revealed short-term effectors for CCE and generally its activity is sufficiently high so that it would not be a rate limiting reaction. However Yeh and Leveille (110) suggested that in fasted chicks a limited availability of free CoA because of the rapid rise in hepatic long chain acyl-CoA esters could limit the activity of CCE. Such a hypothesis has not been proposed for other species.

Glucose-6-phosphate dehydrogenase and malic enzyme. De novo fatty acid synthesis requires reducing equivalents in the form of NADPH.

In rat liver and adipose tissue the production of NADPH results from the flow of G6P through the hexose monophosphate shunt and via the conversion of malate to pyruvate. In contrast to the rat, chick liver has low hexose shunt activity and depends heavily on malate-pyruvate cycle. The two key enzymes involved are glucose-6-phosphate dehydrogenase (G6PD) for the hexose shunt and malic enzyme (ME) for the malate utilization (11, 15). At one time the activities of G6PD and ME and the subsequent utilization of NADPH were considered a determining force in driving fatty acid synthesis (111). However further studies have demonstrated that the activities of G6PD and ME can change independent of fatty acid synthesis rates and probably respond to total tissue NADPH demands (112-114).

In adipose tissue undergoing reasonable rates of fatty acid synthesis, McLean (115) found that G6PD activity was not functioning at maximal capacity. The tissue G6PD activity could be stimulated by addition of acetate as a fatty acid precursor or by addition of phenazine methosulphate, an electron acceptor (115).

G6P is not only substrate for G6PD but can also be metabolized via glycolysis or utilized for glycogen synthesis. The determining factor in flow of G6P through G6PD does not appear to be substrate limitation because the hepatic concentration of G6P is three times higher than G6PD K_m (15). Taketa and Wantanabe (21) have isolated monomer, dimer and tetramer forms of G6PD from rat liver cytosol. NADP stimulated dimer formation and the dimer exhibited sigmoidal kinetics for NADP. They proposed that the

physiologically active form of G6PD was the dimer, and that as NADPH levels rose the structural NADP component of the dimer was replaced with NADPH thereby inactivating the enzyme (21). Thus high rates of NADPH utilization would promote activation of G6PD and flow of G6P through the hexose shunt (21). Such utilization occurs in rats with high carbohydrate intakes and low polyunsaturated fat diets (112, 113).

Both G6PD and ME activities fluctuate with dietary and normonal alterations. Fasted and alloxan-diabetic rats displayed reduced hepatic and adipose tissue G6PD and ME activities (15, 116, 117). Upon refeeding fasted rats a high glucose diet both enzymes rebound in activity to the point of "overshooting" the levels found in unfasted rats maintained on a similar diet (111, 114, 118). An apparent greater increase in G6PD and ME resulted from a second fasting-refeeding period (119). Although both enzymes were reduced in liver of rats fed high fat diets (113), diets high in polyunsaturated fatty acids precipitated the reduction with far greater efficacy (112, 113). Goodridge (120) was unable to observe a decrease in liver ME in chicks fed diets containing 10 or 15% corn oil. In contrast Yet et al. clearly demonstrated chicks did respond to increased amounts of dietary fat by lowering ME activity (107).

In addition to response to dietary fat, G6PD activity was shown to be very sensitive to the amount of glucose consumed by rats each day (121). This response may be due to greater availability of glucose per se, a metabolite of glucose or the product of enhanced

insulin release. Insulin injections markedly elevated G6PD activity in rat liver but this effect was overshadowed by an increased food intake caused by insulin (116, 122). The type of dietary carbohydrate (e.g. glucose, fructose) may also influence the activity of the enzymes and may lead to independent responses between rat adipose tissue and liver (15). For example, in conjunction with accelerated rates of liver fatty acid synthesis with fructose and sucrose feeding was an increase in G6PD activity above that seen in rats fed glucose (123, 124). However under these conditions adipose tissue G6PD activity was unchanged (124).

G6PD and ME do respond differently to dietary protein (125). As previously stated refeeding a high carbohydrate diet to fasted rats caused an "overshoot" in the activities of both enzymes. However if only carbohydrate is refed G6PD activity does not increase (114) whereas ME activity did increase even on extremely low protein diets (126).

G6PD and ME both appear to fulfill the following criteria established by Weber and associates (63) to determine if in vitro enzyme activity increases represent an actual rise in enzyme amount and not catalytic efficiency: a) blockage of rise in activity by protein synthesis inhibitors; b) increased incorporation of amino acids into purified enzyme protein, and c) immunological evidence for elevated amounts of purified enzyme.

Puromycin injection prevented the rise in G6PD activity upon refeeding rats a high carbohydrate diet (111). Similarly, injection

of 8-azaguanine blocked the overshoot in G6PD activity, but did not prevent G6PD from returning to prefasted normal level (127). Since 8-azaguanine inhibits transcription, the G6PD overshoot presumably involves additional RNA synthesis.

Recently studies using antibodies against rat liver G6PD revealed that the enzyme from rats fed a standard pellet diet or high carbohydrate diet reacted identically to antibody precipitation (118). Therefore the higher activity in rats fed the high carbohydrate diet was considered a difference in concentration and not a change in enzyme catalytic efficiency (118). From the rate of incorporation of ^{14}C-amino acids into immunoprecipitated liver G6PD, Garcia and Holten (128) demonstrated the elevation in G6PD upon refeeding fasted rats was the product of enhanced enzyme synthesis. Injection of glucagon during the refeeding period dampened the increase in G6PD activity by reducing its synthesis but without influencing the rate of degradation (128).

Similarly quantitative immunoprecipitation of ME in rats refed a high carbohydrate diet demonstrated that the higher enzyme activity was associated with greater ^{14}C-leucine incorporation into the enzyme protein (117, 125). Thyroxine treatment which enhances ME activity in rats fed chow, also accelerated ^{14}C-leucine incorporation into ME protein (125). Quantitatively Murphy and Walker (117) demonstrated that thyroxine treatment led to a four fold rise in ME synthesis and a six fold rise in specific activity. In neonatal

chicks fed a commercial diet the rate of ME synthesis rose 54 times while local activity increased 63 fold (13).

Both ME and G6PD appear to increase their activities primarily by accelerated rates of enzyme protein synthesis. The G6PD and ME activities of liver and adipose tissue do not determine rates of fatty acid synthesis but most likely change in activity in response to NADPH demands. The modulators of G6PD and ME synthesis have been proposed to include insulin, unsaturated fatty acids, glucose and cAMP (15, 113, 114, 118, 128). However characterization of the repressors and stimulators remains to be ascertained.

REGULATORY ASPECTS OF LONG CHAIN ACYL-CoA ESTERS

Rats and chickens that were fasted or fasted and refed a fat diet displayed very low rates of fatty acid synthesis and significant elevations in total hepatic long chain acyl-CoA thioester concentrations (19, 31, 97, 100, 129). In addition to the influence of acyl-CoA thioesters exert on acetyl-CoA carboxylase activity discussed previously, these derivatives have been implicated in negatively affecting two mitochondrial transport systems: a) ATP/ADP translocase and b) citrate efflux (11, 36).

Adenine nucleotide translocase is an inner mitochondrial enzyme which catalyzes exclusively a molecule-for-molecule exchange of ADP and ATP between the cytosol and mitochondria (11). In this

way the energy state of the two compartments remain interconnected. The translocation of adenine nucleotides across the inner mitochondrial membrane can be inhibited by atractylate, bongkrekic acid, and long chain fatty acyl-CoA esters (11, 80). The acyl-CoA inhibitory action cannot be mimicked by free fatty acids or carnitine derivatives. Uptake of ^{14}C-ADP by isolated rat and guinea pig liver mitochondria was inhibited by CoA derivatives of myristic, palmitic, stearic and oleic acids. Linoleyl-CoA also inhibited translocation but with considerably less effectiveness (130). Guinea pig mitochondria. Octanoyl-CoA was without effect with mitochondria of either species. Kinetic analyses of ADP uptake indicated palmitoyl-CoA inhibition was reversible and competitive in both rat and guinea pigs.

Mitochondria isolated by differential centrifugation from the livers of alloxan-diabetic rats or monkeys, and of hibernating ground squirrels showed very low capacities for ^{32}P-ATP exchange and ^{14}C-ADP translocation. These low rates were reversed by adding 5mM D, L-carnitine or 15 mg albumin which apparently facilitated removal of the fatty acyl-CoA esters by forming carnitine derivatives (131). ^{14}C-ADP translocation was inhibited 92% by 3mM fatty acyl-CoA esters but in all cases 5mM carnitine addition readily reversed the inhibition (131). This was taken as evidence that the site of acyl-CoA inhibition is at the inner mitochondrial membrane and the inhibition is reversible (131).

Recently translocase activity was quantitated in ischemic and non-ischemic regions of dog heart (132). Within 15 minutes ischemic areas had significantly less translocase activity than non-ischemic regions. Concomittantly there was a two-fold increase in total tissue acyl-CoA concentration in ischemic hearts. Translocase activity continued to sharply decline at 30 and 60 minutes while acyl-CoA content rose linearly.

Mitochondria isolated from livers of fasted or alloxan-diabetic rats contained twice the amount of acid insoluble CoA levels relative to carbohydrate-fed animals (133). Whether these CoA derivatives are intramitochondrial, specifically bound to mitochondrial sites, or nonspecifically attached during mitochondrial isolation has yet to be established. In addition to having elevated acyl-CoA contents fasted or fasted-refed (fat diet) rats had considerably greater intramitochondrial ATP/ADP and lower NAD/NADH ratios than animals fed a carbohydrate diet (31). These changes are consistent with the inhibitory effect long chain fatty acids appear to exert on pyruvate metabolism.

In conjunction with adenine nucleotide translocase control by fatty acyl-CoAs they also appear to affect citrate or tricarboxylic acid transport from the mitochondria (133).

Citrate efflux from mitochondria in nonruminant animals is essential for the transfer of mitochondrial acetyl-units into the cytosol for utilization in de novo fatty acid synthesis (11). Citrate exit from the mitochondria supposedly is via a specific carrier

system which functions by exchange diffusion. This means the exit of one citrate molecule is accompanied by the uptake of a tricarboxylic or dicarboxylic acid, e.g. malate (134, 135). Palmitoyl-CoA greatly inhibited the exchange of mitochondrial ^{14}C-citrate with unlabelled media citrate (136). This effect could be prevented by including albumin in the system, was partially reversed by the addition of carnitine and was competitive with citrate (136). The exchange of malate with inorganic phosphate (media) was also inhibited by palmitoyl-CoA but the K_i was three times greater for all dicarboxylic acid systems than tricarboxylic systems (136).

Using the citrate exchange technique, Cheema-Dhadli and Halperin (133) found that mitochondria isolated from diabetic or fasted rats had a K_m for citrate transport which was two-fold greater than mitochondria from fed rats. Interestingly V_{max} for citrate transport did not differ among the various states. Concomittantly the fasted and diabetic rats had twice the amount of mitochondrial acid-insoluble CoA content (133).

The inhibition of adenine nucleotide translocase and citrate transporter by long chain fatty acyl-CoA derivatives is compatible with known in vivo changes in total tissue acyl-CoA content which occur during fasting, fat-feeding and diabetes. In addition these changes are associated with low rates of de novo fatty acid synthesis and consistent with control of pyruvate oxidation at the level of pyruvate dehydrogenase (15, 82).

Yeh and Leveille (129) proposed that free fatty acid acylation with free CoA can limit the amount of free available CoA for citrate cleavage enzyme function and thereby limit rates of lipogenesis. This contention is supported by the knowledge that rat liver fatty acid activating enzyme has a lower K_m for CoA than does citrate cleavage enzyme (129, 137). Increased plasma free fatty acid levels are found in feeding high fat diets to chicks and rats (100, 129) and in short-term fasting in chicks (110). The elevated plasma free fatty acid levels were accompanied by a large increase in hepatic long chain acyl-CoA derivatives and significantly less free CoA (100, 129).

The quantitative significance of each mechanism is unknown but obviously long chain fatty acyl-CoA compounds play a widespread role in controlling de novo fatty acid synthesis.

THE ROLE OF REDOX STATE IN CONTROL OF FATTY ACID SYNTHESIS

The rate at which reducing equivalents are utilized during de novo fatty acid synthesis in liver and adipose tissue may be an important regulator of the rate of lipogenesis (138). Diabetic, fasted and fasted-fat refed rats display low rates of hepatic and adipose fatty acid synthesis and high lactate/pyruvate cytosolic ratios; whereas rats fed a high carbohydrate diet or fasted-carbohydrate refed animals have

lower lactate/pyruvate cytosolic ratios, rapid glycolytic flux and high rates of fatty acid synthesis (31, 46).

Adipose tissue taken from fasted rats displayed high lactate/pyruvate ratios and low rates of glucose conversion to fatty acids (46). The addition of acetate reduced the concentration of lactate and led to accelereated rates of lipogenesis. The implication is that fasting does not prevent acetate utilization for fatty acid synthesis in rat adipose tissue but rather exerts a greater influence on glucose degradation to acetate. The elevated lactate/pyruvate ratio presumably represents an increased cytosolic NADH/NAD ratio which would slow glycolytic flux markedly at the level of glyceraldehyde phosphate dehydrogenase (46). A rise in cytosolic NADH generally is associated with a rise in mitrochondrial NADH (31). Thus the amount of active pyruvate dehydrogenase would be lessened and pyruvate conversion to acetate would be affected (79).

In the liver of fasted rats the level of acyl-CoA esters is increased along with the rate of β-oxidation (100, 139). The net effect in the liver of fasted rats is a more reduced cellular state and blockage of glucose conversion to acetate (31, 46).

Control of fatty acid synthesis through changes in redox state of liver was demonstrated in rats fed diets containing butanediol (140). Liver slices of animals fed butanediol had depressed rates of glucose conversion to fatty acids, but acetate incorporation was unimpaired (140). In conjunction with lower fatty acid synthesis rates, rats fed butanediol had markedly elevated cytoplasmic

NADH/NAD ratios in the liver (141). Purportedly a high NADH/NAD ratio slowed flux through glyceraldehyde-3-phosphate dehydrogenase because of inadequate NAD supplies (140).

Cytosolic NADH/NAD and NADPH/NADP pools are closely integrated and vary in concert (31, 142). Thus as the cytosolic NADH level increases, then the proportion of NADPH rises. The amount of NADPH has extreme implications with control of pentose shunt activity. Glucose-6-phosphate dehydrogenase requires NADP for activity (116). Thus during high rates of lipogenesis more NADPH is oxidized (31) and hexose shunt flux is accelerated (36). Conversely enhanced fatty acid degradation and increased NADH-NADPH levels in liver will reduce both hexose shunt flow (31) and glycolytic rates with the net effect of reduction in fatty acid synthesis.

RELATIONSHIP AMONG SPECIES BETWEEN NUTRITIONAL STATE AND FATTY ACID SYNTHESIS

Rats. The rat synthesizes fatty acids both in the liver and adipose tissue. The proportion that each tissue contributes to overall lipogenesis has not definitively been ascertained and may vary with the type of diet (123) and pattern of dietary consumption (143). In the non meal-eating animal both organs can generally be considered to contribute equally to overall fatty acid synthesis.

Masaro and associates (144) demonstrated several years ago that liver slices from rats consuming a carbohydrate-free diet

displayed very little capacity to incorporate ^{14}C-glucose into fatty acids. Hepatic incorporation of glucose and acetate into fatty acids both in vitro and in vivo was negatively correlated to the content of dietary fat. As little as 2.5% fat effectively depressed liver lipogenesis rates (145). This relationship existed in the presence of constant carbohydrate intake and irrespective of the degree of saturation of the dietary fat (145).

Like the liver, in vitro lipogenesis in rat epididymal adipose tissue, as quantitated by differences in CO_2 release, was markedly lowered by diets containing 48% of calories as fat (146). Adipose tissue slices taken from rats that ate a diet with 14% fat (corn oil, lard or coconut oil) incorporated 50% less ^{14}C-glucose into fatty acids than animals fed a 2% corn oil diet (147). The reduced rates of hepatic and adipose fatty acid synthesis were paralleled by similar reductions in lipogenic enzymes (147, 148).

A distinction exists between long chain triglycerides and medium chain triglycerides as to their influence on rates of lipogenesis (147). Rats that consumed diets containing 14% fat mostly as lard, corn oil, or coconut oil all displayed greatly reduced rates of hepatic fatty acid synthesis (147) whereas comparable levels of medium chain triglycerides had no depressive action on lipogenesis in comparison to a low fat basal diet. The inability of medium chain triglycerides to exert an effect on lipogenesis was attributed to the mode of absorption and rapid uptake and oxidation by liver (147).

Fasting reduces the rate of both liver and adipose tissue fatty acid synthesis in a manner similar to feeding diets high in fat (8, 31, 32). A few days of food deprivation greatly lessened the amount of ^{14}C-acetate, -glucose or -fructose recovered in hepatic fatty acids (149). A 48 hour fast reduced the rate of incorporation of ^{14}C-acetate into liver fatty acids by 90% and lowered immensely the rate of ^{14}C-pyruvate incorporation into adipose tissue fatty acids (8). Fasting for 72 hours nearly abolished liver fatty acid synthetase activity, and resulted in glucose-6-phosphate dehydrogenase and malic enzyme activities which were only one-third and one-half respectively that of the fed state (8, 148, 149).

The rate of lipogenesis was rapidly restored by refeeding, and in many instances "overshot" the original rates (8, 111, 113). Within five hours after initiation of refeeding a fat-free diet to rats fasted 48 hours, the rate of adipose fatty acid synthesis had increased 20-fold and by 24 hours adipose lipogenesis had plateaued at a rate 100-fold higher than the fasting rate (8). The extent of the rise upon refeeding depended on the type of diet refed (8). For example refeeding a fat-free, high carbohydrate diet for 40 hours resulted in a 90-fold rise over fasting in the in vitro rate of hepatic fatty acid synthesis from acetate (8) while realimentation with a lab-chow diet caused only a 15-fold increase in lipogenesis (8). The variation in rates can be explained on the basis of type of carbohydrate and level of fat in the two diets. Lipogenic enzymes also underwent large increases in activity in the liver. However activity

changes lagged behind the rise in liver fatty acid synthesis rates by eight hours which suggested the level of enzyme activity at least initially was not limiting (150). The maximal rate of fatty acid synthesis attainable under refeeding conditions may however be restricted by the level of enzyme in particular fatty acid synthetase (19).

Training rats to consume their daily food during a two or three hour period each day is a form of fasting and refeeding which precipitates a number of metabolic changes (143). Meal-trained rats (250-275 g) will consume about 80% as much food as nibbling counterparts and yet gain weight at the same rate (143). Thus these animals appear to be more efficient in body weight gain and energy gain (151). The explanation for greater efficiency of food utilization under these conditions remains unclear but apparently is not related to reduced basal metabolic rate (143).

One major change in meal-fed rats is a hypertrophy of stomach and small intestine. Because of the enlarged small intestine, the total absorptive capacity for glucose is markedly increased, but per gram of intestine no difference exists between nibblers and meal-eaters (143).

Clearly certain metabolic shifts must occur to handle the large, rapid influx of nutrients from the gut. Meal-trained rats display a greater ability than nibblers to clear blood glucose (152, 153) following an oral or intraperitoneal glucose load. This improved clearance capability is the product of higher circulating

insulin levels both in fed and fasted states and of greater tissue sensitivity to insulin (152). A comparison of liver, adipose and muscle tissues indicates that adipose tissue undergoes the greatest adaptive metabolic change in meal-eating rats (143).

In the meal-fed rat liver metabolism of glucose does not appear to differ drastically from the nibbling animal (68, 154). The liver of both the meal-eating rat and nibbling rat fasted 22 hours and refed two hours contain about the same amount of glycogen. Synthesis rates of liver glycogen may differ between nibblers and meal-eaters depending upon the initial level of hepatic glycogen (143, 154). Rats adapted to meal-eating do not show changes in hepatic activities of glucokinase, pyruvate kinase, α-glycerophosphate dehydrogenase or acetyl-CoA carboxylase (68) relative to their nibbling counterparts. Following the consumption of a meal, the rates of liver fatty acid synthesis rapidly rise such that within two hours the rates are five times pre-meal values (33, 34). Although fatty acid synthesis rates, immediately after a meal, are several fold higher in livers of meal-trained rats than in nibbling animals fasted 22 hours and refed two hours, the rate of lipogenesis in meal-fed rat liver does not reach that of the fed nibbler until five hours after meal initiation (111). The rate of hepatic fatty acid synthesis in meal-eating rats gradually returns to pre-meal values over the subsequent 22 hour period of food deprivation (33, 34).

In muscle of meal-trained rats the activity of hexokinase is about 20% above that in nibblers (68). This probably is related to the higher circulating insulin levels found in meal-eaters (152). Consistent with higher hexokinase activity in muscle, was a greater accumulation of glycogen upon realimentation by meal-eaters relative to fasted (22 hours) nibblers refed for two hours (154). Although hexokinase activity was elevated in meal-eating rats, pyruvate kinase and α-glycerophosphate dehydrogenase activities were unchanged by meal pattern (68).

During adaptation to the meal-eating regimen, the rate of adipose tissue fatty acid synthesis after a meal increased over that found in nibblers. (155). While the rate of lipogenesis was accelerating, the activity of several lipogenic enzymes was decreasing and reached a minimum at five days (155). This suggests the initial rise in lipogenesis rates in adipose tissue of meal-eating rats is not restricted by level of enzyme machinery. When rats were fully adapted to a meal-eating program, adipose tissue hexokinase, and acetyl-CoA carboxylase activities were four and ten times greater than in nibbling rats (68). In addition pyruvate kinase, pyruvate carboxylase and α-glycerophosphate dehydrogenase activities were significantly elevated in meal-fed rats (88). In accordance with large increases in glycolytic and lipogenic enzyme activities in adipose tissue, the rates of fatty acid synthesis in adipose tissue both before and after a meal were well above those found in nibbling rats (143, 154). This large rise in

lipogenesis in adipose tissue correlates well with better glucose tolerance and higher circulating insulin levels in meal-eating rats (152, 153). Glycogen stores in adipose greatly increased above those found in nibbling animals after only five days of adaptation to meal-eating and reached a point in fully adapted animals of being several fold higher (after a meal) than in nibbling rats (143, 154, 155). However prior to the meal glycogen concentrations in adipose were very similar to those found in fed or fasted nibbling rats (154). The significance of large glycogen reserves may lie in a need for the production of α-glycerophosphate for triglyceride synthesis after completion of the daily eating period (156).

The rate of fatty acid synthesis in adipose tissue of meal-trained rats was shown to be inhibited by dietary fat, but the adipose tissue may be less sensitive to dietary fat control than in the nibbling rat (157). Increasing the level of dietary fat from 10 to 20% resulted in an 80% decline in the rate of fatty acid synthesis in adipose tissue of nibbling rats, but only a 50% decline in meal-eating animals (157). A level of 30% dietary fat was needed to depress the rate of fatty acid synthesis in meal-fed rats to the same level as achieved with 20% fat in nibbling rats (157). In conjunction with reduced rates of fatty acid synthesis were depressed activities for adipose glucose-6-phosphate dehydrogenase and malic enzyme (157).

Mice. Like rats, the primary sites of fatty acid synthesis in mice are liver and adipose tissue. These organs also tend to vary

the rates of lipogenesis in response to dietary manipulations in a manner similar to rats. The percent ^{14}C-acetate recovered in fatty acids of liver slices obtained from mice fed a diet containing 15% corn oil was only 30% that recovered from mice fed a fat-free diet (158). Similarly feeding mice a diet with 15% safflower oil or triolein for five days almost completely abolished in vitro hepatic fatty acid synthesis (159). Simultaneously fat feeding was associated with a highly significant reduction in the activities of hepatic fatty acid synthetase, citrate cleavage enzyme, and glucose-6-phosphate dehydrogenase (7, 159).

The influence of dietary fat on adipose lipogenesis rates in mice is less well defined. The in vitro rate of ^{14}C-pyruvate conversion into mouse adipose tissue fatty acids was not altered by the inclusion of 4% coconut oil in a fat-free diet (7), but the addition of 2% linoleate dramatically dropped the in vitro rate of fatty acid synthesis (7). By determining the amount of dietary U^{14}C-glucose incorporated into epididymal fat pads of mice, Jansen et al. (160) found little difference in the rate of lipogenesis by mice fed 1 or 5% corn oil; but 10 and 20% dietary fat reduced the conversion of glucose to fatty acids by 50%. In comparison to the liver, their data indicated that de novo fatty acid synthesis in adipose tissue of mice was less sensitive to dietary fat inhibition. These experiments are subject to criticism because of the long time period (60 minutes) between administration of the ^{14}C-glucose and the removal

of fat pads, and for not correcting for differences in the specific radioactivity of the glucose pool (33, 160).

Mice that are on an intermittent fasting (24 hours): refeeding (48 hours) schedule show no change in body composition relative to ad libitum fed mice (161). However relative rates of fatty acid synthesis in adipose and liver tissue varied considerably. On the day of fasting liver slices had only 6% of the capacity of ad libitum fed mice to synthesize fatty acids: upon refeeding this capacity increased to 400% on the first day and 247% on the second day. The adipose tissue on the day of fast had 374% the capacity of the controls and this rose to 927 and 1100% on days one and two of refeeding (161). Hepatic malic enzyme activity remained unchanged on days of fasting and feedings, while adipose malic enzyme significantly increased upon refeeding (161).

Swine. The primary site of lipogenesis in the pig is the adipose tissue (162). When the levels of dietary corn oil were 1, 4, 7, 10 and 13%, the rate of fatty acid synthesis from glucose and the activities of malic enzyme and citrate cleavage enzyme in pig adipose tissue were depressed in a linear fashion (109, 163). The reduction in rates of lipogenesis was minimal between 1 and 4% dietary fat, while the greatest decline resulted with the increase from 10 to 13% corn oil.

Feeding weanling pigs isocaloric diets containing various amounts of corn oil caused a significant depression in malic enzyme and citrate cleavage enzyme activities as well as marked reduction

in adipose fatty acid synthesis rates (108, 163). In both studies the drop in enzyme activities was less pronounced than the decline in rate of fatty acid synthesis (108, 109, 164).

The adipose tissue of pigs responded to fasting (2-7 days) with a depressed rate of fatty acid synthesis in adipose tissue, and a concomittant drop in the activity of glucose-6-phosphate dehydrogenase, malic enzyme, citrate cleavage enzyme and acetyl-CoA carboxylase (162). Refeeding a low fat diet restored the in vitro rate of lipogenesis and associated enzymes except for the activity of citrate cleavage enzyme. In contrast refeeding a high protein diet or high fat diet prevented the restoration of lipogenesis by nearly 50% (162). As noted with feeding fat-containing diets, the response of lipogenic enzymes to fasting and refeeding was less dramatic than the observed changes in fatty acid synthesis rate. This may suggest that under such conditions initially short-term regulators are more important in controlling rates of lipogenesis and that enzyme levels are adequate and change in level as a secondary response to diet.

Chicken. Unlike the pig, chickens synthesize most of their fatty acids in the liver (164). Some conflict has existed as to whether or not dietary fat lowers hepatic lipogenesis (120, 165). Goodridge (120) fed up to 15% corn oil to growing chicks and found no reduction in the rate of hepatic fatty acid synthesis. However Yeh et al. demonstrated both in vitro and in vivo that growing chicks fed diets containing 10 or 20% corn oil displayed markedly depressed rates of lipogenesis (107). Furthermore feeding a high carbohydrate

diet to chicks previously fed a high fat diet resulted in a four-fold increase in hepatic malic enzyme activity and a two-fold increase in citrate cleavage enzyme activity plus a slight increase in hexose monophosphate shunt dehydrogenases (166). This further substantiated the adaptive nature of fatty acid synthesis.

Young growing chicks adapted to a meal-feeding regimen were unable to consume sufficient food to grow at a rate comparable to nibbling counterparts (167). However meal-feeding older chickens (900 g) did not result in a reduced body weight gain relative to nibbling animals but the meal-eating chickens did eat significantly less food (168).

In the meal-trained rat adipose tissue becomes the major site of lipogenesis and this is reflected in elevated lipogenic enzyme activities and lipogenic capacity (143). However in the meal-trained chicken the activities of hepatic malic enzyme and fatty acid synthetase were not elevated above those in nibbling chicks (143). Just prior to the meal the rate of liver fatty acid synthesis was very low and increased 50-fold within one hour after the meal (143). This great change in lipogenesis without an elevation in enzyme activity reflects transient change in substrate availability and indicates the level of enzyme is not a rate determining factor.

DE NOVO FAT SYNTHESIS AND TYPE OF DIETARY FAT

De novo fatty acid synthesis is inhibited in several species by the addition of fat to a diet (15, 145, 157-160, 163, 166). Evidence has been accumulating from rat and mouse studies which indicates that polyunsaturated fats are more effective than saturated fats in depressing hepatic and adipose lipogenesis (7-10).

Reiser and associates found that when 30% of the carbohydrate in a fat-free diet of rats was replaced with various triglycerides, rat liver fatty acid synthesis rates were differentially inhibited by the fat (169). In relation to the fat-free diet, tributyrin, tricaproin and tricaprylin had little effect on the in vivo rate of hepatic fatty acid synthesis while tricaprin, trilaurin, trimyristin and tripalmitin caused a three to five fold decline in the amount of ^{14}C-acetate recovered in fatty acids. Although lard and palmitoyl-diolein suppressed fatty acid synthesis nearly 13-fold, the percent ^{14}C-recovered in fatty acids was still twice that observed with trilinolein or safflower oil containing diets (169). The high linoleate diets resulted in negligible rates of hepatic fatty acid synthesis (169).

After five days of supplementing a high carbohydrate with 15% tripalmitin or triolein, the amount of ^{14}C-acetate incorporated by rat liver slices was less (not statistically significant) than slices from a group fed a fat-free diet. However a 15% safflower oil diet reduced the in vitro rate of fat synthesis by 65% (159). Concomittantly the activities of hepatic fatty acid synthetase,

citrate cleavage enzyme, malic enzyme, and glucose-6-phosphate dehydrogenase were consistently lowest in rats fed the safflower oil diet (159). Unlike the rat, 15% tripalmitin, triolein, or safflower oil were all effective in significantly depressing the rate of acetate incorporation into mouse liver slices. However safflower oil invariably precipitated the lowest lipogenic enzyme activities (159).

Consistent with these effects of safflower oil in rats at relatively high fat intakes were the observations of Wiegand et al. (148) who found a negative linear relationship between the activity of rat liver fatty acid synthetase and the quantity of dietary safflower oil (ranges 2.5-15%). In contrast cocoa butter was without effect on fatty acid synthetase activity until a dietary level of 15% was reached. At this point the reduction in synthetase activity was comparable to the 2.5% safflower oil diet (148).

In opposition to the previous cited work, Tepperman and Tepperman reported that 10% dietary corn oil and hydrogenated coconut oil were equally effective in depressing in vitro liver fatty acid synthesis (113). In addition when vegetable oil, hydrogenated vegetable oil, lard or corn oil was added to a high carbohydrate diet at a level of 15%, all fat sources precipitated a marked reduction in rat liver fatty acid synthesis relative to a fat-free diet. Lard and corn oil appeared to have the most efficient depressive action (145). Although the rates of fatty acid synthesis in these studies were altered equally by saturated or unsaturated dietary fat, saturated fat diets were associated with significantly

higher hepatic hexose monophosphate dehydrogenase activities and generally malic enzyme activity (112, 113, 147). The activities of hepatic glucose-6-phosphate dehydrogenase and malic enzyme have been negatively correlated to the intake of linoleate and linolenate (112). The reason(s) for maintenance of high glucose-6-phosphate dehydrogenase activity in the presence of high saturated fat diets has been attributed to a requirement of NADPH in desaturation and elongation for the synthesis of polyunsaturated acids (170).

A recent study had indicated that a high safflower oil-containing diet was more effective in significantly reducing rat liver fatty acid synthesis than comparable levels of tallow (171). However tallow had a greater inhibiting influence on adipose lipogenesis (171). In accordance with the apparent site shift in fat synthesis was a large rise in plasma triglyceride levels and a 50% decline in the activities of adipose fatty acid synthetase and acetyl-CoA carboxylase in rats fed tallow (171). Data for chicks indicated no inhibitory advantage of dietary polyunsaturated or saturated fat on hepatic fatty acid synthesis or associated enzymes (171). On the other hand tallow-fed pigs displayed significantly higher rates of adipose fatty acid synthesis and fatty acid synthetase activity than pigs receiving the safflower oil diet which agreed with the effect of tallow on rat adipose tissue (171).

There seems to be some confusion at high dietary fat levels as to the specific action of polyunsaturated fat on affecting

hepatic fatty acid synthesis. In part these discrepancies may be attributed to the very high levels of dietary fat, variations in lipid digestibility (103), and species variation (171).

Considerable evidence in mice and rats fed low levels (3%) of pure methyl esters of fatty acids has been accumulating which suggests that only polyunsaturated fatty acids are capable of lowering hepatic lipogenic enzyme activities and rates of fatty acid synthesis (7-10). Switching mice from a chow diet to a high carbohydrate, fat-free diet resulted in a greatly enhanced liver fatty acid synthetase activity (10-fold higher at 12 days and 20-fold greater at 25 days). The addition of 4% corn oil or 2% methyl linoleate at 18 days immediately reduced fatty acid synthetase activity such that within three days the activity was only three to five times above values of the animals fed chow diet (7). In contrast the inclusion of 4% hydrogenated coconut oil or 2% palmitate or oleate did not alter hepatic fatty acid synthetase activity (7).

Methyl linolenate and arachidonate also possess the ability to specifically depress hepatic fatty acid synthesis when added to a high carbohydrate, fat-free diet (8-10). The removal of essential fatty acids from a high sucrose diet resulted in a significant increase in rat liver glucose-6-phosphate dehydrogenase in only four days, and by seven days both glucose-6-phosphate dehydrogenase and fatty acid synthetase were elevated to near maximum (172). The oral administration (100 mg) of methyl linoleate, linolenate or arachidonate caused a significant drop in both enzymes within two days and

within four days all activities were comparable to essential fatty acid adequate control rats (172). Similarly linoleate intubation to rats for three days appeared to be more effective than palmitate, myristate, or oleate in depressing fatty acid synthetase or citrate cleavage enzyme (9). All dietary methyl esters examined except oleate reduced the activity of citrate cleavage enzyme, fatty acid synthetase and acetyl-CoA carboxylase (9). Linolenate and arachidonate had the greatest depressive influence on all hepatic enzyme activities (9, 172). Interestingly palmitoleic acid resulted in enzyme activity patterns identical to dietary methyl linoleate (9).

Gozukara et al. (121) proposed that the only mechanism by which polyunsaturated fats altered lipogenesis in rat liver was via a reduction in carbohydrate intake. However when rats were pair-fed a fat-free diet and subsequently intubated with oleate or linolenate precipitated a decline in hepatic glucose-6-phosphate dehydrogenase, fatty acid synthetase, and citrate cleavage enzyme (10). Gozukara et al. (121) based their conclusions only on changes in glucose-6-phosphate dehydrogenase activity which may not always reflect the response of other lipogenic enzymes (112, 170).

The ability of polyunsaturated fatty acids to control adipose tissue lipogenesis of mice and rats is less well defined than in liver. At high dietary levels both safflower oil and tallow reduced rat adipose tissue fatty acid synthesis but safflower oil permitted significantly higher rates of lipogenesis than did tallow (171). A similar phenomenon was found in swine adipose tissue (171). In contrast

mice maintained on a high sucrose plus 2% hydrogenated coconut oil for 18 days had rates of pyruvate incorporation into fatty acids by epididymal fat pad slices which were two to three times greater than mice fed a chow diet (7). Replacing the coconut oil with linoleate immediately led to a sharp decline in lipogenesis which was comparable to the rats fed chow within two days (7). Oleate also lowered fatty acid synthesis rates but at a much slower rate, while palmitate addition had no effect (7).

Adipocytes isolated from essential fatty acid deficient rats incorporated 11 times more ^{14}C-glucose per milligram triglyceride than did cells from adequate animals. Adding 5% hydrogenated coconut oil to the deficient diet for seven days did not lower the amount of ^{14}C per milligram of triglyceride, but adding 2.3% methyl linoleate reduced the rate of lipogenesis to normal (173). Adipocytes isolated from rats maintained for five weeks on a diet with 10% hydrogenated coconut oil incorporated six times more glucose into fatty acids (per milligram protein) than did adipocytes from rats fed a 10% safflower oil diet. Adipocytes from rats fed hydrogenated coconut oil or a fat-free diet for four months still incorporated more glucose into fatty acids per unit weight of lipid than did adipocytes from safflower oil diet controls. The degree of difference between coconut oil and safflower oil groups was much less than at five weeks. When the data were expressed as glucose incorporated per milligram protein, no statistical differences existed among dietary treatments in adipocyte

lipogenesis rates. The adipocytes isolated from either the rats of fat-free or hydrogenated coconut oil did tend to have higher rates than controls. The younger essential fatty acid deficient rats, regardless of the mode of data expression had a significantly increased rate of lipid synthesis (174). Essential fatty acid deficient rats have smaller body weights and presumably smaller fat cells. Therefore an admitted restriction of the previous two studies is that the rate of glucose conversion to fatty acids may differ because of differences in adipocyte size, number and triglyceride content (175). Thus an erroneous conclusion may result if data are expressed as glucose converted fatty acids per unit weight of triglyceride. Obviously less triglyceride per cell will result in a high ratio.

In comparing rats which differed in body weight by 100 grams, a product of restricted feeding, Hubbard and Matthew (176) discovered that the lighter animals had twice the number of adipocytes per 100 milligrams adipose tissue and as much as three times the number of adipocytes per gram triglyceride. Therefore the data of Du and Kruger (173) are subject to erroneous conclusions because expressing ^{14}C-glucose incorporation into fatty acids per unit triglyceride means that each unit of triglyceride from essential fatty acid deficient rats may represent three times the number of adipocytes. Ideally rates of fatty acid synthesis in adipocytes should be reported on a per cell basis. Recognizing this problem, Demeyer et al. (174) expressed their data both as per unit triglyceride and per unit

cellular protein and found no significant differences in older rats among fat-free, hydrogenated coconut oil, or safflower oil treatments. However the absolute values still showed fat-free and hydrogenated coconut oil treatments having rates of lipogenesis considerably greater than safflower oil-fed rats. Du and Kruger (173) still maintained that the essential fatty acid deficient rats continued to have a sevenfold greater rate of fatty acid synthesis based on protein content of adipocytes.

Contrary to in vivo data, fatty acid synthesis in chick hepatocytes (177), rat hepatocytes (178); and skin fibroblasts (179) is most effectively inhibited by stearate and palmitate and least influenced by linoleate and arachidonate.

The degree of inhibition of incorporation of tritium from water into rat hepatocyte fatty acids elicited by 500 μM fatty acids bound to 1% albumin after 60 minutes followed a pattern of stearate >oleate >linoleate >palmitate >myristate (178). In chick hepatocytes 500 μM palmitate in 2% albumin had little effect on C^{14}-acetate incorporation into fatty acids, whereas stearate inhibited fat synthesis by 84% (177). Skin fibroblasts exposed for 0.5 minutes to 5 μM albumin bound-fatty acids followed by a 10 minute incubation with ^{14}C-acetate displayed a marked reduction in lipogenesis rate (179). Stearate and palmitate inhibited fatty acid synthesis in these cells by 67 and 48% respectively. Linoleate and arachidonate were less effective, inhibiting synthesis by 26 and 30% respectively (179).

The changes in lipogenesis did not correlate with alterations in citrate or acid-insoluble CoA concentrations. A lack of correlation with these parameters has also been cited in adipose tissue (88).

The short time of exposure of the various types of cells to the albumin bound-fatty acids indicates a very short term type regulation of carbon flux and not a reduction in total enzyme concentration. In support of the cell culture data, Numa and associates (92) reported that purified rat liver acetyl-CoA carboxylase has a lower K_i for stearoyl-CoA (0.33 μM) than for either palmitoyl-CoA (0.91 μM) or oleyl-CoA (0.67 μM).

The marked effectiveness of stearate in prohibiting lipogenesis was suggested to be the result of a reduced rate of stearate utilization or an enhanced rate of linoleate oxidation by the liver (177, 178). Chick hepatocytes re-esterified albumin-bound palmitate to triglyceride several times faster than stearate, and oxidized palmitate to CO_2 at twice the rate (177).

The conflict between cell cultures and in vivo experiments (7-10) in relation to those fatty acids which are most inhibitory to lipogenesis is perplexing. Such conflicting evidence strengthens the need for finding a mechanistic explanation as to why polyunsaturated fatty acids appear to specifically reduce hepatic lipogenesis in essential fatty acid deprived animals. Furthermore, assessment of polyunsaturates' influence on fatty acid synthesis in essential fatty acid adequate animals becomes imperative.

The role of essential fatty acids in affecting lipogenesis in adipose tissue appears to be less well defined and more ambiguous than the data for liver tissue. In both organs, particularly adipose, one must be aware that a nonphysiological state of essential fatty acid deficiency is frequently used as a baseline. Thus results become more difficult to interpret.

PARAMETERS RELEVANT TO FAT METABOLISM AFFECTED BY DIETARY POLYUNSATURATED FATS

Hepatic fatty acid composition. Essential fatty acids are fatty acids which cannot be synthesized by the animal and thus are dietary requirements for maximal growth and prevention of skin dermatitis (11, 180). They are considered to include linoleate ($C_{18:2}$), α-linolenate ($C_{18:3}$) and arachidonate ($C_{20:4}$). Linolenate purportedly promoted growth in rats when added to a fat-free diet but was not as effective as linoleate or arachidonate. Linolenate did not prevent dermatitis in rats (180). Maximal growth and minimal dermal scores were obtained with arachidonate (180). However linoleate can be converted via desaturation and elongation to arachidonate (180). Thus linoleate will meet the arachidonate requirement if provided in sufficient amounts (1-2% dietary energy).

Rats and mice deprived of essential fatty acids undergo many changes in hepatic fatty acid composition. Most notable is an accumulation of eicosatrienoic (5, 8, 11-$C_{20:3}$), palmitoleic (9-$C_{16:1}$) and

and oleic (9-$C_{18:1}$) acids (7-9, 181). After only five days of feeding mice an essential fatty acid free diet, the linoleate content of the liver fell from 20% to 5% of the total hepatic fatty acids, by 19 days the linoleate content of the liver was less than 1% of all fatty acids. In addition the palmitoleic and oleic acid content of these livers had increased threefold while stearate and arachidonate content fell drastically (7). Comparable, but less dramatic changes occured in mouse adipose tissue (7).

As the level of linoleate, linolenate and arachidonate increased from 0 to 3.75% of dietary calories, the percentage of hepatic eicosatrienoic acid linearly declined (180). Arachidonate was the most effective acid in this respect (183). Increasing the dietary level of linoleate, linolenate or arachidonate resulted in elevated hepatic levels of the respective acids and their derivatives (180).

Rats which were previously maintained on a chow diet and then fasted for 48 hours displayed a fourfold reduction in total hepatic fatty acid content and a fivefold drop in total hepatic linoleate content (8). Upon refeeding a high carbohydrate (linoleate-free) diet, the percent hepatic linoleate declined dramatically. Part of this decline can be attributed to dilution by the tremendous increase in total hepatic saturated fatty acids which resulted from refeeding (8, 182). At 10 and 23 hours post-feeding the total hepatic content of linoleate was about 35% and 50% lower than the fasting level and within 48 hours of realimentation with

the fat-free diet, hepatic linoleate content was near zero (8). In contrast adipose tissue displayed very little change in fatty acid composition during the 48 hours refeeding period.

Bartley and Abraham (159) observed that mice and rats fed diets containing 15% safflower oil had a hepatic linoleate content several times greater than animals fed fat-free diets, or tripalmitin or tiolein containing diets. In addition the livers of mice and rats fed tripalmitin, triolein or fat-free diets had elevated amounts of palmitoleic and oleic acids (149). The increased proportion of palmitoleic and oleic acids is believed to represent de novo synthesis (8).

Depletion of liver linoleate appears to be associated with a large increase in hepatic fatty acid synthesis when mice and rats are fed a high carbohydrate diet. On the other hand supplementation of a high carbohydrate diet with pure methyl linoleate returns hepatic fatty acid synthesis and linoleate levels towards normal (7, 10). However this does not prove cause and effect relationship. High hepatic linoleate concentrations in mice and rats fed a 15% safflower oil diet were associated with very low rates of lipogenesis (159), but 15% tripalmitin and triolein diets also significantly depressed (relative to a fat-free regimen) hepatic rates of fatty acid synthesis in mice while the linoleate and arachidonate levels (per gram liver) were comparable to mice fed a fat-free diet (159). This was not the case in rats where only safflower oil precipitated a significant reduction in hepatic lipogenesis (159). Therefore

although high linoleate concentrations in the liver may be affiliated with reduced rates of lipogenesis, this is not always true, (depending upon species) and in fact the two parameters may function independently.

Desaturase activity. The elevation in eicosatrienoic ($C_{20:3}$), palmitoleic ($C_{16:1}$), and oleic ($C_{18:1}$) acid concentrations, which accompany low dietary intakes of essential fatty acids, may be an attempt to maintain vital functions (e.g. membrane structure) requiring unsaturated fatty acids (170, 183). The de novo synthesis of these unsaturated fatty acids requires the enzyme, fatty acid desaturase, and a fatty acid elongation system (11).

Three different desaturase enzymes have been proposed: a) the delta-9 desaturase removes hydrogen from carbons 9, 10 of palmitate and stearate to yield palmitoleate (9-$C_{16:1}$) and oleate (9-$C_{18:1}$0; b) delta-6 desaturase removes hydrogen from carbons 6, 7 of oleate (9-$C_{18:1}$), linoleate (9, 12-$C_{18:2}$), and linolenate (9, 12, 15-$C_{18:3}$) to form the eicosatrienoate precursor 6, 9-$C_{18:2}$, the arachidonate precursor 6, 9, 12-$C_{18:3}$, and the acid 6, 9, 12, 15-$C_{18:4}$, respectively; and c) delta-5 desaturase introduces a double bond between carbons 5, 6 of C_{20} acids to yield eicosatrienoate and arachidonate. The substrates of delta-6 desaturase are reported to be competitive with one another but the enzyme has greatest affinity for linolenate. Generally hepatic 6-desaturation exceeds 9-desaturation (170). Therefore delta-9 desaturase has been proposed to be the rate limiting reaction in in vivo production of eicosatrienoate from oleate (186). The desaturase system is a microsomal oxygenase

of liver and adipose tissue which requires NADPH as a coreductant (11), and uses the acyl-CoA derivative not the free acid as substrate. Mammalian and bird desaturases cannot remove hydrogen atoms from the sixth or third carbon atom from the methyl end of fatty acid. Thus animals fed linoleate-free diets can only synthesize 9-$C_{16:1}$, 9-$C_{18:1}$, 6, 9-$C_{18:2}$ and 5, 8, 11-$C_{20:3}$ polyenoic acids this would account for the abnormal fatty acid composition of liver and adipose tissue.

Wahle (187) has examined the differences in activity of delta-9 desaturase among the sheep, rat and chicken. Microsomes from sheep liver had very low capacity to convert stearate to oleate while the chicken had very high delta-9 desaturase capabilities. The rat liver microsomes desaturated stearate at about half the rate of chicken liver microsomes. In contrast rat and chicken adipose (perinephric) microsomes had similar desaturation rates, while sheep perinephric adipose tissue had about twice the capacity of either rat or chicken. Sheep subcutaneous fat possessed the greatest desaturase activity. Data from sheep liver and adipose microsomes indicate that the delta-9 desaturase prefers endogenous fatty acids and not exogenous long chain fatty acids (187). The delta-6 and delta-9 desaturase systems apparently are adaptable and vary independently in activity with different dietary regimens (1970). Depriving rats of a chow diet for 24 hours nearly abolished delta-9 desaturation but had little effect on delta-6 desaturation. Refeeding resulted in a marked rise in both

desaturase systems with delta-9 having the most dramatic rise (170). Refeeding an all glucose (48 hours) diet induced delta-9 but not delta-6 desaturation; refeeding 95% casein diet stimulated delta-6 desaturation tremendously with no effect on delta-9 desaturation (170). Sheep mammary gland delta-9 desaturase varied with lactation demands. Immediately post-partum the activity was high but dropped precipitously as weaning approached (187). A fat-free diet precipitated a many fold increase in delta-9 desaturation in mice and rats (170, 188) relative to a chow diet. Concomittant with this was a shift in hepatic fatty acid composition in favor of palmitoleic and oleic acids as previously discussed. Inclusion of 15% triolein or tristearin partially (50%) prevented the tremendous rise in delta-9 desaturase activity. However 15% safflower oil added to a fat-free diet nearly abolished delta-9 desaturation (188). Utilizing a 20% fat diet and various combinations of hydrogenated coconut oil and safflower oil, delta-9 desaturation in rat liver was found to linearly decrease as the dietary content of safflower oil increased (170). Thus as the intake of linoleate increases the synthesis of eicosatrienoate is lessened because flux through the rate limiting delta-9 desaturation step is reduced (170, 188).

With the use of protein synthesis inhibitors Inkpen et al. (170) demonstrated that the adaptive rise in desaturase activity upon refeeding required protein synthesis. Furthermore Oshino and Sato (189) in studies utilizing cycloheximide and actinomycin D

have stated that desaturase activity is controlled by the level of enzyme protein.

Cytochrome b_5, which is an integral part of the microsomal desaturase system, was stimulated by stearyl-CoA in rats fed a fat-free diet to yield a greater rate of oxidation. Similarly liver microsomes from rats fed safflower oil did not have an accelerated rate of cytochrome b_5 oxidation (204). Since hepatic cytochrome b_5 levels between the two treatments were similar, the influence of safflower oil on desaturase activity may partially be related to the lower rate of b_5 oxidation (188).

Interestingly, among the species studied the primary lipogenic tissues have the greatest desaturase activity. Also in non-ruminants those dietary and hormonal factors (e.g. insulin) which influence rates of lipogenesis similarly affect delta-9 desaturation rates (170, 188) which is consistent with Wahle's (187) conclusion that desaturase systems preferentially use endogenous fatty acids.

Wahle (187) has proposed some type of link between fatty acid synthetase and desaturase activities. Most likely such a link reflects the quantity of endogenous substrate for desaturation, although the possibility of desaturase end-product stimulation cannot be eliminated.

Membranes. Phospholipids are a fundamental part of eukaryote organelle membranes (190) and in essential fatty acid adequate animals the phospholipids contain a large percentage of linoleate and

arachidonate (8, 180). In the absence of dietary linoleate, arachidonate or linolenate; polyenoic acids can be synthesized from oleate and utilized for phospholipid synthesis. If oleate derived polyenoic acids are utilized, the membrane lipid composition shifts to a greater proportion of palmitoleate, oleate, and eicosatrienoate (8).

Mice and rats fed a fat-free diet for five days or fasted for 48 hours and refed a fat-free diet for 48 hours were found to greatly increase the relative amount of hepatic palmitoleate and oleate while the quantity of linoleate and arachidonate fell to a negligible amount (7, 8, 159). The lipid composition of liver nuclei, mitochondria and microsomal membranes displayed a pattern similar to whole tissue (8). Although less dramatic than in liver, animals fed low essential fatty acid diets also have abnormal fatty acid compositions in heart, adipose and total carcass (7). Aberrations in membrane fatty acid composition occur long before visible essential fatty acid deficiency symptoms (8).

As previously discussed, in association with lipid compositional changes in rat liver membranes was an enhanced rate of fatty acid synthesis (8) and an elevated delta-9 desaturase activity (170). Whether the accelerated rate of hepatic fatty acid synthesis associated with low linoleate containing diets (7-10) is a consequence of membrane compositional alterations or simply that the two events are independent phenomenons remains to be ascertained.

Long term studies with rats fed diets low in essential fatty acids have indicated that certain membrane enzymes and mitochondrial

transport may undergo changes in kinetic behavior (191, 192). Alterations in membrane function, transport or enzyme properties (e.g. ATPase) potentially could affect the overall energy metabolism of an animal and contribute to the varying effects dietary fats exert on lipogenesis. Plasma membranes of erythrocytes of rats fed diets varying in content of polyunsaturated fat contained the same amount of cholesterol and total phospholipid but the degree of unsaturation in the membrane was lower in those rats fed diets low in essential fatty acids (192). Such a change influences membrane fluidity which purportedly alters the inetic properties of (Na^+, K^+)-ATPase (192). In addition, variations in membrane lipid composition have been suggested to alter hormone receptor conformation, thereby affecting hormone interaction with cell membranes (174). However adipocytes isolated from essential fatty acid adequate and deficient rats showed the same relative increase in the rate of carbon dioxide production and fatty acid synthesis with added insulin (173).

Hepatic mitochondria isolated from rats fed a fat-free diet were reported to be in a swollen state (193). In contrast Haeffner and Privett (191) found that as the level of arachidonate in a rat diet increased, the rate of mitochondrial anion and cation translocation was enhanced when measured by swelling agents such as glutathione, phosphate, and ammonium chloride under alkali conditions.

The activities of liver mitochondrial glutamate dehydrogenase, ß-hydroxybutyrate dehydrogenase, and cytochrome C oxidase were

significantly higher in rats fed archidonate rich diets rather than hydrogenated coconut oil (191). Similarly mitochondrial ATPase activity in rats fed diets high in polyunsaturated fat was higher than in animals fed comparable diets with saturated fat (191, 194).

Plasma membrane (Na^+, K^+)-ATPase allosteric properties have been reported to be affected by membrane lipid composition and degree of saturation (192). Total (Na^+, K^+)-ATPase activity of rat erythrocytes, heart, kidney and brain microsomes was not altered by membrane fluidity changes but the regulatory mechanisms (e.g. Hill coefficient) were affected (192).

The influence these membrane alterations exert on the energy balance of an animal remain to be ascertained. Since mitochondrial properties have been most consistently altered by diets varying in degree of unsaturated fat (191-194), the function of pyruvate dehydrogenase, adenine translocase and/or citrate transport may contribute to the control of fatty acid synthesis by various dietary fats.

Albumin binding of fatty acids. Plasma free fatty acids are generally bound to albumin (195). At physiological concentrations of free fatty acids, this binding involves hydrophobic and electrostatic interactions without gross conformational changes in albumin (195). Albumin possesses various binding sites for free fatty acids and the acids appear to compete for the sites with each acid differing in affinity for albumin (196). The order of binding strength for long chain fatty acids reportedly is oleate >stearate >linoleate

>palmitate >myristate for human albumin (196). In support of this the perfused rat liver was shown to take up linoleate more readily than stearate (197).

Hepatic uptake of individual free fatty acids in humans varies with the type of acid (198, 199) such that the order of fractional splanchnic uptake per minute has been reported to be palmitoleate >linolenate >linoleate >arachidonate >oleate >palmitate >stearate. The fractional uptake by liver for oleate in male humans determined by isotope infusion was 80% greater for oleate than stearate (200). Similarly the fractional turnover rate for arachidonate in liver was determined to be 50% higher than that of oleate (199).

The greater fractional turnover rates in the splanchnic area of human subjects for unsaturated acids agrees well with their affinity for albumin (196). The physiological significance of polyunsaturated fatty acid uptake by the liver very likely depends on their plasma concentration. However the higher values are consistent with the in vivo inhibition of de novo hepatic fatty acid synthesis in rats and mice (7-10).

Although it is tempting to propose that polyunsaturated fatty acids (e.g. linoleate) can be extracted by the liver more readily than saturated acids and potentially shift the composition of hepatic long chain acyl-CoA in favor of polyunsaturated acids, not all data agree with the previous results. Perfused rat and dog livers were reported to extract all acids at similar rates (201, 202). Soler-Argilaga et al. (197) proposed that methodology in these studies

may not have been adequately sensitive to detect differences in dogs (201) but the conflicting rat data could not be resolved (202). Hagenfeldt and Wahren have reported that much of the high rate of arachidonate fractional turnover could partially be due to exchange of the radioactive label with plasma and endothelial cells (199). Therefore at this point methodological problems make clear interpretation difficult.

Turnover of polyunsaturated fatty acids from adipose. Unsaturated fatty acids may not only be extracted by the liver more readily but in addition there is evidence accumulating which suggests polyunsaturated fats are mobilized (turnover) more rapidly than saturated fats (203).

When rats were fed a diet with 30% safflower oil for eight weeks and then fasted for 72 hours, they lost a greater percentage of dry weight and two to three times more fat (most as linoleate) than animals under comparable conditions fed a lard diet. During the fast, blood glucose levels fell precipitiously and to a much lower level for the safflower oil group than the lard treatment (203). Similarly rats that consumed the safflower oil diet had the greatest rise in plasma ketone concentrations. These data suggest linoleate was mobilized and oxidized more rapidly than saturated fatty acids.

Rats fed for six weeks either a 20% lard or corn oil diet had similar weight gains but considerably different fat pad in vitro

lipolysis rates. The corn oil-fed animals had a rate of fat pad lipolysis which was 50% greater than the rats consuming lard (204).

Contrary to these previous data, DePury and Collins (205) found that essential fatty acid deficient rats possessed nearly twice the hepatic triglyceride content as essential fatty acid adequate rats. In addition the deficient animals had significantly higher serum free fatty acid concentrations. The animals in question had consumed the deficient diet for several weeks and had visible signs of dermatitis. Their body weights were substantially lower than adequate rats. Difficulty arises in examining fat metabolism parameters in animals with such body weight and physiological differences (176).

In summary there exists evidence that animals adapted to high linoleate diets have greater rates of lipolysis. In addition linoleate may be bound with the least affinity to albumin, and extracted by the liver at a greater rate than saturated fatty acids.

Prostaglandins. Prostaglandins (PG) are varied in structure and are synthesized in vivo from essential fatty acids, most notably arachidonate (206, 207). These compounds are purported to have hormone-like actions and/or may be secondary intracellular messengers related in some fashion to cAMP production (208). PGE_1 and PGE_2 are the prostaglandins studied most extensively in liver and adipose tissue as possible effectors of carbohydrate and lipid metabolism.

The dependence of prostaglandins on essential fatty acids permits speculation as to prostaglandin involvement in fat synthesis

as affected by the level of dietary essential fatty acid (205, 10).

The picture that emerges as to the influence of PGE_1 and PGE_2 on hepatic cAMP and ultimately carbohydrate and lipid metabolism is quite confusing. Lemberg and coworkers (209) found an elevated perfusate glucose concentration with PGE_1 perfusion, which was comparable to norepinephrine infusion PGE_1 and norepinephrine did not have an additive mechanism. Of possible importance was the observation that perfusion per se caused a marked release of glucose which presents the feasibility of cell damage.

One minute after intraportal injection into rats of 40 mg of PGE_1 and PGE_2, a 60% increase in hepatic cAMP occured (208). The response to PGE_1 and PGE_2 relative to tissue cAMP level was slower and much less extensive than an injection of 0.025 mg of glucagon. The addition of 20 μM PGE_1 to an in vitro hepatic adenylate cyclase assay doubled the rate of cAMP production (208). However, intraportal injection of PGE_1 and PGE_2 simultaneously with glucagon greatly prevented the rise in liver cAMP after only 15 seconds. Inhibition by PGE_1 was dependent on glucagon concentration and appeared to be competitive. Since PGE_1 had no inhibitory action on phosphodiesterase the possibility exists that glucagon and prostaglandins compete for the same binding sites. In addition PGE_1 prohibited glucagon's inactivation of glucogen synthetase (208). Of interest would have been insulin plus PGE_1 injections and their influence on carbohydrate metabolism in the liver.

Relative to insulin and protaglandins, the intravenous infusion of PGE_1 and PGE_2 (10 μg/min) lowered basal serum insulin and greatly depressed insulin response to intravenous glucose treatment. Such action was similar to the mechanism of epinephrine on insulin release, but considerably less extensive (210). The response of insulin release to prostaglandins has been found to be quite variable and depends on species, state of animals, etc. (210). One particular problem with prostaglandin injections are changes in blood pressure and blood flow. This makes interpretation of long term (minutes) studies difficult. DeRubertis and associates (208) suggested that PGE_1 and PGE_2 may function as feedback regulators on controlling glucagon's action on the liver. A similar idea has been proposed for adipose tissue (211).

Contrary to the previous reports, infusion of PGE_1 (100 μg/hr) did not significantly change hepatic cAMP during a 60 minute time period. In addition PGE_1 perfusion had no effect on perfusate free fatty acid, cAMP or glucose concentrations. PGE_1 was also without effect when administered to fasted rats. Simultaneous perfusion of epinephrine and PGE_1 following a 15-minute epinephrine treatment had no significant influence on hepatic cAMP levels although there was a trend downward (212). Levine concluded that if PGE_1 influences hepatic lipogenesis or gluconeogenesis, it does so independently of cAMP activity. A rise in hepatic PGE_1 and/or PGE_2 with increased dietary linoleate could theoretically elevate the liver concentration of cAMP and negatively influence the action of insulin (208, 212).

These possibilities are consistent with the inhibitory role of linoleate and arachidonate on liver lipogenesis, but do not explain the similar inhibitory action of linolenate which is not a precursor for prostaglandins (7-10). If PGE_1 and/or PGE_2 alter lipogenesis in the liver, the exact role and mechanism has yet to be elucidated.

The role of prostaglandins in adipose tissue appears to be more definitive. PGE_1 and PGE_2 are synthesized in adipose tissue from cis-8,11,14-eicosatrienoic acid and arachidonate respectively (213, 214). Both of these acids are derivatives of linoleic acid. The fat cell PG-synthetase appears to be membrane-associated (214). Therefore Dalton and Hope (213) presented the possibility that membrane phospholipids were the source of the $C_{20:3}$ and $C_{20:4}$ precursors of prostaglandins. As supportive evidence the analyses of fat cell lipids revealed most of the $C_{20:3}$ and $C_{20:4}$ acids were associated with phospholipid with only small amounts found in neutral lipid. These data are expressed as percent of total fatty acid and do not represent absolute amounts.

In adipose tissue PGE_1 and PGE_2 have been proposed to attenuate hormonally induced cAMP production (211, 213). Elevated adipose content of cAMP precipitated by catecholamines, theophylline, thyroxine, etc. leads to activation of hormone-sensitive lipase via a phosphorylation mechanism requiring a protein kinase (12). The result is an accelerated rate of lipolysis with free fatty acid and glycerol release into peripheral blood. To prevent exessive rates of lipolysis attenuation of this system would seem reasonable.

Whether or not prostaglandins in vivo specifically regulate adipose response to various hormonal factors via control of cAMP levels, remains to be conclusively demonstrated. Dalton and Hope (215) could barely detect prostaglandins in freshly isolated fat cells of rats. This was well below the concentration of 140 μM required to prevent theophylline-induced cAMP accumulation (213). If prostaglandins are realistic regulators then the site of control (adenyl cyclase vs. phosphodiesterase) becomes a question. Currently prostaglandins are thought to exert regulation on adenyl cyclase. However a prostaglandin effect on adenyl cyclase in cell homogenates has not been demonstrated (215).

Because linoleate and arachidonate are immediate precursors of PGE_1 and PGE_2 and because these prostaglandins appear to be involved in preventing cAMP accumulation, several workers have speculated that low dietary intakes of essential fatty acids may alter lipolysis and lipogenesis rates (10, 174, 205, 214). Essential fatty acid deficient rats (12 weeks) had substantially greater serum free fatty acid levels than adequate animals (205). Fat pads from deficient rats released much less PGE_1 and PGE_2 into an incubation media than did rats in an adequate state (214). The basal rate of in vitro glycerol release per gram of fat pad tissue was 50% greater for essential fatty acid deficient rats. Purportedly this was the product of elevated adipose cAMP due to a lack of PGE_1 and/or PGE_2. Adding PGE_1 to the media caused a slight and comparable decline in glycerol release rates for both adequate and inadequate states.

Epinephrine inclusion led to a 23% and 50% increase in lipolysis in adequate and deficient animals respectively. In both conditions inclusion of PGE_1 with epinephrine prevented the stimulus (214). These studies are complicated by the fact that essential fatty acid deficient animals are smaller and have smaller adipocytes. Therefore, per gram of tissue, the smaller rats likely have more adipocytes and greater rates of lipolysis per gram tissue (176). When glycerol release (lipolysis) from adipocytes was expressed per milligram protein, adequate and inadequate rats showed no significant differences (174). The data were quite variable but based on mean values adequate animals actually had 50% higher rate of glycerol release into media. Fain (211) has found that when lipolysis rates were expressed on a per cell basis, differences between essential fatty acid adequate and inadequate animals did not exist.

The role of PGE in attenuating lipolysis and the requirement of linoleate for prostaglandin synthesis does not fit with the observations that rats fed diets containing safflower oil mobilize more lipid and generate more ketones during fasting or upon norepinephrine treatment than rats fed diets with lard (203). However if diets high in linoleate do promote adipose tissue prostaglandin synthesis and reduce rates of lipolysis, this may be in accord with the observations that pigs and rats fed diets high in safflower oil have higher rates of adipose lipogenesis than animals fed high lard diets (171). Further work is required to clarify the role of PGE_1 and PGE_2 in hepatic and adipose lipogenesis.

PART II

DIFFERENTIAL EFFECTS OF DIETARY METHYL ESTERS OF LONG CHAIN SATURATED AND POLYUNSATURATED FATTY ACIDS ON RAT LIVER AND ADIPOSE TISSUE LIPOGENESIS

INTRODUCTION

Many individuals with hyperlipoproteinemia appear to respond to an increased proportion of dietary fat as polyunsaturated fat by displaying reduced blood triglyceride levels (1). The mechanism of polyunsaturated fatty acids still has not been elucidated. However, a specific inhibition of fatty acid synthesis in human liver, the primary site of de novo fatty acid synthesis, exerted by polunsaturated fatty acids would offer a potential explanation for lowered blood triglyceride concentrations.

Rats and mice have been the animal models most commonly used to study the mode of action of dietary fat on liver and adipose tissue lipogenesis (7-9, 121, 148, 172). Early work indicated that high fat diets of either predominately saturated or unsaturated fatty acid composition could precipitate marked depressions in rat and mouse liver and adipose tissue fatty acid synthesis (95, 169). Recently a differential response to high polyunsaturated fatty acids or saturated fat diets was reported to occur in rat adipose and liver lipogenesis (171). That is the rates of fatty acid synthesis in the liver were more depressed by polyunsaturated fatty acids while in adipose tissue the tallow diet was associated with lower rates of fatty acid synthesis.

Using high fat diets of mixed amounts of saturation presents problems in interpretation because the mode of action of specific fatty acids cannot be differentiated. By utilizing low dietary levels of pure fatty acid esters one can attribute specific mechanisms of action to particular fatty acids.

With this approach Allmann and Gibson (7) reported that supplementing a fat-free diet with 2% linoleate precipitated a rapid fall in mouse liver fatty acid synthetase activity. However, the inclusion of palmitate or oleate in the diet had no inhibitory influence. Rats also respond quickly to small amounts of dietary polyunsaturated fat by displaying significant reductions in liver fatty acid synthetase, acetyl-CoA carboxylase, citrate cleavage enzyme and glucose-6-phosphate dehydrogenase. Again, palmitate and oleate had no suppressive action (9, 10, 172).

The activities of lipogenic enzymes do not always reflect rates of fatty acid synthesis of a tissue (113). Yet little attempt has been made to correlate changes in liver lipogenic enzyme activities precipitated by polyunsaturated fatty acids to rates of hepatic fatty acid synthesis. In mice a small amount of dietary linoleate did lead to a decline in fatty acid synthesis in a liver supernatant preparation (7), whereas similar amounts of oleate and palmitate had no depressive effect. Similar comparisons with various fatty acids at low dietary intakes have not been made in rats.

Even though adipose tissue represents 50-70% of the total body de novo fatty acid synthesis in rats (154), its response to

various fatty acid methyl esters has not been significantly investigated. Supplementing a fat-free diet with linoleate or oleate was associated with a reduction in the in vitro rate of adipose tissue lipogenesis of mice. While linoleate exhibited the greatest inhibitory effect, palmitate had no depressive action (7). Only isolated adipocyte data exists for the effect of low levels of specific dietary fatty acids on lipogenesis rates in rat adipose tissue. Although there is some suggestion of an inhibitory effect of linoleate, the data are susceptible to a problem of fat cell size differences between essential fatty acid adequate and deficient rats (173, 174, 176).

Studies comparing methyl esters of saturated and unsaturated acids as well as the respective triglycerides have unfortunately overlooked differences in digestibility as a factor in explaining differential effects on lipogenesis (7-10). Additionally, cell culture and isolated hepatocyte (177-179) data indicate stearate is the most potent inhibitor of fatty acid synthesis and yet no in vivo data is available for this acid. Since little information is available on rat liver and adipose tissue fatty acid synthesis rates, we have quantitated both in vitro and in vivo rates of fatty acid synthesis in rat liver and adipose tissue and at the same time examined the activities of various lipogenic enzymes as affected by palmitate, stearate, oleate, linoleate and linolenate. Since variations in absorption may be a factor in explaining responses to various fattay acids, the apparent digestibilities of all methyl esters were determined.

METHODS

General animal handling. A high carbohydrate: fat-free basal diet (FF-diet) (Table 1) was supplemented with methyl esters of palmitate ($C_{16:0}$), stearate ($C_{18:0}$), oleate ($C_{18:1}$), linoleate ($C_{18:2}$) or linolenate ($C_{18:3}$). In experiments 1-3 esters of 99% purity were added as 3% of the daily FF-diet consumption. In experiment 4, $^{C}16:0$ was increased to 7% of the daily FF-diet intake in order to compensate for its poor digestibility. To avoid rancidity the esters were mixed into the diet daily. The animals were housed in individual stainless steel cages and rats had free access to water.

Eight male Sprague-Dawley rats (100g) per treatment were adapted to a three-hour per day meal-eating regimen (900-1200 hours). This protocol was adopted to facilitate control of carbohydrate intake among treatments. During the adaptation phase all rats received the basal diet plus 2-3% safflower oil. This minimized the unphysiological state of essential fatty acid deprivation. Following adaptation to meal-feeding (8-10 days), all animals were meal-fed the FF-diet for seven days. On the eighth day animals were matched for body weight and food intake and assigned to blocks adopting the randomized complete block design described by Steel and Torrie (217). Each block contained three or four animals depending on the number of treatments in the experiment and each experiment utilized eight blocks of animals. Each block of animals was allotted 85% of the average amount of fat-free diet consumed by those animals during the previous

TABLE 1

Fat free basal diet composition

Ingredient	Parts
Carbohydrate[1]	72.0
Casein	20.0
Nonnutritive fiber[2]	3.0
D, L-methionine	0.3
Choline chloride	0.3
Vitamin mix[3]	0.4
Mineral mix[4]	4.0
	100.0

[1]Glucose was utilized except in experiment 2 in which sucrose replaced glucose.

[2]Solka-floc. Brown Company, Berlin, New Hampshire.

[3]Vitamin mixture was that described by Yeh & Leveille (216).

[4]Rat mineral mix #4164. Teklad Test Diets, 2826 Latham Drive, Madison, Wisconsin.

seven days. Increases in food allotted were permitted when all animals in each block completed all the diet in the three hour period. Controlling carbohydrate consumption would hopefully eliminate the criticism that dietary fat depresses lipogenesis by reducing carbohydrate intake (121). After seven days of supplementation of the FF-diet with the respective esters, all animals were killed one hour following their last meal and liver and adipose tissue removed.

Fecal lipid extraction. During the seven day period of ester supplementation the animals were transferred to metabolic cages and feces were collected in order to determine the apparent digestibility of the methyl esters. Although this procedure does not permit exact calculation of the amount of absorption of each specific acid, it does permit an estimate of the apparent degree of digestion of the respective esters. Duplicate 1.0g samples of ground, dried feces were suspended in 10.0 ml 1.0 N HCl and extracted with two 15 ml volumes of chloroform:methanol (2:1). The chloroform phase was removed and dried in pre-weighed aluminum pans. The quantity of lipid was determined gravimetrically.

Enzyme assays. Immediately upon removal, liver and adipose tissue were homogenized in cold KCl (0.15 M), $MgCl_2$ (1.0 mM) and n-acetyl-cysteine (10 mM) buffer, pH 7.6. Following centrifugation at 100,000 x g for 40 minutes, the supernatant was used for quantitation of enzyme activities. Fatty acid synthetase activity was determined by following the rate of NADPH oxidation (218). Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and NADP-malic enzyme

(EC 1.1.1.40) activities were quantitated by the rate of NADP reduction (107, 219). Protein content of the supernatant fraction was quantitated by the method of Lowry et al. (220).

In vitro fatty acid synthesis. The rate of fatty acid synthesis in experiments 1 and 2 was determined by incubating 100-200 mg liver slices and pieces of adipose tissue in 3.0 ml Krebs-Ringer buffer (37^{o}) containing 0.10 units porcine insulin per ml and 100mM glucose for liver and 10mM glucose for adipose tissue (154). U-^{14}C glucose was added at a concentration of 0.1 μCi per ml buffer. In experiment 3 fatty acid synthesis was quantitated in liver slices and adipose tissue pieces using a double labelled design. $^{3}H_2O$ incorporation avoids possible differences in specific activity of fatty acid precursor pools which may result from the dietary treatments. Therefore, liver slices were incubated in Krebs-Ringer buffer (37^{o}) containing 50 μCi $^{3}H_2O$ and 0.03 μCi U-^{14}C glucose per ml buffer. Adipose tissue was incubated in Krebs-Ringer buffer (37^{o}) containing 50 μCi $^{3}H_2O$ and 0.01 μCi U-^{14}C glucose.

After two hours the tissue slices were removed and saponified. Following extraction of nonsaponifiable compounds with 3-5 ml washings of petroleum ether, the alcoholic-KOH phase was acidified with HCl and the fatty acids extracted with 3-5 ml washings of petroleum ether. The extracted fatty acids were counted in scintillation fluid.

In vivo fatty acid synthesis. In experiment 4 the in vivo rate of fatty acid synthesis was ascertained by determining the amount of $^{3}H_2O$ incorporated into liver and adipose tissue fatty acids. Each

rat was injected intraperitoneally with 1.5 mCi $^{3}H_2O$ in 0.5 ml physiological saline. The animals were killed 10 minutes post-injection. Following killing, liver and adipose tissue were rapidly removed and weighed. Epididymal fat samples (200-300 mg) were deposited directly into 30% KOH for saponification. Livers were homogenized in an equal volume of water and 0.5 ml aliquots were removed for saponification. Following extraction of fatty acids, the amount of ^{3}H in fatty acids was quantitated by liquid scintillation counting. The scintillation fluid contained 4.0g scintillant dissolved in 230 ml absolute ethanol and toluene to one liter. Plasam free fatty acids were extracted and quantitated according to the procedure described by Ko and Royer (221).

Statistics. All data were statistically evaluated by means of analysis of variance for randomized complete-block design. Treatment differences were ascertained using Tukey's t-test procedure (217).

RESULTS

Experiment 1. After three days of supplementing $C_{18:3}$ to the basal diet, food consumption became depressed. This adverse effect on appetite was attributed to rapid lipid peroxidation of residual ester in food cups which reduced diet palitability. Precautions were taken to minimize rancidity and subsequently food intake rapidly improved.

Additon of $C_{18:0}$ or $C_{18:3}$ to the fat-free diet at a level of 3% of the daily food intake had no influence on liver or epididymal

fat pad weights nor on total weight gain (Table 2). In comparison to rats pair-fed the FF-diet or FF+3% $C_{18:0}$ diet, dietary $C_{18:3}$ precipitated a significant drop in hepatic fatty acid synthetase and glucose-6-phosphate dehydrogenase activities while having no effect on malic enzyme activity. In contrast to $C_{18:3}$, supplementation with $C_{18:0}$ actually elevated hepatic fatty acid synthetase, glucose-6-phosphate dehydrogenase and malic enzyme activities over those observed in rats fed the FF-diet (Table 2). In association with changes in hepatic lipogenic enzyme activities rats fed $C_{18:3}$ had a tremendous reduction in U-^{14}C glucose incorporation into fatty acids by liver slices. Although these data substantiate earlier conclusions (7-9) that polyunsaturated fatty acids specifically inhibit hepatic fatty acid synthesis, interpretations become less conclusive after consideration is given to the very poor digestibility of $C_{18:0}$ (35%) relative to $C_{18:3}$ (89%) (Table 2).

Unlike the liver, lipogenic enzyme activities and the rates of fatty acid synthesis in rat epididymal adipose tissue were unaffected by dietary methyl ester supplementation (Table 2).

Experiment 2. Rats fed the FF or FF+$C_{16:0}$ diets differed very little in final body weights, weight gain, liver weights or epididymal fat pad weight (Table 3). However, rats fed the FF+$C_{18:2}$ diet tended to have greater weight gains and heavier fat pads. After considering the great difference in apparent digestibility (Table 3) between $C_{16:0}$ (40%) and $C_{18:3}$ (87%), these parameters seem to be

Par

Bod
Mg
Dai
Est
Liv
Epi

Enz

TABLE 2

Methyl stearate vs. linolenate - Influence on lipogenesis and lipogenic enzymes

(Experiment 1)

Parameter	Treatment		
	Basal	+$C_{18:3}$	+$C_{18:0}$
Body wgt., g	169	172	171
Wgt. gain, g	13	15	15
Daily food intake, g	11.8	11.5	11.8
Ester digestibility, %	---	89	35
Liver wgt., g	6.7 ± 0.2	6.7 ± 0.3	6.9 ± 0.2
Epididymal fat wgt., g	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
Enzyme Activities[2]			
Liver:			
FAS[3]	11 ± 1[1b]	5 ± 0.6[a]	15 ± 1[c]
G6PD[4]	97 ± 12[b]	37 ± 3[a]	121 ± 1[c]
ME[4]	15 ± 12[b]	14 ± 5[a]	21 ± 5[b]
Adipose:			
FAS[3]	38 ± 5[a]	39 ± 5[a]	42 ± 4[a]
G6PD[4]	135 ± 10[a]	109 ± 17[a]	118 ± 16[a]
ME[4]	147 ± 24[a]	168 ± 23[a]	191 ± 19[a]
Fatty Acid Synthesis[5]			
Liver[6]	300 ± 16[b]	151 ± 24[a]	381 ± 42[b]
Adipose[6]	3071 ± 467[a]	2765 ± 481[a]	4052 ± 947[a]

[1]Those values with different superscript letters are significantly different ($P < 0.05$).

[2]Mean ± SEM n=4.

[3]Nanomoles NADPH oxidized min^{-1} mg^{-1} protein at 37°. FAS = fatty acid synthetase.

[4]Nanomoles NADP reduced min^{-1} mg^{-1} protein at 25°. G6PD = glucose-6-phosphate dehydrogenase, ME = malic enzyme.

[5]Mean ± SEM n=8.

[6]Nanomoles U-^{14}C-glucose incorporated into fatty acids per 100 mg wet tissue per 2 hrs. at 37°.

TABLE 3

Methyl palmitate vs. linoleate - Influence on lipogenesis and lipogenic enzymes

(Experiment 2)

Parameter	Treatment		
	Basal	+$C_{18:2}$	+$C_{16:0}$
Body wgt., g	200 ± 5	208 ± 5	199 ± 4
Wgt. gain, g	18 ± 1	26 ± 2	19 ± 1
Daily food intake, g	13.2	13.2	13.2
Ester digestibility, %	---	87	40
Liver wgt., g	9.3 ± 0.2	9.0 ± 0.2	9.4 ± 0.2
Epididymal fat wgt., g	1.3 ± 0.1	1.5 ± 0.1	1.3 ± 0.1
Enzyme Activities[2]			
Liver:			
FAS[3]	30 ± 6[a1]	18 ± 4[a]	24 ± 6[a]
G6PD[4]	107 ± 12[b]	67 ± 5[a]	123 ± 14[b]
ME[4]	53 ± 8[b]	32 ± 4[a]	57 ± 7[b]
Adipose:			
FAS[3]	41 ± 7[a]	41 ± 6[a]	40 ± 4[a]
G6PD[4]	209 ± 41[a]	182 ± 34[a]	190 ± 39[a]
ME[4]	218 ± 29[a]	197 ± 14[a]	216 ± 20[a]
Fatty Acid Synthesis[2]			
Liver[5]	159 ± 29[a]	121 ± 23[a]	131 ± 14[a]
Adipose[5]	2081 ± 209[a]	1876 ± 160[a]	2132 ± 244[a]

[1]Values with different superscript letters are significantly different ($P < 0.05$).

[2]Mean ± SEM n=8.

[3]Nanomoles NADPH oxidized min^{-1} mg^{-1} protein at 37°. FAS = fatty acid synthetase.

[4]Nanomoles NADP reduced min^{-1} mg^{-1} protein at 25°. G6PD = glucose-6-phosphate dehydrogenase, ME = malic enzyme.

[5]Nanomoles U-^{14}C-glucose incorporated into fatty acids per 100 mg wet tissue per 2 hrs. at 37°.

reasonable products of differences in energy intake and/or essential fatty acid status.

In this experiment sucrose replaced glucose as the source of carbohydrate. Therefore, the activities of the hepatic lipogenic enzymes are higher than in experiment 1 (123). Even though rats fed the FF+$C_{18:2}$ diet had hepatic fatty acid synthetase activity 40% below that of the basal group, neither dietary $C_{18:2}$ nor $C_{16:0}$ signifcantly altered liver fatty acid synthetase activity (Table 3) according to Tukey's t-test analysis (217). Hepatic activities of glucose-6-phosphate dehydrogenase and malic enzyme were significantly depressed when the FF+$C_{18:2}$ diet was fed but not when the FF-diet was supplemented with $C_{16:0}$ (Table 3). Rates of in vitro hepatic fatty acid synthesis were slightly lower in animals fed methyl esters (Table 3), but like fatty acid synthetase activity these differences were not significantly reduced by dietary $C_{18:2}$. Like $C_{18:0}$, methyl $C_{16:0}$ was very poorly digested (40%) and may partially explain the lack of influence on the hepatic activities of lipogenic enzymes.

As in experiment 1, $C_{18:2}$ or $C_{16:0}$ supplementation to a fat-free diet had no influence on adipose fatty acid synthetase, glucose-6-phosphate dehydrogenase or malic enzyme activities. Similarly, in vitro U-^{14}C glucose incorporation into adipose tissue fatty acids was not impaired by dietary esters of $C_{18:2}$ or $C_{16:0}$ (Table 3).

Experiment 3. In order to investigate the digestibility of of $C_{18:1}$ and its role in controlling lipogenesis, and to re-examine the effect of $C_{18:2}$ on fatty acid synthesis, these esters were

TABLE 4

Methyl oleate vs. linoleate - Influence on lipogenesis and lipogenic enzymes

(Experiment 3)

	Treatments[1]		
	Basal	$+C_{18:2}$	$+C_{18:1}$
Body wgt., g	191 ± 5	198 ± 4	196 ± 5
Wgt. gain, g	28 ± 2	34 ± 0.8	30 ± 2
Daily food intake, g	13.6	13.8	13.6
Ester digestibility, %	---	87	88
Liver wgt., g	7.4 ± 0.2[b2]	7.6 ± 0.2[b]	7.4 ± 0.2[b]
Epididymal fat wgt., g	1.8 ± 0.1	2.1 ± 0.1	2.0 ± 0.1
Plasma FFA, μeq/L	541 ± 44	552 ± 38	580 ± 56
Enzyme Activities			
Liver:			
FAS[3]	16 ± 2[c]	10 ± 1[b]	14 ± 1[c]
G6PD[4]	142 ± 15[c]	86 ± 7[b]	142 ± 8[c]
ME[4]	36 ± 2[c]	25 ± 3[b]	37 ± 3[c]
Adipose:			
FAS[3]	32 ± 2[b]	31 ± 3[b]	33 ± 3[b]
G6PD[4]	140 ± 13[b]	136 ± 11[b]	124 ± 5[b]
ME[4]	221 ± 13[b]	221 ± 10[b]	209 ± 7[b]
In vitro Fatty Acid Synthesis			
Liver:			
U-^{14}C-glucose[5]	183 ± 22[c]	135 ± 17[b]	159 ± 19[b,c]
$^{3}H_2O$[6]	1642 ± 180[c]	1375 ± 136[b]	1539 ± 174[b,c]
Adipose:			
U-^{14}C-glucose[5]	1808 ± 138[b]	1821 ± 112[b]	1600 ± 148[b]
$^{3}H_2O$[6]	11,584 ± 1032[b]	12,278 ± 933[b]	9877 ± 863[b]

[1] Mean ± SEM, n=8.

[2] Values with different superscript letters are significantly different ($P < 0.05$).

[3] Nanomoles NADPH oxidized min^{-1} mg^{-1} protein at 37°. FAS = fatty acid synthetase.

[4] Nanomoles NADP reduced min^{-1} mg^{-1} protein at 25°. G6PD = glucose-6-phosphate dehydrogenase, ME = malic enzyme.

[5] Nanomoles U-^{14}C-glucose incorporated into fatty acids per 100mg wet tissue per 2 hrs. at 37°.

[6] Dpm ^{3}H-incorporated into fatty acids per 100mg wet tissue per 2 hrs at 37°.

supplemented for seven days to a fat-free basal diet in a manner similar to experiments 1 and 2. Digestibility of $C_{18:1}$ was comparable to $C_{18:2}$ (Table 4). In addition $C_{18:2}$ and $C_{18:1}$ supplementation resulted in similar epididymal fat pad weights which were slightly heavier than in basal rats. Weight gain was the greatest for rats fed $C_{18:2}$ (Table 4). Dietary methyl ester supplementation did not significantly alter liver weights (Table 4).

Both dietary $C_{18:1}$ and $C_{18:2}$ lowered the activity of hepatic fatty acid synthetase but only the effect of $C_{18:2}$ attained statistical significance (Table 4). As observed in experiments 1 and 2, only the essential fatty acid, $C_{18:2}$, significantly depressed glucose-6-phosphate dehydrogenase or malic enzyme activity in the liver. Unlike experiment 2, dietary $C_{18:2}$ significantly impaired the rate of U-^{14}C glucose and $^{3}H_2O$ incorporation into hepatic fatty acids. Supplementation of the basal diet with $C_{18:1}$ yielded intermediate rates of fatty acid synthesis by liver slices based on either U-^{14}C glucose or $^{3}H_2O$ incorporation (Table 4). Both U-^{14}C glucose and $^{3}H_2O$ produced comparable results indicating that the fatty acid precursor specific activity in the liver slices was not influenced by dietary treatment.

As in previous experiments, rates of fatty acid synthesis and activities of lipogenic enzymes in epididymal adipose tissue were not influenced by the source of dietary fatty acid (Table 4).

An elevation in plasma free fatty acids (FFA) may be associated with a reduced rate of hepatic lipogenesis (110). Therefore, blood samples were obtained at the time of sacrifice in order to

determine if differences in plasma free fatty acid concentrations could potentially explain the marked inhibition of $C_{18:2}$ on hepatic lipogenesis. However, plasma FFA did not differ in concentration among the treatments (Table 4). The lack of difference in total concentration of the plasma FFA does not necessarily preclude the possibility of an increase in concentration of liver tissue long chain free fatty acids or their CoA derivatives (129).

Experiment 4. The first three experiments indicated two points which required further clarification: a) the lack of influence of $C_{16:0}$ on hepatic lipogenesis might be partially explained by its poor digestibility (Table 3); (b) $C_{18:3}$ appeared to inhibit hepatic fatty acid synthesis and associated enzymes more effectively than $C_{18:2}$, however these esters were not compared within the same experiment. Therefore, experiment 4 was conducted to determine if, after correction for low digestibility, dietary $C_{16:0}$ could impair hepatic lipogenesis. In additon dietary $C_{18:2}$ and $C_{18:3}$ were compared for influence on hepatic fatty acid synthesis rate.

The amount of each ester absorbed daily was not significantly different among fatty acid types (Table 5). Weight gain did not differ significantly among dietary treatments, but the rats fed fatty acids tended to gain slightly more weight than the control animals (Table 5) which was in accord with the higher energy intakes and/or adequate essential fatty acid status of these animals. Clearly the inclusion of 3% $C_{18:2}$ or $C_{18:3}$ in a fat-free diet markedly and significantly depressed the activities of hepatic fatty acid synthetase,

TABLE 5

Influence of methyl palmitate, linoleate and linolenate on fatty acid synthesis and hepatic lipogenic enzymes

(Experiment 4)

	Treatments[1]			
	Basal	+7% $C_{16:0}$	+3% $C_{18:2}$	+3% $C_{18:3}$
Final body wgt., g	166 ± 2	172 ± 2	171 ± 3	170 ± 2
Wgt. gain, g	16 ± 1	20 ± 1	19 ± 2	19 ± 2
Daily food intake - g	11.1 ± 0.2	11.0 ± 0.2	10.9 ± 0.3	10.9 ± 0.2
Daily ester absorbed, mg	---	320 ± 6	296 ± 7	294 ± 6
Liver wgt., g	6.7 ± 0.1	7.5 ± 0.3	7.1 ± 0.1	7.3 ± 0.2
Hepatic enzyme activities:				
FAS[3]	16 ± 1[c2]	17 ± 1[c]	8 ± 1[b]	6 ± 1[b]
G6PD[4]	132 ± 7[c]	172 ± 14[d]	48 ± 6[b]	41 ± 1[b]
ME[4]	58 ± 5[c]	68 ± 3[c]	30 ± 3[b]	25 ± 2[b]
In vivo fatty acid synthesis:				
Liver[5]	4,218 ± 387[c]	4,576 ± 137[c]	1,948 ± 281[b]	1,704 ± 257[b]
Adipose[5]	10,616 ± 3257[b]	11,226 ± 1913[b]	9,760 ± 3811[b]	10,099 ± 2411[b]

[1]Mean ± SEM, n=8.

[2]Those values with different superscript letters are significantly different ($P < 0.05$).

[3]Nanomoles NADPH oxidized min^{-1} mg^{-1} protein at 37°. FAS = fatty acid synthetase.

[4]Nanomoles NADP reduced min^{-1} mg^{-1} protein at 25°. G6PD = glucose-6-phosphate dehydrogenase, ME = malic enzyme.

[5]Dpm tritium incorporated into fatty acids per gram tissue per 10 minutes.

glucose-6-phosphate dehydrogenase and malic enzyme as well as the in vivo rate of fatty acid synthesis in liver (Table 5). Even when the amount of $C_{16:0}$ absorbed was equivalent to that of $C_{18:2}$ or $C_{18:3}$, the saturated acid had no inhibitory action on hepatic fatty acid synthetase activity or rate of fatty acid synthesis. In fact $C_{16:0}$ resulted in an enhanced glucose-6-phosphate dehydrogenase activity in the liver. Again as in experiments 1-3, adipose tissue lipogenesis was unaltered by the type of fatty acid added to the basal diet. Apparently only polyunsaturated fatty acids impair hepatic fatty acid synthesis when provided at low levels in an otherwise fat-free diet. In addition $C_{18:2}$ and $C_{18:3}$ were equally efficient in eliciting these results.

DISCUSSION

Our experimental design was such that the average daily intake of carbohydrate was identical among rats of different treatments. This design was in response to the conclusion by Gozukara et al. (121) that the depression in lipogenesis elicited by polyunsaturated fatty acids is the product of reduced carbohydrate intake caused by adding fat to the diet. In addition our protocol permitted attributing any differences in lipogenic activity of liver and adipose tissue solely to dietary fatty acid.

The apparent digestibility coefficients determined for various long chain fatty acid methyl esters clearly indicate that dramatic

differences exist between the uptake of $C_{16:0}$ or $C_{18:0}$ and $C_{18:2}$ or $C_{18:3}$ (Tables 2-4). In light of the very poor digestibility of methyl $C_{16:0}$, interpreting earlier studies which compared the effects of $C_{16:0}$ to $C_{18:2}$ or $C_{18:3}$ on liver lipogenic enzyme activities becomes very difficult (7-9). Since methyl $C_{18:1}$ has a degree of digestion comparable to $C_{18:2}$ or $C_{18:3}$, these comparisons are more meaningful (10). Lack of consideration of variations in digestibility of saturated and unsaturated pure triglycerides may also contribute to the lack of inhibitory response of hepatic fatty acid synthesis noted in rats fed tripalmitin or triolein (103, 159).

$C_{18:0}$ and $C_{16:0}$ rather than unsaturated fatty acids have been reported to be more potent inhibitors of fatty acid synthesis in isolated chick hepatocytes (177), rat hepatocytes (178) and human skin fibroblasts (179). However, there is a paucity of information regarding the effect of dietary $C_{18:0}$ on lipogenesis in vivo. In the present study dietary $C_{18:0}$ was associated with a significant increase in hepatic fatty acid synthetase, glucose-6-phosphate dehydrogenase and malic enzyme activities over both fat-free and $C_{18:3}$ treatments, while the in vitro rate of liver fatty acid synthesis was comparable to the animals fed the FF-diet and over twice that of the $C_{18:3}$ group (Table 2). The lack of inhibitory influence of $C_{18:0}$ and the apparent conflict between cell cultures and in vivo data can at least be partially attributed to the very poor, apparent absorption (35%) of $C_{18:0}$. Future experiments should be designed to compensate for the poor

digestibility of $C_{18:0}$ before sound conclusions about its inhibitory efficacy can be made in vivo.

The arguments of Gozukara et al. (121) were based on changes in hepatic glucose-6-phosphate dehydrogenase activity which was assumed to reflect rates of fatty acid synthesis. Lipogenic enzyme activities, particularly glucose-6-phosphate dehydrogenase and malic enzyme, do not always positively correlate with rates of fatty acid synthesis (113). This was demonstrated in experiments 1 and 4 in which rates of hepatic fatty acid synthesis were not significantly elevated by 3% $C_{18:0}$ or 7% $C_{16:0}$ over rats fed a FF-diet, but $C_{18:0}$ was associated with a significant rise in liver glucose-6-phosphate dehydrogenase, malic enzymes and fatty acid synthetase activities (Table 2) and $C_{16:0}$ resulted in a significant increase in glucose-6-phosphate dehydrogenase activity (Table 5). Century (112) has found that the activity of glucose-6-phosphate dehydrogenase and malic enzyme in rat liver is inversely related to the dietary content of polyunsaturated fat. Furthermore, Tepperman and Tepperman (113) demonstrated that rats fed high saturated fat or unsaturated fat diets had comparable rates of in vitro hepatic fatty acid synthesis, but that animals on the saturated fat diet possessed greater hexose-monophosphate dehydrogenase activity. High glucose-6-phosphate dehydrogenase and malic enzyme activities in liver of rats fed saturated fats probably are in response to an elevation in desaturase and elongation activities for monene acid formation (113, 170).

Since the activity of various lipogenic enzymes cannot always be extrapolated to rates of fatty acid synthesis, our studies have expanded earlier work in examining both enzyme activities and in vitro and in vivo rates of fatty acid synthesis in liver and adipose tissue of rats. Low dietary levels of $C_{18:2}$ and $C_{18:3}$ very effectively inhibit liver lipogenic enzyme activities (Tables 2-5) and support the conclusion that dietary polyunsaturated fatty acids are more effective than saturated fatty acids in precipitating a dimunition in hepatic lipogenic enzyme activities (9, 10, 172) in rats and mice. For example, the consumption of a high carbohydrate diet containing 2.5% safflower oil caused a significant decline in rat liver fatty acid synthetase activity in seven days relative to rats fed a FF-diet. In contrast supplementing the FF-diet with cocoa butter had no substantial effect on fatty acid synthetase activity until the dietary content reached 15% (148). Unlike lipogenic enzymes, the in vitro rate of fatty acid synthesis appeared to be more variable in response to dietary fatty acids (Tables 2-4).

Even though $C_{18:1}$ was ineffective in depressing liver lipogenic enzyme activities (Table 4) its addition to the basal diet did reduce the rate of fatty acid synthesis to a level intermediate to basal or saturated fatty acid and polyunsaturated fatty acid treatments. The enzyme effect of $C_{18:1}$ in liver agrees with previous rat and mouse data and its intermediate action on lipogenesis concurs with observations in rats fed low fat diets (222).

The failure of 3% added $C_{16:0}$ to reduce hepatic lipogenesis agrees with its effect found in mice fed 2% $C_{16:0}$ (7). However, both of these experiments are complicated by the low digestibilities of $C_{16:0}$ (Table 2). In experiment 4 the level of $C_{16:0}$ supplementation was increased such that the amount of $C_{16:0}$ apparently absorbed was similar to $C_{18:2}$ and $C_{18:3}$ (Table 5). Under these conditions the rate of in vivo fatty acid synthesis and the activities of fatty acid synthetase, glucose-6-phosphate dehydrogenase and malic enzyme were not depressed below the FF-group (Table 5). Only $C_{18:2}$ and $C_{18:3}$ were effective in lowering these rat liver lipogenic enzyme activities as well as depressing hepatic in vivo fatty acid synthesis by more than 50% (Table 5).

Our research with the effect of various fatty acids on adipose tissue (Table 2-5) fatty acid synthesis represents the first of such comparisons in rats.

Generally the addition of fat to a low-fat diet will lead to a drop in adipose tissue lipogenesis rates (171). However, in this study the in vitro and in vivo rate of fatty acid synthesis and the activitities of lipogenic enzymes of adipose tissue from meal-fed rats of comparable body weights remained unchanged with dietary fat supplementation regardless of the type of fat utilized (Tables 2-5). This is in conflict with data obtained with adipocytes isolated from rats fed a fat-free diet or a high carbohydrate diet containing hydrogenated coconut oil for several weeks. Under these conditions the rates of fatty acid synthesis from glucose were many times

greater in adipocytes from rats fed hydrogenated coconut oil than for cells from essential fatty acid adequate animals (173, 174). In both instances the essential fatty acid deficient rats had smaller body weights and presumably smaller fat cells. Therefore, a restriction of the previous two studies is that the rate of glucose conversion to fatty acids may differ because of differences in adipocyte size, number and triglyceride content (176).

The meal-eating protocol and level of dietary fat adopted for these experiments may have contributed to the inability to detect a depression in adipose tissue lipogenesis. Rats adapted to meal-eating have sizable increases in adipose and liver tissue fatty acid synthesis rates, with adipose and liver constituting 90% and 10% of total fatty acid synthesis, respectively. Thus, meal-eating rats may be less sensitive to dietary fat control of lipogenesis than nibblers.

The stimulation in adipose tissue lipogenesis which occurs after consumption of the daily meal may well be related to the rapid absorption of glucose and subsequent production of α-glycerolphosphate in adipose tissue. This in turn provides for fatty acid esterification and reversal of potential inhibition by elevated levels of tissue free fatty acids or CoA derivatives (156). The level of dietary fat (3%) in our studies may have been too low to exert an inhibitory effect in adipose tissue because the influx of glucose and production of glyceride-glycerol is sufficiently large to permit rapid esterification of incoming fatty acids generated by lipoprotein lipase. Such action

would prevent the free fatty acids or their CoA derivatives from inhibiting rates of adipose lipogenesis.

Our data demonstrate that when utilizing various fatty acid methyl esters for examining their effect and mode of action on liver and adipose lipogenesis differences in digestibility between saturated and unsaturated acids must not be ignored. The inhibition of rat liver fatty acid synthesis by low dietary levels of $C_{18:2}$ or $C_{18:3}$ appears to be very efficient and specific for these acids since there were no differences in carbohydrate intake among fat-free, saturated fat or unsaturated fat groups, and because amounts of absorbed $C_{16:0}$ or $C_{18:1}$ equivalent to $C_{18:2}$ ro $C_{18:3}$ had little or no influence on liver lipogenesis. In general the activity of fatty acid synthetase in rats fed $C_{18:2}$ or $C_{18:3}$ correlated well with rates of fatty acid synthesis in the liver. Unlike the liver, adipose tissue fatty acid synthesis and associated enzymes in meal-fed rats was totally unaffected by all fatty acids investigated.

PART III

SPECIFIC INHIBITION OF HEPATIC FATTY ACID SYNTHESIS EXERTED BY DIETARY LINOLEATE AND LINOLENATE IN ESSENTIAL FATTY ACID ADEQUATE RATS

---

[1]Reproduced by permission of LIPIDS from an article entitled Specific Inhibition of Hepatic Fatty Acid Synthesis Exerted by Dietary Linoleate and Linolenate in Essential Fatty Acid Adequate Rats by S. D. Clarke, D. R. Romsos, and G. A. Leveille.

INTRODUCTION

Ingestion of large amounts of either unsaturated or saturated fat sources markedly inhibit rat liver and adipose tissue fatty acid synthesis (148, 157, 171). Unsaturated fatty acids may be more effective in inhibiting rat liver lipogenesis than are saturated fatty acids (148, 171) but detecting the mode of action of this specific effect may be masked when using high dietary levels of fat.

When linoleate ($C_{18:2}$), linolenate ($C_{18:3}$) and arachidonate ($C_{20:4}$) were supplemented to a fat-free diet of rats and mice, they exerted a specific inhibitory action on hepatic lipogenesis (8-10, 172). Furthermore, the oral administration of methyl $C_{18:2}$, $C_{18:3}$ or $C_{20:4}$ to rats fed a fat-free diet for seven days caused a significant decline in liver glucose-6-phosphate dehydrogenase and fatty acid synthetase activities within two days (172).

Feeding an essential fatty acid deficient diet to rats leads to marked changes in total liver and hepatic organelle fatty acid composition (8, 191). Rats previously fasted for 48 hours displayed large increases in mitochondrial, microsomal and nuclear content of palmitoleic and oleic ($C_{18:1}$) acids and decreases in $C_{18:2}$ and $C_{20:4}$ content within 12 to 18 hours after refeeding a fat-free diet (8). In addition the ability of rat liver mitochondria to translocate anions and cations, as measured by swelling properties, and the

activities of glutamate dehydrogenase, β-hydroxybutyrate dehydrogenase and cytochrome C oxidase appeared to be lowest in rats fed diets low in essential fatty acids for prolonged periods of time (191). Most earlier studies examining the effect of various dietary fatty acids on rat liver and adipose tissue lipogenesis rates have involved supplementation of essential fatty acid deficient diets (8-10, 172) which are known to greatly alter liver organelle fatty acid composition (8, 191) and possibly mitochondrial function (191). To avoid this abnormal state, the experiments in this report were designed to investigate the ability of dietary $C_{16:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ to inhibit rat liver and adipose tissue lipogenesis when supplemented to essential fatty acid adequate diets.

METHODS

Gozukara et al. (121) have proposed that polyunsaturated fatty acids exert an inhibitory action on rat liver fatty acid synthesis by precipitating a depression in carbohydrate intake. Therefore, the male Sprague-Dawley rats utilized in these studies were adapted to a three hour per day (900-1200 hrs) meal-eating regimen in order to facilitate equalization of food intake among treatments. A high glucose-casein basal diet (Table 1) containing either 2.5% safflower oil (experiment 1) or 1% safflower oil (experiment 2) was utilized to ensure adequate essential fatty acid status (180). Following adaptation to meal-eating, blocks of four animals per block, carefully matched

TABLE 1

Basal diet ingredients

Ingredient	Percent	
	Exper. 1	Exper. 2
Glucose	69.5	71.0
Casein	20.0	20.0
Nonnutritive fiber[a]	3.0	3.0
Mineral mix[b]	4.0	4.0
Vitamin mix[c]	0.4	0.4
Choline-chloride	0.3	0.3
Methionine	0.3	0.3
Safflower oil	2.5	1.0

[a]Solka-floc, Brown Company, Berlin, New Hampshire 03570.

[b]Rat mineral mix #4164, Teklad Test Diets, 2826 Latham Dr., Madison, Wisconsin 53713

[c]See Y. Y. Yeh and G. A. Leveille, (216).

for body weight and food intakes, were assigned to respective treatments. Within each block of animals food intake was constant among treatments. Methyl esters of palmitate ($C_{16:0}$), $C_{18:1}$, $C_{18:2}$ or $C_{18:3}$ were supplemented to the basal diet as 3% of the daily food allotment. In experiment 1 the esters were mixed into the diet daily and fed for seven days prior to sacrifice. In experiment 2 the esters were intubated prior to each meal for 10 days before sacrifice.

In addition to the animals supplemented with various esters for seven or 10 days, a second set of rats in each experiment was concurrently maintained on the basal diet until the day of sacrifice. These animals were then fed one meal containing 3% (experiment 1) or 5% (experiment 2) of the day's food allotment as methyl $C_{18:3}$.

Liver and adipose tissue fatty acid synthesis rates were ascertained by intraperitoneal injection of 1.5 mCi ^3H_2O (33). Glucose-6-phosphate dehydrogenase (EC 1.1.1.49), NADP-malic enzyme (EC 1.1.1.40) and fatty acid synthetase activities were quantitated as described previously (107, 218, 219). Protein content of supernatant fractions was determined by the method of Lowry et al. (220).

To determine the digestibility of $C_{16:0}$ the rats (experiment 1) were placed in metabolic cages for days 2-6 of the ester feeding period and feces were collected. The feces were extracted using 1 N HCl and chloroform:methanol.

The data were analyzed with analysis of variance for a randomized complete block design and treatment differences were ascertained

by Duncan's multiply range (experiment 1) and Tukey's t-test (experiment 2). Data for the one meal of $C_{18:3}$ were compared to basal groups using a student t-test (217).

RESULTS AND DISCUSSION

Growth parameters. Because the animals in these experiments (Tables 2-5) were pair-fed, no differences existed in food or carbohydrate intakes. Final body weight and total weight gain for seven days were comparable among treatments after seven days (Table 2) but 10 days of ester supplementation resulted in greater body weight and weight gain (Table 3). The heavier fat pad weights for the $C_{18:1}$ and $C_{18:2}$ groups (Table 2) are in accord with slightly greater energy intakes of these animals due to the high digestibility of $C_{18:1}$ and $C_{18:2}$ (223). Although the digestibility of $C_{16:0}$ was poor (57%), the inclusion of 2.5% safflower oil did improve the value over that determined in a fat-free (FF) diet (223).

Liver. Dietary $C_{18:2}$ and $C_{18:3}$ were very effective in depressing fatty acid synthetase and glucose-6-phosphate dehydrogenase activities (Tables 2 and 3), with $C_{18:2}$ and $C_{18:3}$ having equal efficacy (Table 3). When the basal diet contained 2.5% safflower oil, $C_{18:2}$ supplementation depressed malic enzyme activity the most but was only significantly lower than the $C_{18:1}$ group (Table 2). The specific action of dietary $C_{18:2}$ on liver lipogenic enzyme activities in essential

TABLE 2

Effect of $C_{16:0}$, $C_{18:1}$ or $C_{18:2}$ supplementation to essential fatty acid adequate diet (2.5% safflower oil) on rat liver lipogenesis

	Dietary Fatty Acid			
	Basal	+3% $C_{16:0}$	+3% $C_{18:1}$	+3% $C_{18:2}$
Body wgt., g[1]	149 ± 2	151 ± 3	151 ± 3	151 ± 2
Total wgt. gain, g	21 ± 1	22 ± 1	23 ± 1	23 ± 1
Daily food intake, g	10.5	10.5	10.5	10.5
Ester digestibility, %	---	57	88[2]	87[2]
Liver wgt., g	6.6 ± 0.2	6.5 ± 0.2	6.7 ± 0.2	6.8 ± 0.1
Epididymal fat wgt., g	0.82 ± 0.08[3ab]	0.74 ± 0.05[ab]	1.03 ± 0.04[b]	0.98 ± 0.05[a,b]
Enzyme activities[1]:				
FAS[4]	6.7 ± 0.7[b]	6.7 ± 0.7[b]	7.4 ± 0.7[b]	5.2 ± 0.6[a]
G6PD[5]	65 ± 4[b]	63 ± 10[b]	69 ± 8[b]	43 ± 7[a]
ME[5]	44 ± 2[a,b]	43 ± 8[a,b]	50 ± 5[b]	32 ± 3[a]
In vivo FA-synthesis[6]	7221 ± 1911[a]	10,153 ± 587[a]	10,652 ± 1578[a]	8649 ± 860[a]

[1]Growth parameters and enzyme activities are mean ± SEM, n=8.

[2]Determined by Clarke et al. (223) in ester supplementation of a fat-free diet.

[3]Those values with different superscripts are significantly different ($P < 0.05$).

[4]Nanomoles NADPH oxidized min^{-1} mg $protein^{-1}$ at 37^{o}. FAS = fatty acid synthetase.

[5]Nanomoles NADP reduced min^{-1} mg $protein^{-1}$ at 25^{o}. G6PD = glucose-6-phosphate dehydrogenase, ME = malic enzyme.

[6]Mean ± SEM, n=7; dpm ^{3}H incorporated into fatty acids per g liver in 15 minutes.

TABLE 3

Effect of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ supplements on liver lipogenesis in rats fed an essential fatty acid adequate diet (1% safflower oil)

	Dietary Fatty Acid			
	Basal[1]	+3% $C_{18:1}$[1]	+3% $C_{18:2}$[2]	+3% $C_{18:3}$[2]
Body wgt., g	150 ± 2[3a]	156 ± 2[a,b]	161 ± 2[a]	159 ± 3[a]
Total wgt. gain, g	22 ± 1[a]	27 ± 3[a,b]	29 ± 1[b]	27 ± 2[a,b]
Daily food intake, g	9.6	9.5	9.8	9.5
Liver wgt., g	6.9 ± 0.2[a]	7.1 ± 0.3[a,b]	7.4 ± 0.3[a,b]	7.8 ± 0.2[b]
Enzyme Activities:				
FAS[4]	12.3 ± 0.7[b]	11.3 ± 0.5[b]	7.6 ± 0.5[a]	7.6 ± 0.4[a]
G6PD[5]	110 ± 12[c]	82 ± 6[b]	47 ± 5[a]	50 ± 5[a]
In vivo FA-synthesis[6]	4207 ± 238[c]	3105 ± 234[b]	2455 ± 185[a,b]	1990 ± 107[a]

[1]Mean ± SEM, n=7.

[2]Mean ± SEM, n=9.

[3]Those values with different superscripts are significantly different ($P < 0.05$).

[4]Nanomoles NADPH oxidized min^{-1} mg $protein^{-1}$ at 37°. FAS = fatty acid synthetase.

[5]Nanomoles NADP reduced min^{-1} mg $protein^{-1}$ at 25°. G6PD = glucose-6-phosphate dehydrogenase.

[6]Dpm ^3H incorporated into fatty acids per g liver in 10 minutes.

fatty acid adequate rats is similar to its effect in rats fed a FF-diet supplemented with $C_{18:2}$ (8-10, 172). Contrary to the hepatic enzyme activity pattern in experiment 1, the in vivo rate of ^3H_2O incorporation into hepatic fatty acids showed no statistical change from basal due to dietary fatty acids (Table 2). Such lack of statistical difference was partially the product of large experimental variation as indicated by an experimental coefficient of variation of 44%. Unlike experiment 1, supplementation of a basal diet containing 1% safflower oil (experiment 2) with 3% $C_{18:2}$ or $C_{18:3}$ resulted in a dramatic drop in the rate of ^3H_2O incorporation into liver fatty acids and a significant decline in hepatic fatty acid synthetase and glucose-6-phosphate dehydrogenase activities (Table 3). In addition, $C_{18:1}$ supplementation precipitated a rate of liver fatty acid synthesis and hepatic glucose-6-phosphate dehydrogenase activity intermediate to the basal and basal plus $C_{18:3}$ treatments (Table 3). Such a response for $C_{18:1}$ was not found in experiment 1 or in rats previously fed a FF-diet for 10 days and then intubated with oleate ester for three days (9). However, an intermediate effect of $C_{18:1}$ has been found in rats pair-fed a FF-diet plus $C_{18:1}$ for seven days (172). The inability to detect an effect of $C_{18:1}$ in experiment 1 may be related to the higher level of safflower oil in the basal diet which could have depressed hepatic lipogenesis to such a low point as to mask the influence of $C_{18:1}$. In addition, the longer duration of experiment 2 may have contributed to observing slight differences (9). The higher level of oil in experiment 1 and

the poor digestibility of $C_{16:0}$ may also have prevented detecting an influence of $C_{16:0}$ on liver lipogenesis (Table 2).

Linoleate has been reported to be more effective than $C_{16:0}$ or $C_{18:1}$ but $C_{18:3}$ and $C_{20:4}$ supposedly have the greatest efficacy in dampening fatty acid synthesis (9, 224). Data in Table 3 would suggest this is true but its biological significance requires further elucidation. Using changes in glucose-6-phosphate dehydrogenase activity as an indicator of the rate of rat liver fatty acid synthesis, Gozukara et al. (121) have proposed polyunsaturated fats do not have a specific inhibitory effect on hepatic fatty acid synthesis but rather cause a reduction in carbohydrate intake which in turn lowers the rate of rat liver fatty acid synthesis. Since animals among treatments in these studies had similar basal diet intakes, the action of $C_{18:2}$ and $C_{18:3}$ must be independent of carbohydrate intake (10, 224).

Rat liver glucose-6-phosphate dehydrogenase and malic enzyme activities appear to be closely related to dietary polyunsaturated fat intakes (112). In addition to providing NADPH for fatty acid synthesis, glucose-6-phosphate dehydrogenase and malic enzyme provide reducing equivalents for desaturation and fatty acid chain elongation (11, 170). Dietary $C_{18:2}$ and $C_{18:3}$ not only depress fatty acid synthesis but also reduce the need for NADPH in chain elongation and desaturation. Conceivably, this reduced need for NADPH production could result in lowered enzyme activities (Tables 2 and 3, 170). A similar but less pronounced phenomenon might accompany dietary $C_{18:1}$, thus accounting for the intermediate values seen in experiment 2 (Table 3).

Adipose. Unlike rat liver, supplementing low-fat essential fatty acid adequate diets (Tables 4 and 3) or fat-free diets (223) with $C_{16:0}$, $C_{18:1}$, $C_{18:3}$ or $C_{18:3}$ does not precipitate a change in fatty acid synthetase or glucose-6-phosphate dehydrogenase activities or fatty acid synthesis rates. Rates of adipose tissue fatty acid synthesis in these experiments (Tables 4 and 5) are complicated by large degrees of variation between basal and fatty acid treatments. However, in both experiments the fatty acid supplemented groups within an experiment have very similar rates of lipogenesis. The level of dietary fat (3%) in our studies with meal-fed rats may be too low to exert an inhibitory effect on fatty acid synthesis in adipose tissue. The influx of glucose into adipose tissue and production of glyceride glycerol may be sufficiently large to permit rapid esterification of incoming fatty acids generated by lipoprotein lipase (156). Such action would prevent free fatty acids or their CoA derivatives from exerting an inhibitory influence on adipose tissue lipogenesis rates (129).

One Meal $C_{18:3}$. Two further experiments were conducted in an attempt to explain the specific action of $C_{18:3}$ on rat liver fatty acid synthesis and to ascertain the length of time required to elicit such a response. Although the absolute rates of liver and adipose tissue fatty acid synthesis were lower for the basal plus 3 or 5% $C_{18:3}$ diets, neither level of $C_{18:3}$ significantly depressed rat liver or adipose tissue fatty acid synthesis. Hill et al. (95) found that rats fed ad libitum and intubated with 2.0 ml corn oil displayed a

TABLE 4

Influence of dietary $C_{16:0}$, $C_{18:1}$, $C_{18:2}$ or $C_{18:3}$ on adipose tissue lipogenesis in essential fatty acid adequate rats[1,2]

	Body wgt., g	FAS[4]	G6PD[5]	In vivo FA-synthesis[6]
Experiment 1:				
Basal	142 ± 2[3a]	28 ± 3[a]	128 ± 14[a]	19,329 ± 6600[a]
+$C_{16:0}$	151 ± 3[a]	31 ± 1[a]	128 ± 14[a]	29,972 ± 3485[a]
+$C_{18:1}$	151 ± 3[a]	30 ± 3[a]	144 ± 14[a]	28,409 ± 5796[a]
+$C_{18:2}$	151 ± 2[a]	30 ± 1[a]	128 ± 14[a]	28,221 ± 7491[a]
Experiment 2:				
Basal	150 ± 2[a]	25 ± 9[a]	---	17,567 ± 6004[a]
+$C_{18:1}$	156 ± 2[a,b]	34 ± 3[a]	---	9,704 ± 2927[a]
+$C_{18:2}$	161 ± 2[b]	37 ± 5[a]	---	9,230 ± 2537[a]
+$C_{18:3}$	159 ± 3[b]	36 ± 4[a]	---	9,722 ± 1543[a]

[1]Basal diet contained 2.5% and 1% safflower oil in Experiment 1 and 2 respectively.

[2]Mean ± SEM, n=7.

[3]Those values within an experiment with different superscripts are significantly different ($P < 0.05$).

[4]Nanomoles NADPH oxidized min^{-1} mg $protein^{-1}$ at 37°. FAS = fatty acid synthetase.

[5]Nanomoles NADP reduced min^{-1} mg $protein^{-1}$ at 25°. G6PD = glucose-6-phosphate dehydrogenase.

[6]Dpm ^3H incorporated into fatty acids per g adipose in 15 minutes for Experiment 1 and 10 minutes for Experiment 2.

TABLE 5

Effect of one meal containing $C_{18:3}$ on hepatic and adipose lipogenesis in essential fatty acid adequate rats

	Experiment 1[1]		Experiment 2[1]	
	Basal	+3% $C_{18:3}$[2]	Basal	+5% $C_{18:3}$[2]
Body wgt., g	149 ± 2[3a]	146 ± 6[a]	150 ± 2[a]	160 ± 6[a]
Total wgt. gain, g	21 ± 1[a]	16 ± 1[b]	22 ± 1[a]	18 ± 1[a]
Daily food intake, g	10.5	10.2	9.6	9.6
Liver wgt., g	6.6 ± 0.2[a]	6.4 ± 0.2[a]	6.9 ± 0.2[a]	7.1 ± 0.3[a]
Epididymal fat wgt., mg	818 ± 82[a]	812 ± 89[a]	---	---
Hepatic In vivo FA-synthesis[4]	7221 ± 1911[a]	4630 ± 1943[a]	4207 ± 238[a]	3657 ± 238[a]
Adipose In vivo FA-synthesis[4]	19,329 ± 6600[a]	17,349 ± 7974[a]	17,567 ± 6004[a]	8750 ± 1700[a]

[1]Basal diet contained 2.5% and 1% safflower oil in Experiment 1 and 2 respectively.

[2]Mean ± SEM where n=7 in Experiment 1 and n=6 in Experiment 2.

[3]Those values with different superscripts within an experiment are significantly different ($P < 0.05$).

[4]Dpm ^3H incorporated into fatty acids per g tissue in 15 minutes in Experiment 1 and in 10 minutes in Experiment 2.

tremendous drop in liver slice lipogenesis within three hours and this inhibition continued for several hours. The level of $C_{18:3}$ fed in experiment 2 was 0.75 ml. Not only was the amount of fat consumed substantially less in these experiments, but as previously discussed, the large simultaneous influx of carbohydrate may prevent a significant increase in plasma and tissue free fatty acids. Therefore, the meal-eating animal may be less sensitive to dietary fat inhibition of lipogenesis than is the nibbling rat.

Similar to effects found in rats consuming an essential fatty acid deficient diet, $C_{18:2}$ and $C_{18:3}$ possess a capacity to elicit a specific suppression of rat liver fatty acid synthesis within seven to 10 days (Tables 2 and 3). However, this effect is not immediate since one meal containing 3 or 5% $C_{18:3}$ did not significantly alter the rate of liver fatty acid synthesis (Table 5). The reason for this lack of influence of one meal may be due to very rapid clearance by peripheral (e.g. adipose) tissues of the absorbed fat so that the potential free fatty acid effectors do not reach the liver in significant quantities. Unlike liver tissue, adipose tissue of meal-fed rats consuming essential fatty acid adequate or deficient diets (223) is insensitive to dietary $C_{18:2}$ or $C_{18:3}$. The reason for this observation may be related to the rapid influx of carbohydrate and subsequent production of α-glycerophosphate.

Since animals in these experiments were fed equal amounts of carbohydrate and essential fatty acid adequate diets, the effects

of $C_{18:2}$ and $C_{18:3}$ on liver lipogenesis appear to be specific and independent of carbohydrate intake and essential fatty acid status.

PART IV

INFLUENCE OF DIETARY FATTY ACIDS ON LIVER AND ADIPOSE TISSUE LIPOGENESIS, AND ON LIVER METABOLITES IN MEAL-FED RATS

INTRODUCTION

Lipogenic enzymes and rates of fatty acid synthesis in rat and mouse liver appear to be regulated not only by the level of dietary fat (148, 171) but also by the type of dietary fat (7-10, 148, 171, 172, 222). Wiegand et al. (148) observed that the addition of 15% cocoa butter to a fat-free diet was necessary to achieve a degree of inhibition of rat liver fatty acid synthetase comparable to that obtained with 2.5% safflower oil supplementation. Because diets differing in fat level and lipid composition make it difficult to attribute specific regulatory mechanisms to specific lipid components, the addition of purified methyl esters of various fatty acids has been adopted to investigate the regulatory mode of action of dietary fatty acids (7-10, 178, 222). The high activity of hepatic fatty acid synthetase in mice fed a fat-free diet could be rapidly lowered by the addition of 2% methyl linoleate whereas 2% methyl palmitate supplementation had no inhibitory influence (7). Marked differences exist in digestibility between methyl linoleate and palmitate (222), but even when the absorbed amount of both acids was similar, palmitate still did not reduce fatty acid synthetase activity or in vivo rates of fatty acid synthesis (223). These observations with purified esters of fatty acids concur with the differential effects found with the natural fats, cocoa butter and safflower (148).

In sharp contrast to in vivo dietary studies, stearate and palmitate consistently exert the greatest inhibitory influence on fatty acid synthesis of isolated cells (177-179). However, the little in vivo information available for stearate effects on lipogenesis is confounded by the poor digestibility of stearate relative to unsaturated acids. The current report compared the effects of methyl stearate and linoleate on fatty acid synthesis when similar amounts of both acids had been digested. Frequently the differential effects of various dietary fats on adipose tissue lipogenesis are overlooked and yet in the rat, adipose tissue accounts for a very large portion of total body fat synthesis (203). In the present report the influence of dietary stearate and linoleate on lipogenesis has been investigated in both rat liver and adipose tissue.

Fasting and feeding of high fat diets are associated with low rates of liver and adipose tissue fatty acid synthesis, and are accompanied by increased plasma unesterified fatty acid levels, elevated hepatic long chain acyl CoA concentrations and reduced liver cytosolic NAD/NADH ratios (31, 203, 204, 227). During periods of fasting polyunsaturated fatty acids have been proposed to undergo greater rates of mobilization and oxidation (203, 204). If linoleate and linolenate turnover more rapidly, then changes in the previously mentioned parameters may contribute to reducing rates of hepatic fatty acid synthesis.

As an approach to explaining the efficient inhibitory mechanism of polyunsaturated fatty acids on rat liver fatty acid synthesis, the concentration and composition of plasma unesterified fatty acids, the level of hepatic long chain acyl CoA esters, and liver cytosolic redox state were quantitated in rats fed fat-free or fat-free plus 3% linoleate or linolenate diets.

METHODS

General animal handling. Sprague-Dawley rats were housed individually in stainless steel cages and had free access to water. Prior to the experimental phase all animals were adapted to a three hour per day meal-eating regimen (access to food 800-1100 hrs). During this phase all rats received the basal diet in Table 1 except that 2% safflower oil replaced 2% carbohydrate. Following adaptation to meal-eating, the rats were switched to the fat-free basal diet (Table 1) and fed for an additional seven days. On the eighth day rats were allotted to the treatments as described for each experiment. Methyl esters (99% purity) of stearate ($C_{18:0}$), linoleate ($C_{18:2}$) and linolenate ($C_{18:3}$) were supplemented to the fat-free diet.

Experiment 1. The effects of methyl $C_{18:0}$ and $C_{18:2}$ on rat liver and adipose tissue fatty acid synthesis were examined. When low levels of fat are supplemented to a fat-free diet the effects are independent of carbohydrate intake (10, 222). Therefore, after

TABLE 1

Fat-free basal diet composition

Ingredient	Parts
Glucose	72.0
Casein	20.0
Nonnutritive fiber[1]	3.0
D, L-methionine	0.3
Choline chloride	0.3
Vitamin mix[2]	0.4
Mineral mix[3]	4.0
	100.0

[1]Solka-floc. Brown Company, Berlin, New Hampshire.

[2]Vitamin mixture was that described by Yeh and Leveille (216).

[3]Rat mineral mix #4164. Teklad Test Diets, 2826 Latham Drive, Madison, Wisconsin.

the fat-free feeding period 10 rats per treatment were randomly allotted to the following dietary treatments: a) fat-free diet (FF); (b) FF+8% $C_{18:0}$; (c) FF+3% $C_{18:2}$. Because of the poor digestibility of $C_{18:0}$, an 8% level of supplementation was necessary to achieve an amount absorbed equivalent to 3% $C_{18:2}$. On the first day of fat addition rats were allotted 90% of their average daily intake during the fat-free feeding period. The 90% value was chosen to avoid reduction in food intake among the $C_{18:0}$ rats because of the large amount of additional $C_{18:0}$ necessary for supplementation. Food allotments were increased by 0.5 g increments when all rats finished allotted food in three hours. The dietary fatty acids were added to eight meals as 8% $C_{18:0}$ or 3% $C_{18:2}$ of the daily fat-free diet allotted.

One hour after completion of the eighth meal, in vivo liver and adipose tissue lipogenic rates were quantitated by the amount of ^3H_2O incorporated into fatty acids. Each rat was injected intraperitoneally with 1.5 mCi ^3H_2O in 0.5 ml physiological saline and killed 15 minutes post-injection. The rate of incorporation of ^3H_2O into hepatic fatty acids has been determined to be linear for five to 60 minutes after injection (33, 34). After killing the rats, the livers were removed and placed in cold saline. After weighing, a 1.0g sample was homogenized for enzyme assay while the remainder was homogenized in an equal volume of water. Aliquots of the homogenate (0.5ml) were removed for saponfication in ethanolic KOH (30%). After extraction of the nonsaponifiable matter, the mixture

was acidified and extracted with three 5.0 ml volumes or petroleum ether. The amount of ^{3}H in fatty acids was quantitated by liquid scintillation counting. The scintillation fluid contained 4.0g scintillant dissolved in 230 ml absolute ethanol and toluene to one liter. All visible, removable adipose tissue on the carcass was collected, weighed and homogenized in an equal volume of water. Extraction of fatty acids was handled in a manner similar to liver tissue. Plasma samples were collected at time of kill in order to determine the specific activity of the water. This was used as an index of body water specific activity which permitted calculating micromoles of ^{3}H incorporated into fatty acids per gram tissue. C_2 unit incorporation into fatty acids by liver and adipose was calculated from the $^{3}H_2O$ incorporation (225).

The activities of hepatic fatty acid synthetase and acetyl CoA carboxylase (EC 6.4.1.2) were quantitated from a 100,000 g (40 min) supernatant of a homogenate containing 0.15 M KCl, 1.0 mM $MgCl_2$ and 10 mM n-acetyl-cysteine, pH 7.6 (228, 229). Protein content of the 100,000 g supernatant was determined by the Lowry method (220).

Data were statistically evaluated by means of analysis of variance for completely randomized design. Treatment differences were ascertained using Tukey's t-test procedure (217).

Experiment 2. Rat liver long chain acyl CoA esters were quantitated before and after the sixth meal. Both $C_{18:2}$ and $C_{18:3}$ have similar inhibitory effects on rat liver fatty acid synthesis (222), $C_{18:2}$ was used in the before meal trial and $C_{18:3}$ was

supplemented in the after meal trial. Rats were paired for body weight and food intake and fed the FF diet or FF diet plus 3% $C_{18:2}$ or FF diet plus 3% $C_{18:3}$ for six meals. Before the sixth meal rats were killed by decapitation and blood samples were collected rapidly for plasma free fatty acid composition and concentration analyses. Immediately following the blood collection the livers were exposed and tissue frozen in situ using the freeze clamp technique (230). The time required for blood and tissue collection was less than 20 seconds. One hour after the sixth meal, rats were stunned by a blow to the head, livers rapidly exposed and frozen tissue samples collected in less than 10 seconds by the freeze-clamp method (230). In both trials the liver samples were processed as described by Romsos et al. (230). Long chain acyl CoA concentrations were quantitated by the recycling procedure of Allred and Guy (231).

Plasma samples were extracted for free fatty acids (232), methylated using borotriflouride and methanol (233), and analyzed by gas-liquid chromatography (233). Results are expressed as micrograms per ml plasma.

Treatment differences were determined by a paired t-test analysis (217).

Experiment 3. Three trials were conducted to examine the effects of $C_{18:2}$ and $C_{18:3}$ supplementation on the concentration of liver lactate and pyruvate, and lactate/pyruvate ratio. The preliminary experimental protocol was the same as that previously described. In trials 1 and 2 eight pairs of rats, matched for body weight and food

intake, were fed the FF-diet, FF-diet plus 3% $C_{18:2}$ (trial 1) or FF-diet plus 3% plus 3% $C_{18:3}$ (trial 2) for six meals. Trial 3 involved 10 pairs of rats and fed FF-diet or the FF-diet plus 3% $C_{18:2}$ for seven meals.

One hour after the last meal the rats were stunned by a blow on the head, the liver was rapidly exposed and frozen in situ using the freeze-clamp technique (230). Liver samples were processed by the method of Romsos et al. (230) and lactate and pyruvate quantitated enzymatically with lactate dehydrogenase by the procedures of Bucher et al. (234) and Hohorst (235) respectively. From the concentrations of lactate and pyruvate the ratios of lactate/pyruvate and liver cytosolic NAD/NADH were quantitated (31). Statistically significant differences were determined by a paired t-test analysis (217).

Experiment 4. To obtain information on the effect of $C_{18:2}$ on glycolytic enzyme activities relative to lipogenic enzyme activities in liver, 10 pairs of rats were fed the FF diet or the FF diet plus 3% $C_{18:2}$ for eight meals. The glycolytic enzymes chosen for analysis were glucokinase (EC 2.7.1.2) and pyruvate kinase (EC 2.7.1.40), while citrate cleavage enzyme (EC 4.1.1.40) and fatty acid synthetase were selected as lipogenic enzymes. One hour following completion of the eighth meal the rats were killed by decapitation and the livers were rapidly removed and placed in cold homogenizing buffer. Approximately 1g of liver was homogenized in 0.15 M KCl, 1.0 mM $MgCl_2$, 10 mM n-acetyl-cysteine, and 0.5 mM dithiothrietol (pH 7.6). After 40

minutes of centrifugation at 100,000g the supernatant was utilized for enzymatic analyses. Glucokinase, pyruvate kinase, citrate cleavage enzyme, fatty acid synthetase and acetyl-CoA carboxylase were assayed by the methods of Pilkis (236), Bergmeyer (237), Srere (238), Hsu et al. (228), and Insue and Lowenstein (229) respectively. All enzymes were assayed at 37^{o} and soluble protein was quantitated by the Lowry procedure (220). A paired t-test analysis was utilized to determine treatment differences.

RESULTS

$C_{18:0}$ vs. $C_{18:2}$ effects on lipogenesis. The average daily consumption of fat-free diet over the seven day feeding period was not significantly different among treatments. The differences in diet consumption was a consequence of the random design (see methods) and not an effect attributable to dietary fatty acid supplementation. Because of the poor apparent digestibility (223) of methyl $C_{18:0}$, the level of daily supplementation had to be increased to 8% to reach an amount absorbed comparable to 3% $C_{18:2}$ (Table 2). When enzyme activities were expressed as acetate units utilized min^{-1} mg^{-1} soluble protein at 37^{o}, fatty acid synthetase and acetyl-CoA carboxylase displayed comparable rates. Supplementing 3% $C_{18:2}$ to the fat-free diet very significantly inhibited fatty acid synthetase and acetyl-CoA carboxylase, whereas comparable amounts of absorbed $C_{18:0}$ exerted no inhibitory action on the activity of either enzyme (Table 2).

TABLE 2

Fatty acid synthesis in rats fed a fat-free diet plus 8% $C_{18:0}$ or 3% $C_{18:2}$ (Experiment 1)

Parameter	Dietary Fatty Acid		
	FF	+8% $C_{18:0}$	+3% $C_{18:2}$
Final body wgt., g	193 ± 8	188 ± 7	192 ± 5
Total wgt. gain, g[2]	24 ± 2	26 ± 2	27 ± 1
Food intake, g/day[3]	14 ± 0.7	14.1 ± 0.9	13.7 ± 0.4
Fatty acid absorbed, mg/day[4]	---	395	366
Liver:			
Wgt., g	9.2 ± 0.4	9.8 ± 0.4	9.7 ± 0.4
Fatty acid synthetase[5]	10 ± 0.8[6a]	12 ± 0.8[a]	6 ± 0.8[b]
Acetyl-CoA carboxylase[5]	13 ± 1.5[a]	10 ± 1.5[a]	5 ± 1.5[b]
In vivo FA synthesis:			
nm C_2 units $min^{-1}mg^{-1}$ protein	9.1 ± 0.7[a]	11.4 ± 0.7[a]	6.1 ± 0.7[b]
μm C_2 units, total liver min^{-1}	7.4 ± 0.5[a]	10.2 ± 0.5[b]	5.2 ± 0.5[c]
Adipose:			
Total wgt., g	6.8 ± 0.8[a]	5.4 ± 0.8[a]	6.1 ± 0.8[a]
In vivo FA synthesis:			
μm C_2 units $min^{-1}g^{-1}$	2.7 ± 0.3[a]	2.6 ± 0.3[a]	2.0 ± 0.3[a]
μm C_2 units min^{-1} total	15.2 ± 2.3[a]	11.6 ± 2.3[a]	13.6 ± 2.3[a]
Total FA synthesis distribution:			
% Liver	31 ± 3[a]	49 ± 5[b]	32 ± 4[a]
% Adipose	69 ± 3[a]	51 ± 4[b]	68 ± 3[a]

[1]Mean ± SEM; n=10.

[2]Weight gain during seven day period of ester supplementation.

[3]Fat-free basal diet.

[4]Calculated from digestibility values determined by Clarke et al.[4]

[5]Acetate unit equivalents utilized min^{-1} mg $protein^{-1}$ at 37°.

[6]Those values with different superscripts are significantly different ($P < 0.05$) using Tukey's t-test.

From the incorporation of ^3H, the amount of C_2 units utilized was calculated (225) and has been expressed as nanomoles min^{-1} mg^{-1} protein for comparison to fatty acid synthetase and acetyl-CoA carboxylase activities. $C_{18:2}$ supplementation resulted in a significant depression in fatty acid synthesis rates expressed on the basis of total liver, or per mg protein. In contrast $C_{18:0}$ feeding was associated with a significant increase over fat-free controls in total liver fatty acid synthesis but per mg protein the rates were statistically equivalent to the rates in the fat-free group. Most notable was the similarity within each treatment between fatty acid synthetase and acetyl-CoA carboxylase activities when both were assayed at 37^o (Table 2) and the extremely close relationship between the in vivo rates of acetyl units incorporated into fatty acids and the activities of carboxylase and synthetase (Table 2).

Neither $C_{18:0}$ nor $C_{18:2}$ supplementation exerted a significant inhibitory action on adipose tissue fatty acid synthesis rates either on a per g adipose or on total removable adipose tissue basis (Table 2). Because of the significant increase in total liver fatty acid synthesis and the lower absolute rate of total adipose fatty acid synthesis, dietary $C_{18:0}$ was associated with a marked increase in the contribution of liver to total body fatty acid synthesis (Table 2).

Metabolites. Because $C_{18:0}$ had no inhibitory effect on hepatin fatty acid synthesis, more detailed experiments with $C_{18:0}$ were not deemed necessary. Supplementing a fat-free diet with 3% $C_{18:2}$ for five days did not significantly elevate plasma nonesterified fatty

acid concentration or the amount of circulating free palmitate, stearate or oleate when the samples were collected prior to the sixth meal. However, dietary $C_{18:2}$ was associated with over a fourfold increase in the amount of plasma free linoleate (Table 3). In these same animals hepatic long chain acyl-CoA concentrations before the meal were not significantly different from rats fed a fat-free diet. Similarly, rats fed a 3% $C_{18:3}$ supplemented diet for five days displayed no significant change relative to fat-free controls in hepatic long chain acyl-CoA content after the sixth meal. Although examined in two separate experiments, there was dramatic decline in hepatic long chain acyl-CoA levels within four hours after initiation of meal-eating (Table 3).

Using the lactate/pyruvate ratio as an index of cytosolic redox state of the liver, the concentrations of lactate and pyruvate in the liver of rats fed a fat-free diet with 3% $C_{18:2}$ or $C_{18:3}$ supplementation were quantitated and are summarized in Table 4. Since the response of rat liver lipogenesis to dietary $C_{18:2}$ or $C_{18:3}$ was similar (10, 172, 222), the data of all three experiments were combined and are also presented in Table 4. In no experiment was the hepatic lactate/pyruvate ratio significantly altered by dietary $C_{18:2}$ or $C_{18:3}$. In addition, no consistent change in either lactate or pyruvate concentration occurred among the experiments. The mean values of all three experiments indicate that the addition of 3% $C_{18:2}$ or $C_{18:3}$ does not significantly affect the cytosolic redox state of the liver.

TABLE 3

Liver long chain acyl-CoA concentration and Plasma FFA composition in rats fed a fat-free diet plus 3% $C_{18:2}$ or $C_{18:3}$

(Experiment 2)

	Dietary Fatty Acid			
Parameter[1]	FF	+3% $C_{18:2}$	FF	+3% $C_{18:3}$
Plasma FFA[2] (ug ml^{-1})				
Palmitate	100 ± 20[3a]	119 ± 15[a]		
Stearate	45 ± 6[a]	56 ± 10[a]		
Oleate	78 ± 8[a]	66 ± 6[a]		
Linoleate	7 ± 2[a]	32 ± 3[b]		
TOTAL	226 ± 27[a]	273 ± 18[a]		
Long-chain acyl-CoA:[4]				
Before meal	133 ± 19[a]	127 ± 16[a]	---	---
After meal	---	---	35 ± 5[a]	30 ± 4[a]

[1]Mean ± SEM; n=8.

[2]Samples collected prior to sixth meal.

[3]Values with different superscripts are significantly different ($P < 0.05$) as determined by paired t-test.

[4]Nanomoles per g liver.

TABLE 4

Estimate of liver cytosolic redox state in rats fed a fat-free diet plus 3% $C_{18:2}$ or $C_{18:3}$ (Experiment 3)

Diet Treatment	Parameter			
	Lactate[2]	Pyruvate[2]	Lac/Pyr	NAD/NADH
		Trial 1 (n=8)[4]		
FF	1302 ± 96[3a]	280 ± 33[a]	4.74 ± 0.68[a]	1937
+3% $C_{18:2}$	1665 ± 182[a]	217 ± 13[a]	6.42 ± 0.62[a]	1174
		Trial 2 (n=8)[4]		
FF	1596 ± 87[a]	185 ± 18[a]	8.01 ± 0.60[a]	1044
+3% $C_{18:3}$	1384 ± 61[a]	171 ± 15[a]	8.50 ± 0.24[a]	1113
		Trial 3 (n=10)[5]		
FF	1424 ± 73[a]	396 ± 18[a]	3.63 ± 0.20[a]	2505
+3% $C_{18:2}$	1253 ± 94[a]	319 ± 10[b]	3.89 ± 0.27[a]	2293
		Mean of Trials 1-3		
FF	1441 ± 85[a]	287 ± 61[a]	5.46 ± 1.31[a]	1794
+3% PUFA	1434 ± 121[a]	236 ± 44[a]	6.27 ± 1.33[a]	1482

[1]Mean ± SEM

[2]Nanomoles per g liver.

[3]Values with different superscripts are significantly different ($P < 0.05$) according to paired t-test analysis.

[4]Diet was fed six meals.

[5]Diet was fed seven meals.

Hepatic glycolytic and lipogenic enzyme activities. Clearly supplementing a fat-free diet with only 3% $C_{18:2}$ for seven days will significantly depress hepatic rates of fatty acid synthesis and lipogenic enzyme activities whereas comparable amounts of absorbed saturated fatty acids are without inhibitory influence (Table 2, 7-10, 172, 222). In ascertaining the mechanism of $C_{18:2}$ it must be determined if the effect on lipogenesis is secondary to an inhibition of glycolytic activity. Of two key regulatory enzymes of glycolysis, glucokinase and pyruvate kinase, only pyruvate kinase was significantly depressed by seven days of $C_{18:2}$ supplementation (Table 5). In contrast $C_{18:2}$ resulted in a significant depression in both hepatic fatty acid synthetase and citrate cleavage enzyme activities. In fact the percentage decline was approximately twice that of either glucokinase or pyruvate kinase.

DISCUSSION

In an earlier study (223) the addition of 3% methyl $C_{18:0}$ to a fat-free diet caused no reduction in the rate of fatty acid synthesis. However, the very poor digestibility of methyl $C_{18:0}$ made interpreting these observations difficult. In the experiment presented in Table 2 the level of dietary $C_{18:0}$ was increased to 8% in order to achieve an amount of digested $C_{18:0}$ equivalent to 3% $C_{18:2}$. Even when comparable quantities of $C_{18:0}$ and $C_{18:2}$ were absorbed, $C_{18:0}$ had no

TABLE 5

Changes in rat liver glycolytic and lipogenic enzyme activities after seven days of $C_{18:2}$ supplementation

(Experiment 4)

	Diet	
Parameter[1]	FF	+3% $C_{18:2}$
Final body wgt., g	165 ± 4	172 ± 6
Total wgt. gain, g[2]	13 ± 2[7a]	20 ± 3[b]
Food intake, g/day[3]	12.2	12.2
Liver:		
Wgt. g	7.7 ± 0.3	8.1 ± 0.3
GK[4]	17 ± 3[a]	13 ± 1[a]
PK[5]	496 ± 31[a]	360 ± 34[b]
CCE[5]	47 ± 4[a]	21 ± 3[b]
FAS[6]	19 ± 1[a]	10 ± 1[b]

[1]Mean ± SEM; n=10.

[2]Gain during seven day $C_{18:2}$ supplementation period.

[3]Fat-free basal diet.

[4]Glucokinase activity expressed as nanomoles NADP reduced min^{-1} mg $protein^{-1}$, 37°.

[5]Pyruvate kinase and citrate cleavage enzyme activities expressed as nanomoles NADH oxidized min^{-1} mg $protein^{-1}$, 37°.

[6]Fatty acid synthetase activities expressed as nanomoles NADPH oxidized min^{-1} mg $protein^{-1}$, 37°.

[7]Values with different superscripts are significantly different ($P < 0.05$) according to paired t-test.

depressive action on lipogenic enzyme activities and was actually associated with a significant increase in total liver fatty acid synthesis. These in vivo effects of $C_{18:0}$ are in direct contrast to in vitro data with chick and rat hepatocytes and human skin fibroblasts in which stearic acid depressed rates of fatty acid synthesis with greater efficacy than unsaturated acids (177-179, 226). The marked inhibitory effect of stearic acid in hepatocytes has been partially attributed to the low K_i of purified rat liver acetyl-CoA carboxylase for stearoyl-CoA (177). However, dietary $C_{18:0}$ caused no depression in acetyl-CoA carboxylase activity in rat liver (Table 2), while $C_{18:2}$ lowered the activity by more than 50%.

Although in vitro assays do not necessarily reflect in vivo enzyme activity or rate of substrate flux (36, 155), theoretically the assays for acetyl-CoA carboxylase and fatty acid synthetase do represent total tissue enzyme protein (85, 86). Under our conditions the in vivo rate of acetate unit utilization for hepatic fatty acid synthesis agreed very closely with the in vitro activities of fatty acid synthetase and acetyl-CoA carboxylase. This indicates that at the time de novo fatty acid synthesis was quantitated (4 hrs after meal initiation) synthetase and carboxylase enzymes were functioning at maximal rates and that enzyme activity is indicative of substrate flux. Thus, the level of these enzymes may dictate the maximum in vivo rates of fatty acid synthesis (19). The accuracy of the calculations in Table 2 is substantiated by the close similarity between

our data and in vivo fatty acid synthesis rates reported by others for meal-eating rats (33, 34) and isolated hepatocytes (239).

A very significant portion (50-70%) of the total body de novo fatty acid synthesis of rats is contributed by adipose tissue (Table 2). However, the differential response of adipose tissue to the addition of various fatty acids to a fat-free diet, particularly in vivo studies, has largely been ignored. The total amount of removable adipose tissue did not significantly vary among treatments. This quantity of adipose tissue was only about 3% of the body weight. Although Sprague-Dawley rats of comparable body size have been shown to contain about 12% solvent extractable body fat, much of this is not associated with disectable fat depots. Our values for removable fat are very close to those reported for Osborne-Mendel rats of similar size (240, 241).

Adipose tissue lipogenesis in vivo remained unchanged by the addition of $C_{18:0}$ or $C_{18:2}$ which was consistent with our earlier data (222). In contrast Allmann and Gibson (7) reported that mouse adipose tissue fatty acid synthesis was rapidly depressed by adding 2% $C_{18:2}$ to a low fat diet devoid of essential fatty acids, but the addition of 2% $C_{16:0}$ had no inhibitory effect. This apparent discrepancy in response of mouse and rat adipose tissue to dietary fat may be attributed to: 1) the poor digestibility of $C_{16:0}$ (222); 2) species differences; and/or 3) the meal-eating regimen of our rats (143).

The increased rate of total liver fatty acid synthesis and greater contribution of liver to total body fatty acid synthesis associated with dietary $C_{18:0}$ is in accordance with the apparent site shift observations of Waterman et al. (171). In their comparison of diets high in polyunsaturated fat vs. saturated fat, rats fed a tallow diet were found to possess greater rates of liver fatty acid synthesis whereas the rates of adipose tissue fatty acid synthesis were higher in rats fed a safflower oil containing diet.

Even though adding $C_{18:2}$ to the fat-free diet caused a significant reduction in rates of hepatic lipogenesis of meal-fed rats (Table 2, 7-10, 172, 222) this was not associated with an elevation in plasma free fatty acids or hepatic long chain acyl-CoA concentrations (Table 3). $C_{18:2}$ supplementation did cause a fourfold increase in plasma free linoleate, but had no significant effect on the composition of the remaining fatty acids studied (Table 3). If the inhibition of liver fatty acid synthesis associated with dietary polyunsaturated fatty acids is mediated in some way via their CoA derivatives, then the effects of these derivatives on mitochondrial citrate efflux (131) and acetyl-CoA carboxylase (88, 92) may be more dependent on composition of acyl-CoA's than on concentration. The liver of rats and humans has been shown to remove nonesterified fatty acids from plasma at differential rates and that the rate of uptake was greater for polyunsaturated fatty acids (196, 214). Therefore, the proportion of hepatic linoleoyl-CoA may be expected to increase. However, the CoA ester of linoleic acid prevented citrate efflux from isolated mitochondria with far less

effectiveness than did added CoA derivatives of saturated acids (131). In Addition the K_i of acetyl-CoA carboxylase for unsaturated fatty acids is higher than for the saturated acids (92). Therefore, an inhibition of fatty acid synthesis by an increased proportion of linoleoyl-CoA in vivo does not agree with the observations of isolated systems. Interestingly the observations with isolated systems are consistent with the greater inhibitory effect of $C_{18:0}$ in isolated hepatocytes (177-179).

The physiological significance of long chain fatty acid CoA esters in controlling lipogenesis rates has been criticized by Shafrir and Ruderman (93) who suggested that other intracellular modifiers such as NADH/NAD and acetyl-CoA/CoA ratios, may be more significant factors. If polyunsaturated fatty acids are degraded in the liver more rapidly than saturated fatty acids (203), a greater turnover rate of CoA esters in the liver of rats fed $C_{18:2}$ or $C_{18:3}$ could occur without a significant change in total tissue long chain acyl-CoA content. The potential net effect would be increased acetyl-CoA and NADH levels which would negatively affect pyruvate dehydrogenase activity and pyruvate utilization for fatty acid synthesis (79, 227). Using lactate/pyruvate ratios as an index of cytosol redox potential (31), we found no indication that dietary $C_{18:2}$ or $C_{18:3}$ caused a more reduced state in liver (Table 4). In addition the concentrations of lactate and pyruvate were not consistently altered by dietary $C_{18:2}$ or $C_{18:3}$. The average of all three experiments demonstrates no significant change in lactate or pyruvate

concentrations, the lactate/pyruvate or NAD/NADH ratios (Table 4). A reduction in NAD/NADH ratio caused by fasting, diabetes or high-fat diets has been related to lower rates of lipogenesis and glycolysis (31). The inability to demonstrate such a relationship with 3% added $C_{18:2}$ or $C_{18:3}$ may be due to the high intake of dietary carbohydrate and the insensitivity of the lactate/pyruvate ratio to less dramatic nutritional manipulations. Nevertheless the redox state of hepatic cytosol does not appear to be a major regulatory mechanism to explain the inhibitory effects of polyunsaturated fatty acids on liver fatty acid synthesis.

Fatty acid synthesis from dietary glucose requires an active flow of glucose through glycolysis in order to produce substrate for de novo fat synthesis. All earlier work (7-10, 172, 222) designed to study the effects of polyunsaturated fatty acids on lipogenesis have focused primarily on changes in lipogenic enzyme activities in liver. However, the depression in the activities of fatty acid synthetase, acetyl-CoA carboxylase and citrate cleavage enzyme (Tables 2 & 5, 172), and in rates of fatty acid synthesis (Table 2, 7-10) characteristic of $C_{18:2}$ supplementation of a fat-free diet could be the consequence of a specific inhibition of glycolysis. The data in Table 5 indicate that dietary $C_{18:2}$ had a minimal negative effect on liver glucokinase and pyruvate kinase activities. Only the decline in pyruvate kinase activity was statistically significant and the percentage decline in both glucokinase and pyruvate kinase activities was about half that of citrate cleavage enzyme or fatty acid synthetase (Table 5).

However, glucose flux through glycolysis has not been examined under our experimental conditions. Therefore, a specific or more potent inhibition of glycolytic flow associated with dietary $C_{18:2}$ cannot be totally eliminated at this time. However, glucokinase activity has been noted not to change after three meals of $C_{18:2}$ or $C_{18:3}$ supplementation whereas fatty acid synthetase activity and hepatic fatty acid synthesis were significantly lowered (242). This suggests the inhibitory effect of polyunsaturated fatty acids is primary on the lipogenic machinery and only secondarily on the glycolytic machinery.

In summary dietary $C_{18:2}$ very effectively depressed rat liver fatty acid synthesis whereas comparable amounts of absorbed $C_{18:0}$ had no inhibitory action. This inhibition by $C_{18:2}$ was not the result of increased plasma free fatty acid or hepatic fatty acid CoA ester levels or changes in redox state of the liver. The only detectable metabolite change was more than a fourfold increase in plasma free linoleate concentration. The depression in the activity of two key glycolytic enzymes, glucokinase and pyruvate kinase, was only half that of the lipogenic enzymes citrate cleavage enzyme and fatty acid synthetase. However, without measurements on glycolytic flux rates one cannot ascertain if the effect of polyunsaturated fatty acids is primarily on lipogenesis or glycolysis. The less dramatic decline in activity of glucokinase and pyruvate kinase than in fatty acid synthetase and citrate cleavage enzyme, and the very close relationship between in vivo rates of fatty acid synthesis and activities of acetyl

CoA carboxylase and fatty acid synthetase might indicate that polyunsaturated fatty acids exert their rate regulating effect on hepatic fatty acid synthesis in meal-fed rats by controlling the level of carboxylase, synthetase or both (19).

PART V

CHANGE IN HEPATIC FATTY ACID SYNTHESIS IN MEAL-FED RATS DURING SEVEN DAYS OF FEEDING POLYUNSATURATED FATTY ACIDS

INTRODUCTION

The rate of fatty acid synthesis in mouse and rat liver as well as the activities of several lipogenic enzymes appear to fluctuate with changes in both level and type of dietary fat (8-10, 222). Rats and mice either ad libitum fed or fasted-refed a fat-free diet responded to the addition of small amounts of methyl linoleate, linolenate or arachidonate by displaying significant reductions in the activities of hepatic fatty acid synthetase, acetyl-CoA carboxylase, citrate cleavage enzyme, glucose-6-phosphate dehydrogenase and malic enzyme (8-10). Supplementing a low-fat, high carbohydrate diet with 3% linoleate or linolenate resulted in a very significant decline in the in vivo rate of hepatic fatty acid synthesis and activity of fatty acid synthetase in meal-trained rats (222). The suppression of liver fatty acid synthesis at low levels of dietary polyunsaturated fat intake was not mimicked by the consumption of saturated fatty acids and was independent of carbohydrate intake (222, 223).

A paucity of information is available regarding the differential effects of polyunsaturated and saturated fatty acids on rat adipose tissue lipogenesis. In meal-fed rats adipose tissue fatty acid synthesis and associated enzymes consistently remained unaffected by the addition of either polyunsaturated or saturated fatty acids to a high carbohydrate diet (222, 223).

The mechanism by which polyunsaturated acids regulate liver fatty acid synthesis in rats and mice has yet to be ascertained. In order to attribute specific hepatic changes in de novo fatty acid synthesis to a particular lipid component, purified methyl esters of long chain fatty acids have been used to supplement the fat-free diet of meal-fed rats.

Most earlier investigations (8-10, 222) have examined rat liver fatty acid synthesis and/or hepatic lipogenic enzyme activities after several days of supplementing polyunsaturated fat to the diet. At these time points a new steady-state has likely been achieved which makes characterizing the initial point of inhibition by polyunsaturated fatty acids very difficult. In addition a reduction in the activities of lipogenic enzymes (8-10) and/or fatty acid synthesis (222) caused by dietary polyunsaturated fatty acids could be secondary to a primary reduction in glycolytic flux perhaps via a depression in glucokinase activity.

Therefore the research reported in this communication has investigated the quantitative changes through time in fatty acid synthetase activities and in rates of fatty acid synthesis in vivo in the liver of meal-fed rats following the addition of methyl stearate, linoleate or linolenate to a fat-free diet. In addition, the change in these parameters were examined relative to the fluctuation in glucokinase activity. Our data are consistent with a long-term type regulation exerted specifically by linoleate and linolenate which primarily affects lipogenic machinery.

METHODS

General animal handling. Sprague-Dawley rats (100g) were housed individually in stainless steel cages and had free access to water. Prior to the experimental phase all animals were adapted to a three hour per day meal-eating program with access to food from 800 to 1100 hours. During this phase all rats received the basal diet (Table 1) except that 2% safflower oil replaced 2% carbohydrate. Following adaptation to meal-eating, the rats were switched to the fat-free basal (FF) diet described in Table 1 and fed for an additional seven days. This diet contained 1.0 g BHT per kg diet. On the eighth day rats were allotted to the treatments described in the tables of results. Methyl esters (99% purity) of linoleate ($C_{18:2}$) and linolenate ($C_{18:3}$) were supplemented as 3% of the daily fat-free diet consumption. Methyl esters of palmitate ($C_{16:0}$) and stearate ($C_{18:0}$) were added as 7 and 8% of daily food consumption in order to compensate for their poor digestibility (223). Except in experiment 1, the time of kill for animals began one hour after completion of the meal. On the first day of fat addition to the basal diet rats were given 90% of their average daily intake during the fat-free feeding period. The 90% value was chosen to avoid reduction in food intake among the $C_{16:0}$ and $C_{18:0}$ rats because of the large amount of ester necessary for supplementation. Food was increased at 0.5 g increments on every third day.

In vivo fatty acid synthesis. In experiments 1-4 liver lipogenesis was quantitated by determining the rate of incorporation of

TABLE 1

Fat-free basal diet composition

Ingredient	Parts
Glucose	72.0
Casein	20.0
Nonnutritive fiber[1]	3.0
D, L-methionine	0.3
Choline chloride	0.3
Vitamin mix[2]	0.4
Mineral mix[3]	4.0
	100.0

[1]Solka-floc. Brown Company, Berlin, New Hampshire.

[2]Described by Yeh & Leveille (216).

[3]Rat mineral mix #4164. Teklad Test Diets, 2826 Latham Drive, Madison, Wisconsin.

^3H_2O into long chain fatty acids. Each rat was injected intraperitoneally (IP) with 0.5 ml physiological saline which contained 1.5 mC_i ^3H_2O. Rats were killed 15 minutes after injection. The rate of incorporation of ^3H_2O into hepatic fatty acids has been ascertained to be linear for five to 60 minutes after IP injection (33). After killing by decapitation, the liver was removed, placed in cold saline, weighed, and a piece removed for enzyme assay preparation. The remaining portion was homogenized in an equal volume of water. Aliquots of the homogenate (0.5 ml) were removed for saponification in ethanolic KOH (30%). The nonsaponifiable matter was extracted with two-5.0 ml washes of petroleum ether. The mixture was acidified and extracted with three-5.0 ml volumes of petroleum ether to remove long chain fatty acids. The amount of ^3H in the extracted fatty acids was quantitated by liquid scintillation counting in which the scintillation fluid contained 4.0 g scintillant dissolved in 230 ml absolute ethanol and toluene to one liter. In experiment 2 the rate of lipogenesis was determined basically in the same manner except that each rat received 2.0 mC_i ^3H_2O and 2.5 μC_i 1-^{14}C-acetate in physiological saline via IP injection.

The in vivo rate of lipogenesis for experiments 1 and 2 was expressed as dpm ^3H or ^{14}C incorporated into fatty acids min^{-1} g $liver^{-1}$. In experiments 3 and 4 plasma samples were collected at the time of kill and the specific activity of ^3H_2O in plasma was used as the specific activity of body water. Using the method of

Jungas (225), the nanomoles of C_2-units incorporated into fatty acids min^{-1} are expressed g $liver^{-1}$ and mg soluble $protein^{-1}$.

Enzyme assays. Approximately a 1.0 g liver sample was homogenized in 10.0 ml of cold KCl (0.15M), Mg Cl_2 (1.0mM), n-acetylcysteine (10mM) and dithiothreitol (1.0mM), pH 7.6. The homogenate was centrifuged for 40 min. at 100,000 xg. The supernatant was used to quantitate the activity of glucokinase (EC 2.7.1.2) (236) and fatty acid synthetase (228). The protein concentration of the supernatants was determined by the method of Lowry et al. (220).

Experiments 1-3. These experiments were conducted: a) to determine the number of fat-containing meals which must be consumed before a significant decline in hepatic fatty acid synthesis was observed and b) to compare quantitatively changes in glucokinase and fatty acid synthetase activities with in vivo rates of fatty acid synthesis. In experiment 1 the in vivo rate of liver lipogenesis, and disappearance of FF-diet from stomach and small intestine was examined at four, five, six and eight hours after the animals began eating. Each time point in each treatment involved five animals. Upon killing of the animals the stomach and small intestine contents were removed and dried in pre-weighed aluminum pans. The percentage of diet disappearance is defined as:

$$\left[1 - \frac{\text{grams dry gut contents - grams fatty acid}}{\text{grams FF-diet consumed}} \right] \times 100$$

The lipid was extracted from gut contents and gravimetrically quantitated as described by Clarke et al. (222) for fecal lipid extraction.

Experiment 2 investigated the effects of two and three meals of fatty acid supplementation on rates of liver fatty acid synthesis. The dietary treatments were: a) FF, b) FF plus 8% $C_{18:0}$, c) FF plus 3% $C_{18:2}$ and d) FF plus 3% $C_{18:3}$. $C_{18:0}$ was chosen for evaluation because in vitro data from hepatocytes and fibroblasts suggested it to be the most potent inhibitor of fatty acid synthesis (177-179). $C_{18:2}$ and $C_{18:3}$ were chosen to determine if they possessed equal ability to alter rates of hepatic lipogenesis under identical experimental conditions.

In experiment 3 the FF or FF plus 3% $C_{18:2}$ diets were fed to meal-trained rats for two, three and four meals. The specific activity of plasma ^3H_2O of body water was estimated from plasma. From the in vivo incorporation of ^3H_2O into hepatic fatty acids, the incorporation of C_2-units min^{-1} mg^{-1} protein was calculated (225) after each meal period and compared to the change in C_2-unit utilization by hepatic fatty acid synthetase as determined from NADPH oxidation in vitro (228). In addition changes in glucokinase activity as influenced by dietary $C_{18:2}$ were followed during these time periods.

Experiment 4. This experiment was conducted to examine the effect of removing $C_{18:2}$ from the diet. The dietary treatment were: a) eight meals of FF-diet; b) eight meals of FF plus 3% $C_{18:2}$ diet; c) seven meals of FF plus 3% $C_{18:2}$ followed by one meal of FF-diet; and d) six meals of FF plus 3% $C_{18:2}$ followed by two meals of FF-diet. As performed in experiment 3, the in vivo incorporation of

C_2-units into liver fatty acids was compared to the utilization of C_2-units in vitro by fatty acid synthetase as measured by NADPH oxidation.

Statistics. Experiment 1 was analyzed as a completely randomized 3x4 factorial experiment using an analysis of variance. Dietary fat and time interactions were examined by an F-test. Experiments 2 and 4 were statistically evaluated by means of analysis of variance for completely randomized design and treatment differences were ascertained using Tukey's t-test procedure. Significant treatment mean differences in experiment 3 were ascertained using Student t-test of significance (217).

RESULTS

In the time sequence study of fatty acid synthesis rates following one meal of fatty acid (experiment 1), all groups of animals consumed comparable amounts of carbohydrate (Table 2). The rats fed $C_{16:0}$ ate over twice the quantity of fatty acid as those animals given $C_{18:3}$. However because of the poor digestibility of $C_{16:0}$ (223), the amount of $C_{16:0}$ and $C_{18:3}$ absorbed from the gut should have been similar. The percentage disappearance of FF-diet from the stomach and small intestine increased through time and was not affected by dietary fat (Table 2). The percent extractable fatty acid which disappeared from gut contents was slightly higher for $C_{18:3}$ than for $C_{16:0}$.

TABLE 2

Effect of time after meal and dietary fat on rate of food passage and fatty acid digestibility

(Experiment 1)

Parameter	Dietary fatty Acid		
	FF	+7% $C_{16:0}$	+3% $C_{18:3}$
Food intake, g[1]	10.6 ± 0.8	11.2 ± 0.9	11.2 ± 0.7
Fatty acid intake, mg/day	---	806 ± 50	340 ± 20
	Disappearance of FF-diet, %[2,3]		
Hours after meal initiation:			
4	43 ± 1	39 ± 2	38 ± 3
5	53 ± 1	50 ± 2	47 ± 4
6	61 ± 2	54 ± 3	62 ± 3
8	75 ± 5	68 ± 3	69 ± 3
	Disappearance of fatty acid, %[2]		
Hours after meal initiation:			
4	---	52 ± 14	73 ± 4
5	---	39 ± 3	52 ± 9
6	---	41 ± 1	72 ± 2
8	---	56 ± 4	64 ± 4

[1]Average daily intake of FF-diet by all rats at all times for each treatment.

[2]Mean ± SEM, n=5.

[3]A significant effect ($P < 0.05$) of time on the disappearance of FF-diet from GI-tract.

No marked difference in percent fatty acid disappearance occurred between four and eight hours post-meal initiation (Table 2).

The pattern of in vivo rates of incorporation of $^{3}H_2O$ into liver fatty acids indicates only slight differences in rates of synthesis between four and eight hours after beginning to eat (Table 3). Neither time after the meal nor dietary fatty acid source significantly affected hepatic rates of fatty acid synthesis after one meal (Table 3).

Rats fed two and three meals containing $C_{18:0}$, $C_{18:2}$ or $C_{18:3}$ consumed similar quantities of FF-diet (Tables 4 and 5). Even though the amount of $C_{18:0}$ eaten each day was much higher than either $C_{18:2}$ or $C_{18:3}$, the quantity absorbed, estimated from apparent digestion coefficients (223), was similar among all treatments (Tables 4 and 5). Supplementing $C_{18:0}$, $C_{18:2}$ or $C_{18:3}$ for two meals did not influence glucokinase activity or alter rates of in vivo incorporation of either $^{3}H_2O$ or 1-^{14}C-acetate into hepatic fatty acids (Table 4). Two meals corresponds to 24 hours after consumption of the first fat containing meal.

Consumption of three meals containing $C_{18:2}$ or $C_{18:3}$ (48 hours after eating first fat meal) resulted in a significant depression in hepatic fatty acid synthetase activity relative to rats eating the FF-diet (Table 5). Similarly the in vivo rate of $^{3}H_2O$ incorporation into hepatic fatty acids was significantly lowered by dietary polyunsaturated acids (Table 5). Although $C_{18:0}$ supplementation precipitated an intermediate fatty acid synthetase activity, the rate of fatty acid

TABLE 3

Effect of time and dietary fat on liver fatty acid synthesis after one meal containing fatty acids

(Experiment 1)

Time after meal initiation[1], hrs.	Dietary Fatty Acid			
	FF	+7% $C_{16:0}$	+3% $C_{18:3}$	Ave.
4	569 ± 67[2,3]	504 ± 16	629 ± 70	567 ± 50
5	599 ± 32	592 ± 28	505 ± 50	565 ± 37
6	496 ± 40	494 ± 45	442 ± 20	477 ± 35
8	438 ± 45	513 ± 29	566 ± 31	505 ± 35
Ave.	530 ± 26	524 ± 17	540 ± 26	

[1]Mean ± SEM; n=5.

[2]Dpm ^3H incorporated in vivo into fatty acids min^{-1} g $liver^{-1}$.

[3]No significant effect of dietary fat or time on liver fatty acid synthesis.

TABLE 4

Effect of two meals of fat-free diet containing stearate, linoleate or linolenate on rat liver fatty acid synthesis

(Experiment 2)

Parameter	Dietary Fatty Acid[1]			
	FF	+8% $C_{18:0}$	+3% $C_{18:2}$	+3% $C_{18:3}$
Daily food intake[2], g	12.8 ± 0.6	14.0 ± 0.6	13.4 ± 0.7	13.5 ± 0.6
Fatty acid intake, mg day^{-1}	----	1117 ± 53	400 ± 22	406 ± 18
Fatty acid absorbed[3], mg day^{-1}	----	391	348	361
Liver:				
Wgt., g	7.5 ± 0.2	7.5 ± 0.2	7.7 ± 0.3	7.9 ± 0.2
Glucokinase[4]	24 ± 2	22 ± 1	22 ± 2	22 ± 2
In vivo FA-synthesis[5]				
^3H_2O	780 ± 101	729 ± 65	678 ± 47	689 ± 58
1-^{14}C-acetate	1382 ± 128	1469 ± 272	1357 ± 190	1249 ± 205

[1]Means + SEM; n=10. No significant treatment effect on any parameter. Average initial body weight for all rats was 171 ± 5 g.

[2]Fat-free basal diet only.

[3]Calculated from digestibility values determined by Clarke et al (223).

[4]Nanamoles NADP reduced min^{-1} mg $protein^{-1}$ at 30°.

[5]Dpm of ^3H or ^{14}C incorporated in vivo min^{-1} g $liver^{-1}$.

TABLE 5

Effect of three meals of fat-free diet containing stearate, linoleate, or linolenate on rat liver fatty acid synthesis

(Experiment 2)

Parameter	Dietary Fatty Acid[1]			
	FF	+8% $C_{18:0}$	+3% $C_{18:2}$	+3% $C_{18:3}$
Daily food intake[2], g	13.6 ± 0.6[7a]	13.1 ± 0.8[a]	14.2 ± 0.6[a]	12.9 ± 0.9[a]
Fatty acid intake, mg per day	---	1088 ± 66	425 ± 17	386 ± 26
Fatty acid absorbed[3], mg per day	---	381	370	343
Liver:				
Wgt., g	7.4 ± 0.2	8.0 ± 0.3	7.9 ± 0.2	7.8 ± 0.3
Glucokinase[4]	29 ± 2	27 ± 1	26 ± 2	30 ± 1
Fatty acid synthetase[5]	31 ± 2[c]	27 ± 2[b,c]	24 ± 2[a,b]	21 ± 2[a]
In vivo FA-synthesis[6]:				
^3H_2O	1046 ± 68[b]	1044 ± 92[b]	730 ± 86[a]	658 ± 76[a]
1- ^{14}C-acetate	1919 ± 224[a]	2079 ± 352[a]	1463 ± 301[a]	1368 ± 253[a]

[1]Mean ± SEM; n=10. Average initial body weight of all rats was 171 ± 5g.

[2]Fat-free diet intake only.

[3]Calculated using digestibility coefficients determined by Clarke et al (1976).

[4]Nanomoles NADP reduced min^{-1} mg $protein^{-1}$ at 30°.

[5]Nanomoles NADPH oxidized min^{-1} mg $protein^{-1}$ at 37°.

[6]Dpm of ^3H or ^{14}C incorporated in vivo into fatty acids min^{-1} g $liver^{-1}$.

[7]Those values with different superscript letters are significantly different ($P < 0.05$).

synthesis was identical to the FF-group (Table 5). Unlike $^{3}H_2O$ the incorporation of 1-^{14}C-acetate into hepatic fatty acids was not significantly reduced by $C_{18:2}$ or $C_{18:3}$ but the percentage decline from the FF-group was comparable to $^{3}H_2O$ incorporation (Table 5). This lack of statistical significance may be related to the potentially greater variability in the specific activity of the acetate precursor pool among treatments.

Correcting for the specific activity of $^{3}H_2O$ in rats among treatments, we again observed that the rate of C_2-unit utilization for liver fatty acid synthesis was not significantly depressed until after three meals of $C_{18:2}$ supplementation to FF-diet (Table 6). The greatest inhibition of liver lipogenesis occurred after the fourth meal and at this time the percent reduction relative to FF-diet was comparable to that seen after eight meals of $C_{18:2}$ (Table 7). Unlike experiment 2 neither three nor four meals of $C_{18:2}$ significantly reduced hepatic fatty acid synthetase activity. Furthermore the utilization of C_2-units by fatty acid synthetase, as determined by NADPH oxidation, was higher than the in vivo rate of C_2 utilization for fatty acid synthesis (Table 6). The significant reduction in hepatic fatty acid synthesis was not associated with an inhibition in glucokinase activity by dietary $C_{18:2}$ (Table 6). In fact when glucokinase was assayed at 37^{o}, its activity far exceeded that of fatty acid synthetase (Table 6).

In experiment 4 changes in hepatic fatty acid synthesis rates were examined in rats following removal of $C_{18:2}$ from the diet for

TABLE 6

Change in liver fatty acid synthesis and associated enzyme activities following 3% linoleate addition to fat-free diet

(Experiment 3)

Dietary treatment[1]	No. Meals		
	2	3	4
		Liver wgt., g	
FF	8.2 ± 0.3[6a]	7.6 ± 0.3[a]	8.2 ± 0.3[a]
+3% $C_{18:2}$	8.7 ± 0.3[a]	7.3 ± 0.4[a]	8.6 ± 0.2[a]
		Glucokinase[2]	
FF	75 ± 9[a]	82 ± 9[a]	123 ± 27[a]
+3% $C_{18:2}$	77 ± 9[a]	74 ± 9[a]	82 ± 11[b]
		Fatty acid synthetase[3,4]	
FF	---	18.0 ± 1.5[a]	14.5 ± 1.5[a]
+3% $C_{18:2}$	---	14.5 ± 1.5[a]	11.5 ± 1.0[a]
		In vivo fatty acid synthesis[5] C_2-units mg protein^{-1}	
FF	11.6 ± 0.5[a]	13.6 ± 1.6[a]	13.6 ± 1.7[a]
+3% $C_{18:2}$	10.6 ± 0.7[a]	10.0 ± 0.9[b]	8.4 ± 0.8[b]
		In vivo fatty acid synthesis[5] C_2-units g liver^{-1}	
FF	910 ± 40[a]	888 ± 71[a]	840 ± 60[a]
+3% $C_{18:2}$	805 ± 58[a]	727 ± 57[b]	556 ± 34[b]

[1]Mean ± SEM; n=10 for rats weighing 145 ± 5g.

[2]Nanomoles glucose utilized min^{-1} mg^{-1} protein, 37°.

[3]Nanomoles $H^{14}CO_3$ fixed min^{-1} mg^{-1} protein, 37°.

[4]C_2-unit equivalents utilized min^{-1} mg^{-1} protein, 37°.

[5]Nanomoles C_2-units incorporated in vivo min^{-1}.

[6]Those values with different superscripts for each meal sequence are significantly different ($P < 0.05$).

TABLE 7

Influence of one and two meals of fat-free diet on rat liver fatty acid synthesis after six and seven meals of linoleate supplementation

(Experiment 4)

Parameter	Meal Sequence[1]			
	8-FF	6 $C_{18:2}$ 2-FF	7 $C_{18:2}$ 1-FF	8 $C_{18:2}$
Final Body wgt., g	197 ± 9	206 ± 7	205 ± 6	210 ± 10
Total wgt. gain, g	18 ± 2[a]	26 ± 2[b]	27 ± 2[b]	25 ± 2[b]
Daily food intake[2], g	14.3 ± 0.7	14.5 ± 0.4	14.1 ± 0.6	14.3 ± 0.7
Fatty acid intake, mg per day	---	444 ± 17	425 ± 18	432 ± 22
Liver:				
Wgt., g	9.7 ± 0.4[a]	10.5 ± 0.4[a]	10.2 ± 0.4[a]	10.7 ± 0.4[a]
Glucokinase[3]	18 ± 2[6a]	16 ± 1[a]	14 ± 1[a]	14 ± 1[a]
Fatty acid synthetase[4]	9.5 ± 0.5[b]	7.0 ± 1[a]	5.0 ± 0.5[a]	5.5 ± 0.5[a]
In vivo FA-synthesis[5]	10.4 ± 0.6[b]	7.1 ± 0.6[a]	6.1 ± 0.6[a]	6.2 ± 0.6[a]

[1]Means ± SEM; n=10.

[2]Fat-free diet intake.

[3]Nanomoles NADP reduced min^{-1} mg $protein^{-1}$ at 30°.

[4]Nanomoles C_2-units used min^{-1} mg $protein^{-1}$ at 37°.

[5]Nanomoles C_2-units converted to fatty acids in vivo min^{-1} mg $protein^{-1}$.

[6]Those values with different superscript letters are significantly different ($P < 0.05$) as determined by Tukey's t-test.

one or two meals (Table 7). Even though all animals consumed the same quantity of FF-diet, those supplemented with $C_{18:2}$ gained significantly more weight during the seven day period (Table 7). This likely reflects added energy intake and adequate essential fatty acid status (180).

In vivo incorporation of ^3H_2O into hepatic fatty acids was very effectively inhibited by dietary $C_{18:2}$. Consistent with the observation that three meals of $C_{18:2}$ supplementation were essential to detect inhibition of hepatic lipogenesis (Tables 5 and 6), removing $C_{18:2}$ from the diet for one and two meals did not cause a significant increase in liver fatty acid synthesis (Table 7). Fatty acid synthetase activity was also not significantly elevated after two fat-free meals according to the Tukey t-test of significance (Table 7). However the absolute value was intermediate to eight meals of FF and FF plus $C_{18:2}$, and this change represented a 33% rise over the activity found in rats fed eight meals containing $C_{18:2}$.

In this longer study the C_2-unit utilization by fatty acid synthetase as measured in vitro was nearly identical to that for in vivo hepatic fatty acid synthesis (Table 7). Feeding $C_{18:2}$ for eight meals moderately reduced glucokinase activity but this decline was not marked as that for fatty acid synthetase nor was the depression statistically significant (Table 7).

DISCUSSION

Within four hours after beginning to eat their meal 40% of the food eaten was gone from the stomach and small intestine of meal-trained rats (Table 3) which suggests a large absorptive capacity. Rats trained to consume their daily food in a two or three hour period have been shown to display a 30% increase in absorptive capacity (143). A greater percentage of dietary $C_{18:3}$ disappeared from gut contents than $C_{16:0}$ which was in agreement with differences in apparent digestibilities. However, the percent disappearance (Table 3) for $C_{16:0}$ and for $C_{18:3}$ was higher and lower respectively than values determined by a six day digestion trail (222, 223). This discrepancy probably reflects the duration of experiment and differences in collection methodology.

The inhibitory action of dietary $C_{18:2}$ and $C_{18:3}$ on liver fatty acid synthesis (Tables 5-7) could be caused by a direct inhibition of lipogenic machinery or could be secondary to a reduction in pyruvate production from glucose (60). Supplementing the FF-diet with $C_{18:2}$ or $C_{18:3}$ at no time significantly altered the in vitro activity of glucokinase (Tables 4-7). However $C_{18:2}$ and $C_{18:3}$ was associated with a significant decline in the rate of hepatic fatty acid synthesis after three meals of supplementation (Tables 5 and 6), and with a definite depression in fatty acid synthetase after six meals (Table 7).

Since the liver is freely permeable to glucose, the key initial point and possibly the rate limiting step (37) in hepatic glucose metabolism is its phosphorylation by glucokinase (40). Glucokinase activity was reduced in rats by higher dietary fat levels, and in gerbils was more effectively inhibited by a safflower oil (15%) diet than coconut oil (40). The inability of $C_{18:2}$ or $C_{18:3}$ supplementation to reduce glucokinase activity may be due to the low dietary level. In addition the higher circulating insulin levels in meal-eating rats plus the large influx of glucose after a meal (152) may maintain glucokinase activity even with $C_{18:2}$ and $C_{18:3}$ supplementation.

Admittedly the in vitro activity of glucokinase does not necessarily represent its activity in vivo nor does it yield an indication of in vivo flux of glucose through glucolysis. Nevertheless the total lack of response of an enzyme at the initial step of glucose metabolism at a time when fatty acid synthesis and fatty acid synthetase are significantly declining suggests that dietary $C_{18:2}$ and $C_{18:3}$ specifically affect the function of lipogenic processes.

Clearly in vivo rates of liver fatty acid synthesis in the meal-eating rat are not altered immediately by supplementing to or removing from a high carbohydrate diet low levels of polyunsaturated fatty acids (Tables 3-7). These observations are consistent with earlier observations in which the addition of 5% $C_{18:3}$ to a low fat, high carbohydrate diet for one meal had no inhibitory effect on the in vivo rate of rat liver fatty acid synthesis (222).

The ineffectiveness of one low fat meal to reduce hepatic lipogenesis may not be unusual considering that most of the fatty acid would be transported as triglyceride and taken up by peripheral tissues with minimal escape of unesterified fatty acids. Very low rates of liver fatty acid synthesis were observed in rats within three hours after intubation with 2.0 ml corn oil (95). This apparent discrepancy can likely be attributed to the high fat dosage associated with 2.0 ml corn oil.

The time required before a significant inhibition of hepatic fatty acid synthesis was observed after $C_{18:2}$ and $C_{18:3}$ addition to FF-diet indicates that the regulator is long term in nature.

This is consistent with the hypothesis that the level of acetyl-CoA carboxylase and/or fatty acid synthetase determines the rate of fatty acid synthesis in the liver of rats fed a high carbohydrate diet (19). Potentially $C_{18:2}$ and $C_{18:3}$ could control the level of one or both enzymes via altered synthesis and degradation. The estimated half-life of these enzymes of 30 to 60 hours would be consistent with the time required for inhibition of FA-synthesis (23, 26). The in vitro utilization of C_2-units by liver fatty acid synthetase after six to eight meals of $C_{18:2}$ was nearly identical to the rate of C_2-unit incorporation in vivo into liver fatty acids (Table 7). Such close correlation tends to substantiate the conclusion that the level of hepatic fatty acid synthetase enzyme determines the rate of fatty acid synthesis in rats fed high carbohydrate diets (19).

An alternative possibility is that the regulator is short term in nature, e.g. $C_{18:2}$ or $C_{18:3}$ per se or a derivative. However a minimum of two meals of $C_{18:2}$ or $C_{18:3}$ is needed to effectively alter the composition of the liver in favor of the specific inhibitor (8). The location of action of such a short term effector could at acetyl-CoA carboxylase or citrate efflux.

The rate of fatty acid synthesis not only depends on the rate of malonyl-CoA utilization but also on the rate of its production by acetyl-CoA carboxylase (35). The data from three and four meals of $C_{18:2}$ supplementation (Table 6) indicate that during the transition to a new steady-state, the depression in fatty acid synthesis preceded any decline in fatty acid synthetase activity. Furthermore the in vitro rate of utilization of C_2-units by hepatic fatty acid synthetase at these times exceeded that utilized for in vivo fatty acid synthesis (Table 6). Fatty acid synthetase activity in vivo may not necessarily be as high as that in vitro but the enzyme purportedly follows Michaelis-Menten kinetics and does not display allosteric regulation in vivo (35). Therefore our data suggest the initial point of inhibitory action of $C_{18:2}$ and $C_{18:3}$ on liver fatty acid synthesis in meal-fed rats is via the production and not the utilization of malonyl-CoA.

PART VI

SUMMARY AND CONCLUSIONS

SUMMATIONS

An elevation in blood triglycerides and cholesterol in humans has received wide acceptance as a warning sign for cardiovascular problems. One approach to controlling hypertriglyceridemia has been the dietary manipulation of reducing the proportion of saturated fat (e.g. butter, beef tallow, lard) and increasing the amount of polyunsaturated fat (e.g. corn oil, safflower oil) (1-5). The validity of this approach has not been universally accepted (4, 5) and a mechanism of action of polyunsaturated fats has not been elucidated.

Initial observations with mice and rats fed high fat diets indicated that perhaps polyunsaturated fats were more effective than saturated fats in suppressing liver and adipose tissue lipogenic enzyme activities and de novo rates of fatty acid synthesis (158, 159, 169). Such an effect in man, who synthesizes fatty acids primarily in the liver, would have significant implications. A reduction in the amount of de novo fat synthesis in the liver precipitated by dietary polyunsaturated fatty acids could mean less triglyceride transport via blood and hence a lowered blood triglyceride level. However because of the high dietary fat levels and because of variation in fatty acid composition of dietary fats, earlier studies were unable to explain the mechanism of action by which polyunsaturated fats affect rates of lipogenesis. One method adopted to avoid

these problems was to supplement a fat-free diet of mice and rats with low levels of pure esters of individual fatty acids (7-10). However differences attributed to individual fatty acids in affecting liver and adipose lipogenesis were overshadowed by the following oversights: a) variation among experiments and treatments in the type and amount of carbohydrate consumed by animals, (b) differences among methyl esters of fatty acids in digestibility, (c) the assumption that lipogenic enzyme activities reflected rates of fatty acid synthesis and (d) failure to adequately examine the influence of dietary fatty acids on adipose tissue lipogenesis.

Therefore the primary objective of this research was to re-evaluate the contention that low levels of dietary polyunsaturated fatty acids specifically and effectively inhibit liver and adipose tissue fatty acid synthesis and associated enzymes in the meal-eating rat. Particular attention was given to possible differences in digestibility among the fatty acid methyl esters utilized, and attention was directed to correlating in vivo rates of fatty acid synthesis to in vitro lipogenic enzyme activities. The second phase of experimentation investigated parameters which could potentially explain the inhibition of fatty acid synthesis by dietary fat, specifically linoleate and linolenate.

Supplementing a fat-free diet for seven days with 3% $C_{18:2}$ or $C_{18:3}$ resulted in a significant decline in the activities of hepatic fatty acid synthetase, glucose-6-phosphate dehydrogenase and malic enzyme. In general these acids were associated with a significant

reduction in the in vitro rate of liver fatty acid synthesis (Part II, Tables 2-4). In contrast 3% $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$ supplements were all ineffective in reducing rates of fatty acid synthesis in rat liver. Similarly $C_{16:0}$ and $C_{18:1}$ did not lower the activities of lipogenic enzymes while $C_{18:0}$ actually led to a significant rise in hepatic fatty acid synthetase, glucose-6-phosphate dehydrogenase and malic enzyme (Part II, Tables 2-4). However the marked differences in digestibilities among $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ (40, 35, 88, 87 and 89%, respectively) greatly confounded the interpretation of the effects of $C_{16:0}$ and $C_{18:0}$. When the level of $C_{16:0}$ and $C_{18:0}$ added to the fat-free diet was raised such that the quantity of acid absorbed was comparable to $C_{18:2}$ and $C_{18:3}$, both dietary saturated acids were still unable to reduce the in vivo rate of ^3H_2O incorporation into liver fatty acids. The activities of hepatic glucose-6-phosphate dehydrogenase, malic enzyme, fatty acid synthetase or acetyl-CoA carboxylase (Part II, Table 5, Part IV, Table 2) were also unaffected by $C_{16:0}$ and $C_{18:0}$. Since all animals among treatments consumed the same amount of carbohydrate and protein, the inhibition of hepatic lipogenesis must be specific for $C_{18:2}$ and $C_{18:3}$.

At no time did any of the added fatty acid methyl esters lead to a reduction in adipose tissue lipogenesis (in vitro or in vivo) or lipogenic enzyme activities (Part II, Tables 2-4, Part III, Table 4; Part IV, Table 2). The rapid influx of carbohydrate and higher circulating insulin levels associated with these meal-trained rats would lead to high rates of adipose tissue fatty acid esterification

(152, 156). Therefore the small amount of absorbed fat may not significantly affect the rate of adipose fatty acid synthesis.

Examining the effects of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ on lipogenesis in rats fed a fat-free diet results in a comparison between essential fatty acid deficient rats and essential fatty acid adequate rats. Since an essential fatty acid deficient status leads to marked changes in total liver and hepatic organelle fatty acid composition as well as aberrations in mitochondrial function (174, 192), the inhibitory action of $C_{18:2}$ and $C_{18:3}$ could have been the consequence of fulfilling an essential fatty acid requirement. The addition of 3% $C_{18:2}$ and $C_{18:3}$ to a low fat essential fatty acid adequate diet still was associated with a significant drop in liver fatty acid synthetase, glucose-6-phosphate dehydrogenase and malic enzyme activities, whereas $C_{16:0}$ and $C_{18:1}$ had little or no influence on these enzyme activities. Unlike the data from fat-free diet supplementation, adding $C_{18:2}$ for seven days to a diet containing 2.5% safflower oil did not depress the rate of in vivo fatty acid synthesis. However 10 days of 3% $C_{18:2}$ and $C_{18:3}$ addition to a 1% safflower oil diet significantly reduced in vivo hepatic fatty acid synthesis by over 50%. Similarly 10 days of 3% $C_{18:1}$ supplementation to the 1% safflower oil diet significantly reduced the rate of fatty acid synthesis relative to basal, but the rate was well above that of the polyunsaturated fat groups. The discrepancies between the two experiments may be the product of greater variation in the 2.5% safflower oil trial (CV = 44%) or due to the lower level

of fat and longer period of supplementation in the 1% safflower oil trial. Under essential fatty acid adequate conditions $C_{18:2}$ and $C_{18:3}$ continued to inhibit de novo rat liver fatty acid synthesis with extreme efficacy relative to $C_{16:0}$ or $C_{18:1}$. In both essential fatty acid experiments, adipose tissue lipogenesis remained unaffected by the addition of $C_{16:0}$, $C_{18:1}$, $C_{18:2}$ or $C_{18:3}$ to the basal diets.

Polyunsaturated fatty acids have been suggested to turnover from adipose tissue and undergo β-oxidation more rapidly than saturated fatty acids (177, 203). This could lead to an elevation in plasma free fatty acids and/or an increase in long chain acyl-CoA content of liver. Long chain acyl-CoAs play a significant role in the control of citrate efflux and acetyl-CoA carboxylase activity (89, 136). In addition a higher rate of β-oxidation could increase the proportion of hepatic NADH and elevate the lactate/pyruvate ratio causing a reduction in glycolytic flux (36, 60). Even though adding 3% $C_{18:2}$ and $C_{18:3}$ to a fat-free diet for seven days caused a significant decline in the rate of de novo fatty acid synthesis and the activity of lipogenic enzymes of rat liver, these effects were not associated with an altered hepatic lactate/pyruvate ratio, an elevation in plasma free fatty acids, or an increase in long-chain acyl-CoA concentration in the liver (Part IV, Tables 2-4). $C_{18:2}$ supplementation for six meals did cause a fourfold rise in plasma free linoleate concentration, but had no significant effect on the concentration of the remaining long-chain fatty acids (Part

IV, Table 3). The liver of rats and humans was shown to remove circulating polyunsaturated free fatty acids more rapidly than saturated acids (198). Therefore if polyunsaturated fatty acids exert an effect on lipogenesis through long chain acyl-CoA esters, the action must be more dependent on composition of hepatic acyl-CoAs than on concentration.

The very effective inhibition of fatty acid synthesis and lipogenic enzyme activities in the liver of meal-trained rats by dietary $C_{18:2}$ and $C_{18:3}$ may be secondary to a reduction in the rate of glucose flux through glycolysis (60). Rats fed the fat-free diet plus 3% $C_{18:2}$ displayed a 25% reduction in liver glucokinase and pyruvate kinase activities (Part IV, Table 5). However this depression was much less than the 50% drop in fatty acid synthetase and citrate cleavage enzyme activities (Part IV, Table 5). Admittedly glycolytic flux was not quantitated, nevertheless glucokinase activity (often considered the rate limiting step of glycolysis) had a capacity to phosphorylate glucose which greatly exceeded the demands of fatty acid synthetase.

In the seven day feeding trials (Part IV, Table 2; Part V, Table 7) in which the specific activity of body water-^3H_2O was determined, the in vivo rate of C_2-unit incorporation into liver fatty acids was nearly identical to the in vitro rate of C_2-unit utilization by fatty acid synthetase and acetyl-CoA carboxylase. This close correlation after seven days was independent of whether the dietary treatment was fat-free or supplemented with $C_{18:0}$

or $C_{18:2}$. In these meal-trained rats fed the high-carbohydrate diet, fatty acid synthetase and acetyl-CoA carboxylase appear to be functioning at near capacity shortly after the meal and the level of one or both enzymes could determine the rate of fatty acid synthesis (19). Under these conditions the in vitro enzyme activity of fatty acid synthetase and/or acetyl-CoA carboxylase can be used as an indication of substrate flux to fatty acids in the liver.

However after seven days of $C_{18:2}$ feeding the animal very likely has reached a new steady-state. At this point the initial inhibitory point of dietary $C_{18:2}$ may not be detected accurately. Thus of particular importance was the characterization of changes in lipogenic and glycolytic enzymes as well as rates of fatty acid synthesis during the transitional period following the addition of $C_{18:2}$ to the fat-free diet. A minimum of three $C_{18:2}$-containing meals was essential before a significant decline in liver fatty acid synthesis was detectable (Part V, Tables 4-7).

Three meals corresponds to about 48 hours. Such a time span indicates the control of fatty acid synthesis may be long-term type regulation. If in the meal-eating rat the level of fatty acid synthetase and/or acetyl-CoA carboxylase determines the rate of hepatic lipogenesis (19), then an effect of polyunsaturated fatty acids on the rate of synthesis or degradation of the enzymes would be consistent with their estimated half-lifes (109). A quantitative comparison of the in vivo rate of C_2-unit incorporation into hepatic fatty acids to the rate of in vitro utilization by fatty acid synthetase

(Part V, Table 7), revealed that the total enzyme activity was well in excess of the rate of synthesis and the drop in synthesis preceded the fall in synthetase activity (Part V, Table 7). In fact the rate of fatty acid synthesis had reached a point of depression at four meals comparable to the eight meal level (Part V, Table 7) while fatty acid synthetase activity had not significantly declined. Glucokinase activity remained very high during this four meal transition period and at no time was it reduced in activity by 3% $C_{18:2}$ addition (Part IV, Table 5; Part V, Tables 5-7). These data during the achievement of a new steady-state indicate that fatty acid synthetase is not limiting and that the location of inhibition is in production of malonyl-CoA not in its utilization.

In conclusion low levels of dietary methyl $C_{18:2}$ and $C_{18:3}$ consistently reduced the rates of hepatic fatty acid synthesis and lipogenic enzymes in meal-fed rats by 35 to 40%. This specific inhibition was independent of carbohydrate and protein intake, could not be mimicked by comparable amounts of absorbed $C_{16:0}$, $C_{18:0}$ or $C_{18:1}$ and was not a product of an essential fatty acid deficient diet. Adipose tissue from both essential fatty acid deficient and essential fatty acid adequate meal-fed rats maintained high rates of lipogenesis which were unaffected by the addition of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$ or $C_{18:3}$ to the diet. The inability of dietary $C_{18:2}$ to reduce glucokinase activity during a time when hepatic lipogenesis was markedly depressed, the minimal effect of $C_{18:2}$ on pyruvate kinase activity, and the lack of consistent change in

hepatic pyruvate concentration collectively indicate that polyunsaturated fatty acids specifically affect lipogenic mechanisms and not glycolytic activity. Polyunsaturated fatty acid inhibition of lipogenesis in meal-fed rats requires 48 to 72 hours before becoming detectable which suggests long-term type regulation. The nature of the mechanism is not related to the concentration of hepatic long chain acyl-CoA esters, nor to the cytosolic redox state nor apparently to the level of fatty acid synthetase. Since the only other detectable effect of $C_{18:2}$ supplementation was to increase plasma unesterified $C_{18:2}$ by fourfold, inhibition may be related to the composition of the free fatty acids entering the liver or to the acyl-CoA composition.

In speculation, since the activity of acetyl-CoA carboxylase, and citrate transport and adenine translocase systems are all sensitive to acyl-CoA esters; the key to future work may live in correlating acyl-CoA ester composition of the liver to changes in these parameters during the transitional phase. In addition changes in glycolytic flux rates cannot be totally eliminated as a site of polyunsaturated fat action. The insensitivity of adipose tissue to low fat diets would be an interesting point of future investigation involving diet and food composition.

LITERATURE CITED

LITERATURE CITED

1. Harper, H. A. (1974) Physiological Chemistry Lange Medical Publications, Los Altos, California.

2. Faergeman, O. and Havel, R. J. (1975) Metabolism of Cholesteryl esters of rat very low density lipoproteins. J. Clin. Invest. 55, 1210-1218.

3. Spritz, N. and Mishkel, M. M. (1969) Effects of dietary fats on plasma lipids and lipoproteins: an hypothesis for the lipid-lowering effect of unsaturated fatty acids. J. Clin. Invest. 48, 78-85.

4. Keys, A., Grande, F., and Anderson, J. T. (1974) Bias and misrepresentation revisited: Perspective on saturated fat. Amer. J. Clin. Nutr. 27, 188-212.

5. Reiser, R. (1973) Saturated fat in the diet and serum cholesterol concentration: a critical examination of the literature. Amer. J. Clin. Nutr. 26, 524-555.

6. Horlick, L. and Craig, M. B. (1957) Effect of long-chain polyunsaturated and saturated fatty acids on the serum lipids of man. Lancet 2, 566-570.

7. Allmann, D. W. and Gibson, D. M. (1965) Fatty acid synthesis during early linoleic acid deficiency in the mouse. J. Lip. Res. 6, 51-62.

8. Allmann, D. W., Hubbard, D. D., and Gibson, D. M. (1965) Fatty acid synthesis during fat-free refeeding of starved rats. J. Lip. Res. 6, 63-74.

9. Muto, Y. and Gibson, D. M. (1970) Selective dampening of lipogenic enzymes of liver by exogenous polyunsaturated fatty acids. Biochem. Biophys. Res. Comm. 38, 9-15.

10. Musch, K., Ojakian, M. A., and Williams, M. A. (1974) Comparison of a-linolenate and oleate in lowering activity of lipogenic enzymes in rat liver: evidence for a greater effect of dietary linolenate independent of food and carbohydrate intake. Biochim. Biophys. Acta 337, 343-348.

11. Lehninger, A. L. (1970) Biochemistry. Worth Publishers, Inc., New York, New York.

12. Larner, J. (1971) Intermediary Metabolism and Its Regulation. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

13. Silpanta, P. and Goodridge, A. G. (1971) Synthesis and degradation of malic enzyme in chick liver. J. Biol. Chem. 246, 5754-5761.

14. Wieland, O. H. and Portenhauser, R. (1974) Regulation of pyruvate dehydrogenase interconversion in rat liver mitochondria as related to phosphorylation state of intramitochondrial adenine nucleotides. Eur. J. Biochem. 45, 577-588.

15. Romsos, D. R., and Leveille, G. A. (1974) Effect of diet on activity of enzymes involved in fatty acid and cholesterol synthesis. Advan. Lip. Res. 12, 97-146.

16. Berry, M. N., Kun, E., and Werner, H. V. (1973) Regulatory role of reducing-equivalent transfer from substrate to oxygen in the hepatic metabolism of glycerol and sorbitol. Eur. J. Biochem. 33, 407-417.

17. Bailey, E., Taylor, C. B., and Barley, W. (1968) Effect of dietary carbohydrate on hepatic lipogenesis in the rat. Nature (London) 217, 471-472.

18. Fitch, W. M. and Chaikoff, I. L. (1960) Extent and patterns of adaptation of enzyme activities in livers of normal rats fed diets high in glucose and fructose. J. Biol. Chem. 235, 554-557.

19. Guynn, R. W., Veloso, D., and Veech, R. L. (1972) The concentration of malonyl-coenzyme A and the control of fatty acid synthesis in vivo. J. Biol. Chem. 247, 7235-7242.

20. Volpe, J. J. and Vagelos, P. R. (1973) Fatty acid synthetase of mammalian brain, liver and adipose tissue. Regulation of prosthetic group turnover. Biochim. Biophys. Acta 326, 293-303.

21. Taketa, K. and Wantanabe, A. (1970) Interconvertible microheterogeneity of glucose-6-phosphate dehydrogenase in rat liver. Biochim. Biophys. Acta 235, 19-26.

22. Slayton, C. A. and Szepesi, B. (1973) The role of high fat diet in the rerepression of two rat liver enzymes. Proc. Soc. Exp. Biol. Med. 144, 876-881.

23. Craig, M. C. and Porter, J. W. (1973) Synthesis of fatty acid synthetase by isolated liver cells obtained from rats in different nutritional or hormonal states. Arch. Biochem. Biophys. 159, 606-614.

24. Kitajima, K., Tashiro, S. and Numa, S. (1975) Acetyl-coenzyme-A carboxylase in cultured hepatocytes. Eur. J. Biochem. 54, 373-383.

25. Volpe, J. J. and Vagelos, P. R. (1974) Regulation of mannalian fatty-acid synthetase. The roles of carbohydrate and insulin. Proc. Nat. Acad. Sci. 71, 879-893.

26. Craig, M. C., Nepokroeff, C. M., Lakshamanan, M. R., and Porter, J. W. (1972) Effect of dietary change on the rates of synthesis and degradation of fat liver fatty acid synthetase. Arch. Biochem. Biophys. 152, 619-630.

27. Nepokroeff, C. M., Lakshamanan, M. R., Ness, G. C., Muesing, R. A., Kleinsek, D. A., and Porter, J. W. (1974) Coordinated control of rat liver lipogenic enzymes by insulin. Arch. Biochem. Biophys. 162, 340-344.

28. Weber, G., Lea, M. A., Fisher, E. A., and Stamm, N. B. (1966) Regulatory pattern of liver carbohydrate metabolizing enzymes: insulin as inducer of key glycolytic enzymes. Enzymol. Biol. Clin. 7, 11-24.

29. Goodridge, A. G. (1972) Regulation of the activity of acetyl coenzyme A carboxylase by palmitoyl coenzyme A and citrate. J. Biol. Chem. 247, 6946-6952.

30. Taunton, O. D., Stifel, F. B., Greene, H. L., Herman, R. H. (1974) Rapid reciprocal changes in rat hepatic glycolytic enzymes and fructose diphosphatase activities following insulin and glucagon injection. J. Biol. Chem. 249, 7228-7239.

31. Gumaa, K. A., McLean, P. and Greenbaum, A. L. (1971) Compartmentation in relation to metabolic control in liver. Essays Biochem. 7, 39-86.

32. Ballard, F. J. (1972) Effects of fasting and refeeding on the concentrations of glycolytic intermediates and the regulation of lipogenesis in rat adipose tissue in vivo. Biochim. Biophys. Acta 273, 110-118.

33. Lowenstein, J. M. (1971) Effect of (-)-hydroxycitrate on fatty acid synthesis by rat liver in vivo. J. Biol. Chem. 246, 629-632.

34. Sullivan, A. C., Triscari, J., Hamilton, J. G., Miller, O. N. and Wheatley, V. R. (1974) Effect of (-)-hydroxycitrate upon the accumulation of lipid in the rat. Lipids 9, 121-128.

35. Guynn, R. W., Veloso, D., Harris, R. L., Lawson, J. W. R., and Veech, R. L. (1973) Ethanol administration and the relationship of malonyl-coenzyme A concentrations to the rate of fatty acid synthesis in rat liver. Biochem. J. 136, 639-647.

36. Newsholme, E. A. and Start, C. (1973) Regulation in Metabolism. John Wiley and Sons, New York, New York.

37. Niemeyer, H., Ureta, T. and Clark-Turri, L. (1975) Adaptive character of liver glucokinase. Mol. Cell Biochem. 6, 109-126.

38. Greenbaum, A. L., Gumaa, K. A. and McLean, P. (1971) The distribution of hepatic metabolites and the control of the pathways of carbohydrate metabolism in animals of different dietary and hormonal status. Arch. Biochem. Biophys. 143, 617-663.

39. Owen, O. E., Morgan, A. P., Kemp, H. G., Sullivan, J. M., Herrera, M. G. and Cahill, G. F. (1967) Brain metabolism during fasting. J. Clin. Invest. 46, 1589-1595.

40. Niemeyer, H., Perez, N. and Rabajille, E. (1966) Interrelation of actions of glucose insulin, and glucagon on induction of adenosine triphosphate: D-hexose phosphotransferase in rat liver. J. Biol. Chem. 241, 4055-4061.

41. Ureta, T., Gonzalez, C., Lillo, S. and Niemeyer, H. (1971) Comparative studies on glucose phosphorylating isoenzymes of vertebrates. I. The influence of fasting and the nature of the diet on liver glucokinase and hexokinases of rodents. Comp. Biochem. Physiol. 40B, 71-80.

42. Sharma, C., Manjeshwar, R. and Weinhouse, S. (1963) Effects of diet and insulin on glucose adenosine triphosphate phosphotransferases of rat liver. J. Biol. Chem. 238, 3840-3845.

43. Perez, N., Clark-Turri, L., Rabajille, E., and Niemeyer, H. (1964) Regulation of rat liver enzymes by natural components of the diet. J. Biol. Chem. 239, 2420-2426.

44. Seiler, M. W., Hamilton, M. A., Laurio, V., Herrera, M. G. and Hegested, D. M. (1971) Hyperlipemia in the gerbil: effect of diet on serum lipids and hepatic glucokinase. Amer. J. Physiol. 221, 548-553.

45. Weber, G., Lea, M. A. and Stamm, N. B. (1966) Sequential feedback inhibition and regulation of liver carbohydrate metabolism through control of enzyme activity. Advan. Enz. Reg. 6, 101-123.

46. Del Boca, J. and Flatt, J. P. (1969) Fatty acid synthesis from glucose and acetate and the control of lipogenesis in adipose tissue. Eur. J. Biochem. 11, 127-134.

47. Galton, D. J. and Wilson, J. P. D. (1971) The effect of starvation and diabetes on glycolytic enzymes in human adipose tissue. Clin. Sci. 41, 545-553.

48. Hansen, R. J., Pilkis, S. J. and Krahl, M. E. (1970) Effect of insulin on the synthesis in vitro of hexokinase in rat epididymal adipose tissue. Endocrinology 86, 57-65.

49. Anderson, J. W. (1974) Alterations in metabolic fate of glucose in the liver of diabetic animals. Amer. J. Clin. Nutr. 27, 746-750.

50. Purich, D. L., Fromm, H. J. and Rudolph, F. B. (1973) The hexokinases: kinetic, physical, and regulatory properties. Advan. Enzymology 39, 249-326.

51. Knull, H. R., Taylor, W. F., and Wells, W. W. (1973) Effects of energy metabolism on in vitro distribution of hexokinase in brain. J. Biol. Chem. 248, 5414-5417.

52. Bessman, S. P. and Gots, R. E. (1975) The hexokinase receptor theory of insulin action-hormonal control of functional compartmentation. Life Sci. 16, 1215-1226.

53. Borrebaeck, B. (1970) Mitochondrial-bound hexokinase of the rat epididymal adipose tissue and its possible relation to the action of insulin. Biochem. Med. 3, 485-497.

54. Tornheim, K. and Lowenstein, J. M. (1975) The purine nucleotide cycle. Control of phosphofructokinase and glycolytic oscillations in muscle extracts. J. Biol. Chem. 250, 6304-6314.

55. Hofer, H. W. and Furst, M. (1976) Isolation of phosphorylated form of phosphofructokinase from skeletal muscle. FEBS Let. 62, 118-122.

56. Brand, J. A. and Soling, H. D. (1975) Activation and inactivation of rat liver phosphofructokinase by phosphorylation-dephosphorylation. FEBS Let. 57, 163-168.

57. Veech, R. L., Raijman, L. and Krebs, H. (1970) Equilibrium relations between the cytoplasmic adenine nucleotide system and nicotinamide-adenine nucleotide system in rat liver. Biochem. J. 117, 499-503.

58. Denton, R. M., Yorke, R. E., and Randle, P. J. (1966) Measurement of concentrations of metabolites in adipose tissue and effects of insulin, alloxan-diabetes and adrenaline. Biochem. J. 100, 407-419.

59. Start, C. and Newsholme, E. A. (1970) A switch mechanism in the regulation of glycolysis and gluconeogenesis in the rat liver. FEBS Let. 6, 171-173.

60. Gunn, J. M., and Taylor, C. B. (1973) Relationships between concentration of hepatic intermediary metabolites and induction of the key glycolytic enzymes in vivo. Biochem. J. 136, 455-465.

61. Tsai, M. Y. and Kemp, R. G. (1974) Rabbit brain phosphofructokinase. J. Biol. Chem. 249, 6590-6596.

62. Ramadoss, C. S., Uyeda, K., and Johnston, J. M. (1976) Studies on the fatty acid inactivation of phosphofructokinase. J. Biol. Chem. 251, 98-107.

63. Weber, G., Singhal, R. L., Stamm, N. B., Lea, M. A., and Fisher, E. A. (1966) Synchronous behavior pattern of key glycolytic enzymes: glucokinase, phosphofructokinase and pyruvate kinase. Advan. Enz. Reg. 4, 59-83.

64. Orevi, M., Gorin, E., and Shafrin, E. (1972) Adaptive changes of phosphofructokinase and aldolase in adipose tissue. Eur. J. Biochem. 30, 418-426.

65. Dyson, R. D., Cardena, J. M. and Barsotti, R. J. (1975) The reversibility of skeletal muscle pyruvate kinase and an assessment of its capacity to support glycogenesis. J. Biol. Chem. 250, 3316-3321.

66. Seubert, W. and Schoner, W. (1971) The regulation of pyruvate kinase. Cur. Topics Cell. Regul. 3, 237-267.

67. Pogson, C. I. and Denton, R. M. (1967) Effect of alloxan diabetes, starvation and refeeding on glycolytic kinase activities in rat epididymal adipose tissue. Nature (London) 216, 156-157.

68. Chakrabarty, K. and Leveille, G. A. (1968) Influences of periodicity of eating on the activity of various enzymes in adipose tissue, liver and muscle of the rat. J. Nutr. 96, 76-82.

69. Linn, T. C., Petit, F. H. and Reed, L. J. (1969) α-keto acid dehydrogenase complexes, X. Regulation of the activity of the pyruvate dehydrogenase complex from beef kidney mitochondria by phosphorylation and dephosphorylation. Proc. Nat. Acad. Sci. (USA) 62, 234-241.

70. Chiang, P. K. and Sacktor, B. (1975) Control of pyruvate dehydrogenase activity in intact cardiac mitochondria. J. Biol. Chem. 250, 339-348.

71. Hucko, F., Randall, D. D., Roche, T. E., Burgett, M. W., Pelley, J. W. and Reed, L. J. (1972) α-keto acid dehydrogenase compleses, XVII. Kinetic and regulatory properties of pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase from bovine kidney and heart. Arch. Bioch. Biophys. 151, 328-340.

72. Wieland, O., Siess, E., Schulze-Wethmar, E. H., Funcke, H. G. and Winton, B. (1971) Active and inactive forms of phruvate dehydrogenase in rat heart and kidney: effect of diabetes, fasting, and refeeding on pyruvate dehydrogenase interconversion. Arch. Biochem. Biophys. 143, 593-601.

73. Wieland, O. H., Patzelt, C. and Loffler, G. (1972) Active and inactive forms of pyruvate dehydrogenase in rat liver. Eur. J. Biochem. 26, 426-433.

74. Soling, H. D. and Bernard, S. (1971) Interconversion of inactive to active pyruvate dehydrogenase in rat liver after fructose application in vivo. FEBS Let. 13, 201-203.

75. Coore, H. G., Denton, R. M., Martin, B. R., and Randle, P. J. (1971) Regulation of adipose tissue pyruvate dehydrogenase by insulin and other hormones. Biochem. J. 125, 115-127.

76. Martin, B. R., Denton, R. M. Rask, H. T., and Randle, P. J. (1972) Mechanisms regulating adipose tissue pyruvate dehydrogenase. Biochem. J. 129, 763-773).

77. Mukherjee, C. and Jungas, R. L. (1975) Activation of pyruvate dehydrogenase in adipose tissue by insulin. Biochem. J. 148, 229-235.

78. Taylor, S. I., Mukerjee, C., and Jungas, R. L. (1973) Studies on the mechanism of activation of adipose tissue pyruvate dehydrogenase by insulin. J. Biol. Chem. 248, 73-81.

79. Kerbey, A. L., Randle, P. J., Cooper, R. H., Whitehouse, S., Rask, H. T. and Denton, R. M. (1976) Regulation of pyruvate dehydrogenase in rat heart.

80. Wojtczak, L. and Zaluska, H. (1967) Inhibition of translocation of adenine nucleotides through mitochondrial membranes by oleate. Biochem. Biophys. Res. Com. 28, 76-81.

81. Batenburg, J. J. and Olson, M. S. (1975) The inactivation of pyruvate dehydrogenase by fatty acid in isolated rat liver mitochondria. Biochem. Biophys. Res. Com. 66, 533-540.

82. Taylor, S. I., Mukerjee, C. and Jungas, R. L. (1975) Regulation of pyruvate dehydrogenase in isolated rat mitochondria. J. Biol. Chem. 250, 2028-2035.

83. Hucko, F. (1975) The pyruvate dehydrogenase multienzyme complex. Angewandte Chemie. 14, 591-601.

84. Volpe, J. J. and Vagelos, P. R. (1973) Saturated fatty acid biosynthesis and its regulation. Ann. Rev. Biochem. 42, 21-75.

85. Nakanishi, S. and Numa, S. (1970) Purification of rat liver acetyl-CoA carboxylase and immunochemical studies on its synthesis and degradation. Eur. J. Biochem. 16, 161-173.

86. Volpe, J. J. and Marasa, J. C. (1975) Regulation of hepatic fatty acid synthetase in the obese-hyperglycemic mutant mouse. Biochim. Biophys. acta 409, 235-248.

87. Volpe, J. J. and Marasa, J. C. (1975) Hormonal regulation of fatty acid synthetase, acetyl-CoA carboxylase and fatty acid synthesis in mammalian adipose tissue and liver. Biochim. Biophys. Acta 380, 454-472.

88. Halestrap, A. P. and Denton, R. M. (1974) Hormonal regulation of adipose tissue acetyl-coenzyme A carboxylase by changes in the polymeric state of the enzyme. Biochem. J. 142, 365-377.

89. Numa, S., Ringelmann, E. and Lynen, F. (1965) Zur biochemishen funktion des biotins, VIII. Die bindung der kohlensaure in der carbosylierten acetyl-CoA carboxylase. Biochem. Z. 343, 243-247.

90. Gregolin, C., Ryder, E., Warner, R. C., Kleinschmidt, A. K., Chang, H. C. and Lane, M. D. (1968) Liver acetyl coenzyme A carboxylase, II. Further molecular characterization. J. Biol. Chem. 243, 4236-4245.

91. Majerus, P. W. and Kilburn, E. (1969) Acetyl coenzyme A carboxylase. The roles of synthesis and degradation of enzyme levels in rat liver. J. Biol. Chem. 244, 6254-6262.

92. Numa, S., Bortz, W. M., and Lynen, F. (1965) Regulation of fatty acid synthesis at the acetyl-CoA carboxylation step. Advan. Enzyme Reg. 3, 407-423.

93. Shafrin, E. and Ruderman, N. B. (1974) Enzymes of carbohydrate and fat metabolism in anti-insulin serum diabetes, inactivation by free fatty acids and the protective effect of cellular protein. Diabetol. 1, 731-742.

94. Korchak, H. M. and Masoro, E. J. (1964) Free fatty acids as lipogenic inhibitors. Biochim. Biophys. Acta 84, 75-77.

95. 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. Lip. Res. 1, 150-153.

96. Bortz, W. M. (1967) Fat-feeding and cholesterol synthesis. Biochim. Biophys. Acta 137, 533-540.

97. Garland, P. B., Shepherd, D. and Yates, D. W. (1965) Steady-state concentrations of coenzyme A, acetyl-coenzyme A and long-chain fatty acyl-coenzyme A in rat liver mitochondria oxidizing pyruvate. Biochem. J. 97, 587-594.

98. Liou, G. I. and Donaldson, N. E. (1973) Relative activities of acetyl-CoA carboxylase and fatty acid synthetase in chick liver. Effects of dietary fat. Can. J. Biochem. 51, 1029-1033.

99. Dorsey, J. A. and Porter, J. W. (1968) The effect of palmityl coenzyme A on pigeon liver fatty acid synthetase. J. Biol. Chem. 243, 3512-3516.

100. Tubbs, P. K. and Garland, P. B. (1964) Variations in tissue contents of coenzyme A thioesters and possible metabolic implications. Biochem. J. 93, 550-557.

101. Wakil, S. J., Goldman, J. K., Williamson, I. P., and Toomey, R. E. (1966) Stimulation of fatty acid biosynthesis by phosphorylated sugars. Proc. Nat. Acad. Sci. (U.S.A.) 55, 880-887.

102. Kumar, S., Phillips, G. T. and Porter, J. W. (1972) Comparative biochemistry of fatty acid synthesizing enzyme system: A review. Int. J. Biochem. 3, 15-32.

103. Smith, S. (1970) Fatty acid synthesis in mouse liver. Arch. Biochem. Biophys. 136, 112-121.

104. Gibson, D. M., Lyons, R. T., Scott, D. F. and Muto, Y. (1972) Synthesis and degradation of the lipogenic enzymes of rat liver. Advan. Enzyme Reg. 10, 187-204.

105. Kornacker, M. and Lowenstein, J. M. (1963) The relation between rates of citrate cleavage and fatty acid synthesis in rat liver preparations. Biochem. J. 89, 27-35.

106. Srere, P. A. and Foster, D. W. (1967) On the proposed relation of citrate enzymes to fatty acid synthesis and ketosis in starvation. Biochem. Biophys. Res. Com. 26, 556-560.

107. Yeh, Y. Y., Leveille, G. A. and Wiley, J. H. (1970) Influence of dietary lipid on lipogenesis and on the activity of malic enzyme and citrate cleavage enzyme in the liver of growing chicks. J. Nutr. 100, 917-924.

108. Allee, G. L., Baker, D. H. and Leveille, G. A. (1971) Fat utilization and lipogenesis in the young pig. J. Nutr. 101, 1415-1422.

109. Allee, G. L., Baker, D. H. and Leveille, G. A. (1971) Influence of level of dietary fat on adipose tissue lipogenesis and enzymatic activity in the pig. J. Animal Sci. 33, 1248-1254.

110. 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.

111. Tepperman, J. and Tepperman, H. M. (1958) Effects of anticedent food intake pattern on hepatic lipogenesis. Amer. J. Physiol. 193, 55-64.

112. Century, B. (1972) Effect of dietary lipid on various liver enzymes and on in vivo removal of 3, 4-dimethoxy-phenylethylamine, 3, 4-dihydroxyphenylamine, and 5-hydroxytryptophan. J. Nutr. 102, 1067-1078.

113. Tepperman, H. M. and Tepperman, J. (1965) Effect of saturated fat diets on rat liver NADP-linked enzymes. Amer. J. Physiol. 209, 772-780.

114. Taketa, K., Kaneshige, Y., Tanaka, A. and Kosaka, K. (1970) Differential responses of glucose-6-phosphate dehydrogenase and lipogenesis to fat-free diets. Biochem. Med. 4, 531-545.

115. McLean, P. (1965) Carbohydrate metabolism in mammary tissue. III. Factors in the regulation of pathways of glucose catabolism in the mammary gland of the rat. Biochem. Biophys. Acta 37, 296-309.

116. Rudack, D., Chisholm, E. M. and Holten, D. (1971) Rat liver glucose-6-phosphate dehydrogenase. Regulation by carbohydrate diet and insulin. J. Biol. Chem. 246, 1249-1254.

117. Murphy, G. and Walker, D. G. (1974) Enzyme synthesis in the regulation of hepatic "malic" enzyme activity. Biochem. J. 144, 149-160.

118. Peavey, D. E. and Hansen, R. J. (1975) Immunoligical titration of rat liver glucose-6-phosphate dehydrogenase from animals fed high and low carbohydrate diets. Biochem. Biophys. Res. Com. 66, 1106-1112.

119. Szepesi, B. (1973) Studies on the time course of rerepression of two rat liver enzymes. Nutr. Repts. Intern. 8, 415-419.

120. Goodridge, A. G. (1969) The effects of dietary fat on fatty acid synthesis and malic enzyme activity in liver of growing chicks. Can. J. Biochem. 47, 743-746.

121. Gozukara, E. M., Frolich, M. and Holten, D. (1972) The effect of unsaturated fatty acids on the rate of synthesis of rat liver glucose-6-phosphate dehydrogenase. Biochim. Biophys. Acta 286, 155-163.

122. Berdanier, C. D., Szepesi, B., Diachenko, S. and Moser, P. (1971) Effect of tolbutamide and exogenous insulin on the metabolic responses of rats. Proc. Soc. Exper. Biol. Med. 137, 861-867.

123. Romsos, D. R. and Leveille, G. A. (1974) Effect of dietary fructose on in vitro and in vivo fatty acid synthesis in the rat. Biochem. Biophys. Acta 360, 1-11.

124. Chevalier, M. M., Wiley, J. H. and Leveille, G. A. (1972) Effect of dietary fructose on fatty acid synthesis in adipose tissue and liver of the rat. J. Nutr. 102, 337-342.

125. Szepesi, B. and Freedland, A. (1968) Time-course of enzyme adaptation. I. Effects of substituting dietary glucose and fructose at constant concentrations of dietary protein. Can. J. Biochem. 46, 1459-1470.

126. Frenkel, R., Stark, M. J. and Stafford, J. (1972) Increased "malic enzyme" activity during adaptation to a low protein diet. Biochem. Biophys. Res. Com. 49, 1684-1689.

127. Potter, V. R. and Ono, T. (1961) Enzyme patterns in rat liver and Morris hepatoma 5123 during metabolic transitions. Cold Spring Harbor Symp. Quant. Biol. 26, 355-362.

128. Garcia, D. R. and Holten, D. (1975) Inhibition of rat liver glucose-6-phosphate dehydrogenase synthesis by glucagon. J. Biol. Chem. 250, 3960-3965.

129. Yeh, Y. Y., and Leveille, G. A. (1971) Studies on the relationship between lipogenesis and the level of coenzyme A derivatives, lactate and pyruvate in chick liver. J. Nutr. 101, 911-918.

130. Sul, H. S., Shrago, E. and Shug, A. L. (1976) Relationship of phosphoenolpyruvate transport, acyl coenzyme A inhibition of adenine nucleotide translocase and calcium ion efflux in guinea pig heart mitochondria. Arch. Biochem. Biophys. 172, 230-237.

131. Lerner, E., Shug, A. L., Elson, C. and Shrago, E. (1972) Reversible inhibition of adenine nucleotide translocation by long-chain fatty acyl coenzyme A esters in liver mitochondria of diabetic and hibernating animals. J. Biol. Chem. 247, 1513-1519.

132. Shug, A. L., Shrago, E., Bittar, N., Folts, J. D. and Koke, J. R. (1975) Acyl-CoA inhibition of adenine nucleotide translocation in ischemic myocardium. Amer. J. Physiol. 228, 689-692.

133. Cheema-Dhadli, S. and Halperin, M. L. (1973) The role of the mitochondrial citrate transporter in the regulation of fatty acid synthesis: effect of fasting and diabetes. Can. J. Biochem. 51, 1542-1544.

134. Robinson, B. H., Williams, G. R. and Halperin, M. L. (1970) The effects of 2-ethylcitrate tricarboxylate on citrate transport in rat liver mitochondria and fatty acid synthesis in rat white adipose tissue. Eur. J. Biochem. 15, 263-272.

135. Robinson, B. H., Williams, G. H., Halperin, M. L. and Leznoff, C. C. (1971) The sensitivity of the exchange reactions of tricarboxylate, 2-oxoglutarate, and dicarboxylate transporting systems of rat liver mitochondria to inhibition by 2-pentyl malonate, p-dodobenzymalonate and benzene-o, 2, 3-tricarboxylate. Eur. J. Biochem. 20, 65-71.

136. Halperin, M. L., Robinson, B. H. and Fritz, I. B. (1972) Effects of palmitoyl-CoA on citrate and malate transport by rat liver mitochondria. Proc. Nat. Acad. Sci. (U.S.A.) 69, 1003-1007.

137. Plowman, K. M. and Clealand, W. W. (1967) Purification and kinetic studies of the citrate cleavage enzyme. J. Biol. Chem. 242, 4239-4248.

138. Flatt, J. P. (1970) Conversion of carbohydrate to fat in adipose tissue: an energy-yielding and therefore, self-limiting process. J. Lip. Res. 11, 131-144.

139. Krebs, H. A. (1966) The regulation of the release of ketone bodies by the liver. Advan. Enz. Reg. 4, 339-353.

140. Romsos, D. R., Sasse, C. and Leveille, G. A. (1974) Effect of dietary 1, 3-butanediol on in vitro fatty acid synthesis and malic enzyme activity in rat liver and adipose tissue. J. Nutr. 104, 202-209.

141. Mehlman, M. A. and Veech, R. L. (1972) Redox and phosphorylation states and metabolite concentrations in frozen clamped livers of rats fed diets containing l, 3-butanediol and d, l-carnitine. J. Nutr. 102, 45-52.

142. Holten, D., Procsal, D. and Chang, H. L. (1976) Regulation of pentose pathway dehydrogenases by NADP/NADPH ratios. Biochem. Biophys. Res. Com. 68, 436-441.

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

144. Masaro, E. J., Chaikoff, I. L., Chernick, S. S., and Felts, J. M. (1950) Previous nutritional state and glucose conversion to fatty acids in liver slices. J. Biol. Chem. 185, 845-856.

145. Hill, R., Linezasoro, J. M., Chevalier, F. and Chaikoff, I. L. (1958) Regulation of hepatic lipogenesis: the influence of dietary fats. J. Biol. Chem. 233, 305-310.

146. DiGiorgio, J., Bonanno, M., and Hegsted, D. M. (1962) Effect of diet upon the in vitro metabolism of rat epididymal adipose tissue. J. Nutr. 78, 384-392.

147. Wiley, J. A. and Leveille, G. A. (1973) Metabolic consequences of dietary medium-chain triglycerides in the rat. J. Nutr. 103, 829-835.

148. Wiegand, R. D., Rao, G. A. and Reiser, R. (1973) Dietary regulation of fatty acid synthetase and microsomal glycero= phosphate acyltransferase activities in rat liver. J. Nutr. 103, 1414-1424.

149. Derr, R. F. and Zieve, L. (1974) Effect of carbohydrate load by intravenous infusion in fasted rats on liver glucose-6-phosphate dehydrogenase and malic enzyme. J. Nutr. 104, 65-68.

150. Niskikori, K., Iritani, N. and Numa, S. (1973) Levels of acetyl-CoA carboxylase and its effectors in rat liver after short-term fat-free refeeding. FEBS Let. 32, 19-21.

151. Leveille, G. A. (1975) Effect of periodicity of eating and diet composition on enzymatic adaptations. Nutr. 1, 13-21.

152. Wiley, J. H. and Leveille, G. A. (1970) Significance of insulin in the metabolic adaptation of rats to meal ingestion. J. Nutr. 100, 1073-1080.

153. Romsos, D. R. and Leveille, G. A. (1974) Effect of meal frequency and diet composition on glucose tolerance in the rat. J. Nutr. 104, 1503-1512.

154. Leveille, G. A. (1966) Glycogen metabolism in meal-fed rats and chicks and the time sequence of lipogenic enzyme adaptive changes. J. Nutr. 90, 449-459.

155. Armstrong, M. K., Romsos, D. R. and Leveille, G. A. (1976) Time sequence of lipogenic changes in adipose tissue of rats when converted from ad libitum feeding to meal-eating. J. Nutr. 106, 884-891.

156. Leveille, G. A. (1967) Lipogenesis in adipose tissue of meal-fed rats. Can. J. Physiol. Pharm. 45, 201-214.

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

158. Sabine, J. R., McGrath, H. and Abraham, S. (1969) Dietary fat and the inhibition of hepatic lipogenesis in the mouse. J. Nutr. 98, 312-318.

159. Bartley, J. C. and Abraham, S. (1972) Hepatic lipogenesis in fasted re-fed rats and mice: response to dietary fats of differing fatty acid composition. Biochim. Biophys. Acta 280, 258-266.

160. Jansen, G. R., Hutchinson, C. F. and Zanetii, 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.

161. Romsos, D. R. and Leveille, G. A. (1972) Effect of intermittent feeding on body fat content and in vitro lipogenesis in mice. Nutr. Repts. Intern. 5, 131-138.

162. O'Shea, E. K. and Leveille, G. A. (1969) The influence of fasting and refeeding on lipogenesis and enzymatic activity of pig adipose tissue. J. Nutr. 99, 345-352.

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

164. Brady, L., Romsos, D. R. and Leveille, G. A. (1976) In vitro estimation of fatty acid synthesis in the chicken (Gallus domesticus) utilizing $^{3}H_2O$. Comp. Biochem. Physiol. 54B, 403-407.

165. Balnave, D. and Pearce, J. (1969) Adaptation of laying hen (Gallus domesticus) to dietary fat with special reference to changes in liver and ovarian lipid content and liver enzyme. Comp. Biochem. Physiol. 29, 539-546.

166. Madappally, M. M., Paquet, R. J., Mehlman, M. A. and Tobin, R. B. (1971) Gluconeogenic and lipogenic enzyme activities in growing chicks fed high fat and high carbohydrate diets. J. Nutr. 101, 755-760.

167. Griminger, P., Villamil, V. and Fisher, H. (1969) The meal eating response of the chicken-species differences and the role of partial starvation. J. Nutr. 99, 368-374.

168. Muiruri, K. L., Romsos, D. R. and Leveille, G. A. (1975) Influence of meal frequency on in vivo hepatic fatty acid synthesis, lipogenic enzyme activity and glucose tolerance in the chicken. J. Nutr. 105, 963-971.

169. Reiser, R., Williams, M. C., Sorrels, M. F. and Murty, N. L. (1963) Biosynthesis of fatty acids and cholesterol as related to diet fat. Arch. Biochem. Biophys. 102, 276-285.

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

171. Waterman, R. A., Romsos, D. R., Tsai, A. C., Miller, E. R. and Leveille, G. A. (1975) Influence of dietary safflower oil and tallow on growth, plasma lipids, and lipogenesis in rats, pigs, and chicks. Proc. Soc. Exper. Biol. Med. 150, 347-351.

172. 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.

173. Du, J. T. and Kruger, F. A. (1972) Effect of essential fatty acids on fatty acid synthesis in epididymal fat cells of the rat. J. Nutr. 102, 1033-1038.

174. Demeyer, D. I., Tan, W. C. and Privett, O. S. (1974) Effect of essential fatty acid deficiency on lipid metabolism in isolated fat cells of epididymal fat pads of rats. Lipids 9, 1-7.

175. Salan, L. B., Knittle, J. L. and Hirsch, J. (1968) The role of adipose cell size and adipose insulin sensitivity in the carbohydrate intolerance of human obesity. J. Clin. Invest. 47, 153-160.

176. Hubbard, R. W. and Matthew, W. T. (1971) Growth and lipolysis of rat adipose tissue: effect of age, body weight and food intake. J. Lip. Res. 12, 286-293.

177. Goodridge, A. G. (1973) Regulation of fatty acid synthesis in isolated hepatocytes. J. Biol. Chem. 248, 4318-4326.

178. Nilsson, A., Sundler, R. and Akesson, B. (1974) Effect of different albumin-bound fatty acids on fatty acid and cholesterol biosynthesis in rat hepatocytes. FEBS Let. 45, 282-285.

179. Jacobs, R. A. and Majerus, P. W. (1973) The regulation of fatty acid synthesis in human skin fibroblasts. J. Biol. Chem. 248, 8392-8401.

180. Mohrhauser, H. and Holman, R. T. (1963) The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. J. Lip. Res. 4, 151-159.

181. Nunn, L. C. A. and Smedley-McLean, I. (1938) The nature of the fatty acids stored by the liver in the fat-deficiency disease of rats. Biochem. J. 32, 2178-2184.

182. Aoyama, Y., Yoshida, A. and Ashida, K. (1974) Effect of dietary fats and fatty acids on the liver lipid accumulation induced by feeding a protein-repletion diet containing fructose to protein depleted rats. J. Nutr. 105, 741-746.

183. Aees-Jorgensen, E. (1961) Essential fatty acids. Physiol. Rev. 41, 1-48.

184. Sprecher, H. and Lee, C. J. (1975) The absence of an 8-desaturase in rat liver: a re-evaluation of optional pathways for the metabolism of linoleic and linoleic acids. Biochim. Biophys. Acta 388, 113-125.

185. Brenner, R. R. and Peluffo, R. O. (1966) Effect of saturated and unsaturated fatty acids on the desaturation in vitro of palmitic, stearic, oleic, linoleic and linolenic acids. J. Biol. Chem. 241, 5213-5219.

186. Sprecher, H. W. (1972) Regulation of polyunsaturated fatty acid biosynthesis in the rat. Fed. Proc. 31, 1451-1457.

187. Wahle, K. W. J. (1974) Desaturation of long-chain fatty acids by tissue preparations of the sheep, rat and chicken. Comp. Biochem. Physiol. 48B, 87-105.

188. Holloway, C. T. and Holloway, P. W. (1975) Stearyl-CoA desaturase activity in mouse liver microsomes of varying lipid composition. Arch. Biochem. Biophys. 167, 496-504.

189. Oshino, N. and Sato, R. (1972) The dietary control of the microsomal stearyl CoA desaturation enzyme system in rat liver. Arch. Biochem. Biophys. 149, 369-377.

190. Tsukagoshi, N. and Fox, C. F. (1973) Transport system assembly and the mobility of membrane lipids in Esherichia coli. Biochem. 12, 2822-2829.

191. Haeffner, E. W. and Privett, O. S. (1975) Influence of dietary fatty acids on membrane properties and enzyme activities of liver mitochondria of normal and hypophysectomized rats. Lipids 10, 417-425.

192. Farias, R. N., Bloj, B., Morero, R. D., Sinery, F. and Trucco, R. E. (1975) Regulation of allosteric membrane-bound enzymes through changes in membrane lipid composition. Biochim. Biophys. Acta 415, 231-251.

193. Leduc, E. H. and Wilson, J. W. (1963) Alterations in mitochondria of a transplantable hepatoma in essential fatty acid-deficient hosts. J. Cell Biol. 19, 44A-45A.

194. Blakenship, J. W., Elias, I., Taylor, M. and Register, V. D. (1975) The effect of beef tallow and corn oil diets upon rat mitochondrial succinate oxidation and ATPase activity. J. Amer. Oil Chem. Soc. 52, 132.

195. Spector, A. A. (1975) Fatty acid binding to plasma albumin. J. Lipid Res. 16, 165-179.

196. Ashbrook, J. D., Spector, A. A., Santos, E. C. and Fletcher, J. E. (1975) Long chain fatty acid binding to human plasma albumin. J. Biol. Chem 2333-2338.

197. Soler-argilaga, C., Infante, R. and Polonovski, J. (1973) Influence of chain length and degree of unsaturation on plasma free fatty acid uptake by the perfused rat liver. Biochim. Biophys. Acta 326, 167-173.

198. Hagenfeldt, L. (1975) Turnover of individual free fatty acids in man. Fed. Proc. 34, 2246-2249.

199. Hagenfeldt, L. and Wahren, J. (1975) Turnover of plasma-free arachidonic and oleic acids in resting and exercising human subjects. Metab. 24, 799-806.

200. Hagenfeldt, L. and Wahren, J. (1975) Turnover of plasma-free stearic and oleic acids in resting and exercising human subjects. Metab. 24, 1299-1304.

201. Basso, L. V. and Havel, R. J. (1969) Hepatic metabolism of free fatty acids in normal and diabetic dogs. J. Clin. Invest. 49, 537-547.

202. Kohout, M., Kohoutova, B. and Heimberg, M. (1970) The effect of chain length and degree of unsaturation of the fatty acid substrate on the output of triglyceride by the liver. Biochim. Biophys. Acta 210, 177-179.

203. Anderson, R. L. and Boggs, R. W. (1975) Gluconeogenic and hetogenic capacities of lard, safflower oil and tri-undecanoin in fasting rats. J. Nutr. 105, 185-189.

204. Pawar, S. S. and Tidewell, H. C. (1968) Effect of prostaglandin and dietary fats on lipolysis and esterification in rat adipose tissue in vitro. Biochim. Biophys. Acta 164, 167-171.

205. DePury, G. G. and Collins, F. D. (1965) A raised level of free fatty acids in serum of rats deficient in essential fatty acids as a contributing cause of their fatty livers. Biochim. Biophys. Acta 106, 213-214.

206. Dog, D. A., Becrthuis, R. K., Nugteren, D. H. and Vonkeman, H. (1964) The biosynthesis of prostaglandins. Biochim. Biophys. Acta 90, 204-207.

207. Bergstrom, S., Danielson, H. and Samuelson, B. (1964) The enzymatic formation of prostaglandin E_2 from arachidonic acid, prostaglandins and related factors. Biochim. Biophys. Acta 90, 207-210

208. DeRubertis, F. R., Zenser, T. V. and Curnow, R. T. (1974) Inhibition of glucagon-mediated increases in hepatic cyclic adenosine 3^1, 5^1-monophosphate by prostaglandins E_1 and E_2. Endocrin. 5, 93-102.

209. Lemberg, A., Wikinski, R., Szurieta, E. M., Halperin, H., Paglione, A. M., DeNeuman, P., Jauregiu, H. (1971) Effects of prostaglandin E_1 and norephinephrine on glucose and lipid metabolism in isolated perfused rat liver. Biochim. Biophys. Acta 248, 198-204.

210. Robertson, R. P., Gavareski, D. J., Porte, D. and Bierman, E. L. (1973) Prostaglandin E_1: Inhibition of glucose induced insulin secretion in dogs. Clin Res. 21, 219-225.

211. Fain, J. N. (1973) Biochemical aspects of drugs and hormone action on adipose tissue. Pharm. Rev. 25, 67-90.

212. Levine, R. A. (1974) Effect of prostaglandin E_1 on hepatic cyclic AMP activity, carbohydrate and lipid metabolism. Prostaglan. 6, 509-521.

213. Dalton, C. and Hope, H. R. (1973) Prostaglindin inhibition of cAMP generation and its direct relationship to rate of lipolysis in isolated fat cells. Circulation XLVIII Sup. IV, 243-255.

214. Christ, E. J. and Nugteren, D. H. (1970) The biosynthesis and possible function of prostaglandins in adipose tissue. Biochim. Biophys. Acta 218, 296-302.

215. Dalton, C. and Hope, H. (1974) Prostaglandin inhibition of cyclic-AMP accumulation and rate of lipolysis in fat cells. Prostaglan. 7, 319-326.

216. Yeh, Y. Y. and Leveille, G. A. (1969) Effect of dietary protein on hepatic lipogenesis in the growing chick. J. Nutr. 98, 356-366.

217. Steel, R. G. D. and Torrie, J. H. (1960) Principles in Procedures of Statistics. Mc-Graw-Hill Bood Company, New York, New York.

218. Hsu, R. Y., Butterworth, P. H. W. and Porter, J. W. (1969) Methods in Enzymology (J. M. Lowenstein, ed.) Vol. 14, p. 33. Academic Press, New York, New York.

219. Lohr, G. W. and Waller, H. D. (1971) Methods of Enzymatic Analysis (H. V. Bergmeyer, ed.) Vol. II, p. 36. Academic Press, New York, New York.

220. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193, 265-277.

221. Ko, H. and Royer, M. E. (1967) A submicromolar assay for nonpolar acids in plasma and depot fat. Anal. Biochem. 20, 205-214.

222. Clarke, S. D., Romsos, D. R., and Leveille, G. A. (1976) Specific inhibition of hepatic fatty acid synthesis exerted by dietary linoleate and linolenate in essential fatty acid adequate rats. Lipids (in press).

224. Muto, Y., Shinahara, N., Banphothasem, S., Sitoyo, N. and Hosoya, N. (1975) Effect of exogenous polyunsaturated fatty acids on lipogenic enzyme activity in rat liver under fixed carbohydrate consumption. J. Nutr. Sci. Vitaminol. 21, 39-47.

225. Jungas, R. L. (1968) Fatty acid synthesis in adipose tissue incubated in tritiated water. Biochem. J. 7, 3708-3716.

226. Goodridge, A. G., Garay, A. and Silpananta, P. (1974) Regulation of lipogenesis and the total activities of lipogenic enzymes in a primary culture of hepatocytes from pre-natal and early post-natal chicks. J. Biol. Chem. 249, 1469-1475.

227. McLean, P., Gumaa, K. A., and Greenbaum, A. L. (1971) Long chain acyl CoA's, adenine nucleotide translocase and the coordination of the redox state of the cytosolic and mitochondrial compartments. FEBS Let. 17, 345-350.

228. Gibson, D. M. and Hubbard, D. D. (1960) Incorporation of malonyl-CoA into fatty acids by liver in starvation and alloxan-diabetes. Biochem. Biophys. Res. Com 3, 531-535.

229. Inoue, H. and Lowenstein, J. M. (1975) Acetyl coenzyme A carboxylase from rat liver. In: Methods in Enzymology (J. M. Lowenstein, ed.) Vol. 35B, pp. 3-11, Academic Press, New York, New York.

230. Romsos, D. R., Belo, P. S., and Leveille, G. A. (1974) Effect of 1, 3-butanediol on hepatic fatty acid synthesis and metabolite levels in the rat. J. Nutr. 104, 1438-1445.

231. Allred, J. B. and Guy, D. G. (1969) Determination of coenzyme A and acetyl CoA in tissue extracts. Anal. Biochem. 29, 293-300.

232. Hagenfeldt, L. (1966) A gas chromatographic method for determination of individual free fatty acids in plasma. Clin. Chim. Acta 13, 266-268.

233. Morrison, W. R. and Smith, L. M. (1964) Preparation of fatty acid methyl esters and dimethylacetate from lipids with boron flouride-methanol. J. Lip. Res. 5, 600-608.

234. Bucher, T., Czok, R., Lamprecht, W. and Latzko, E. (1965) Pyruvate. In: Methods of Enzymatic Analysis (Bergmeyer, H. V., ed.) pp. 253-259, Academic Press, New York, New York.

235. Hohorst, H. (1965) Lactate. In: Methods of Enzymology (Wood, W. A., ed.) Vol. 42, pp. 31-39, Academic Press, New York, New York.

236. Pilkis, S. J. (1975) Glucokinase in rat liver. In: Methods of Enzymology (Wood, W. A., ed.) Vol. 42, pp. 31-39, Academic Press, New York, New York.

237. Bergmeyer, H. V. (1974) In: Methods of Enzymatic Analysis (Bergmeyer, H. V., ed.) Vol. I, pp. 510-511, Academic Press, New York, New York.

238. Srere, P. A. (1959) The citrate cleavage enzyme. J. Biol. Chem. 234, 2544-2547.

239. Clark, D. G., Rognstad, R. and Katz, J. (1974) Lipogenesis in rat hepatocytes. J. Biol. Chem. 249, 2028-2036.

240. Schemmel, R., Mikelsen, O. and Gill, J. L. (1970) Dietary obesity in rats: body weight and body fat accretion in seven strains of rats. J. Nutr. 100, 1041-1048.

241. Schemmel, R., Mikelsen, O. and Fisher, L. (1973) Body composition and fat depot weights of rats influenced by ration fed dams during lactation and that fed rats after weaning. J. Nutr. 103, 477-487.

242. Clarke, S. D., Romsos, D. R. and Leveille, G. A. (1976) Specific control of hepatic lipogenesis exerted by dietary linoleate and linolenate. Am. Oil Chem. Soc. 53, 146A (Abst.).