THE UNIVERSITY OF CALGARY

THE EFFECT OF MALNUTRITION ON ESSENTIAL FATTY ACID STATUS

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF

MASTER OF SCIENCE

DEPARTMENT OF

MEDICAL SCIENCE

CALGARY, ALBERTA

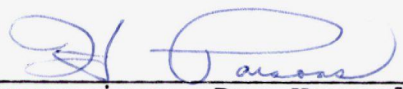
FEBRUARY, 1988

ISBN 0-315-42434-6

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "The Effect of Malnutrition on Essential Fatty Acid Status", submitted by Rebecca Rodgers in partial fulfillment of the requirements for the degree of Master of Science.



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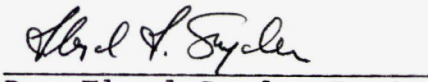

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ABSTRACT

Diseases involving malnutrition are frequently complicated by essential fatty acid (EFA) deficiency. Hypothesizing that in the malnourished state there is increased oxidation of tissue linoleic acid (18:2n-6) to meet energy demands, the purpose of this study was to determine the effect of malnutrition on tissue levels and metabolism of linoleic acid.

Malnutrition was induced in male, weanling rats by restricting food intake to 50% of the amount of a nutritionally complete, semi-synthetic diet consumed by a control group. Three other dietary groups were included: ad libitum rat chow fed controls, an EFA deficient group, and their pair fed but nutritionally complete controls.

Liver, plasma and red blood cell phospholipid fatty acid profiles were determined and compared. Food restricted rats did not have decreased amounts of 18:2n-6 but had increased oleic acid (18:1n-9), an increased 18:1/18:2 ratio and a decreased 18:2/20:4 ratio (20:4n-6 levels remained normal) compared to ad libitum semi-synthetic controls. These changes suggest that the trend towards EFA deficiency had commenced.

ACKNOWLEDGEMENTS

I would like to thank Dr. Howard Parsons for his advice and guidance in the completion of this research project.

Financial support provided by the Natural Sciences and Engineering Research Council, the Medical Research Council and the Alberta Heritage Foundation for Medical Research is appreciatively acknowledged.

This thesis is dedicated, with love, to my husband Scott Bower and to my parents David and Ruth Rodgers in acknowledgement of the support and encouragement they always give me.

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CHAPTER I

INTRODUCTION AND BACKGROUND

A. INTRODUCTION AND RATIONALE

Dietary fat is an important source of energy in the North American diet, supplying 40% of the total daily caloric intake. Its other important function is to provide the essential fatty acids necessary for the integrity of all animal tissue: linoleic acid (18:2n-6), the principal dietary form of essential fatty acid; and arachidonic acid (20:4n-6), the principal functional form (1). Linoleic acid is an essential nutrient because humans cannot synthesize it. No desaturase enzyme exists that can introduce a double bond between the methyl end and the seventh carbon of a fatty acid molecule. The first double bond of linoleate occurs at carbon six (2).

When linoleic acid is not present in the diet in sufficient quantity to meet metabolic requirements, deficiency symptoms develop. At the clinical level, the symptoms include dermatitis, growth retardation, reproductive failure, kidney lesions, hematuria, thrombosis and premature death (3). At the biochemical level, there is a decline of existing linoleate levels; a reduction of the n-6 metabolites 18:3n-6, 20:3n-6 and 20:4n-6; and an increase in the n-9 fatty acid series palmitic, stearic,

palmitoleic, oleic and eicosatrienoic acids; all reflecting a disturbance in the metabolism of essential fatty acids in the desaturation/elongation pathways of the liver microsomes (4).

Diseases involving malnutrition, e.g. protein calorie malnutrition (PCM), cystic fibrosis and anorexia nervosa, are frequently complicated by an essential fatty acid deficiency but this relationship is poorly understood (5,6,7). The accepted indicators of simple essential fatty acid deficiency (i.e. linoleic acid being the sole deficient nutrient in the diet) are decreased linoleate and arachidonate, increased oleate, palmitoleate and eicosatrienoate, and an elevated triene/tetraene ratio (20:3n-9/20:4n-6). The fatty acid profiles seen in the essential fatty acid deficiency accompanying malnutrition, however, do not mimic those of a simple essential fatty acid deficiency. In malnutrition, eicosatrienoic acid is usually not detectable and while linoleate levels remain low, metabolites of linoleate may remain in the normal range, reflecting the differential effect that malnutrition exerts on the desaturation/elongation system of polyunsaturated fatty acids (1).

In both simple and relative essential fatty acid deficiency, however, linoleic acid is always depressed. The contention of this study holds that under conditions of

malnutrition, linoleic acid, like non-essential fatty acids, is oxidized to meet energy demands, thus preventing this essential fatty acid from fulfilling its normal biological functions of cell membrane integrity, prostaglandin and leukotriene synthesis and growth.

When essential fatty acids are the only deficient nutrients in the diet, there is evidence to suggest that essential fatty acids can be spared oxidation and preferentially incorporated into the phospholipid fraction of various tissues (8). However, assuming that the above hypothesis is valid, any conservation mechanism would be overridden in the face of severe malnutrition. Results from this study should provide some answers about the concerted effect of malnutrition and essential fatty acid deficiency on the desaturation/elongation of polyunsaturated fatty acids and the implications of such an effect upon metabolism.

B. OUTLINE OF THESIS

1. Hypothesis

In the malnourished state, there is increased oxidation of tissue linoleic acid.

2. Statement of Objectives

(a) To examine, in an animal model, the effect of malnutrition versus linoleic acid deficiency on the body tissue levels of essential fatty acids.

(b) To determine if the present method of expressing the recommended daily allowance of essential fatty acids as a percentage of total calories is valid in a malnourished state.

(c) To identify the influence of malnutrition on linoleic acid metabolism and requirements.

3. Thesis Organization

This thesis is organized into three chapters. Chapter I provides an introduction to and the rationale behind the thesis. The introdution will review the literature on essential fatty acids, essential fatty acid deficiency, the dietary requirements for essential fatty acids, the function of essential fatty acids and the regulation of fatty acid metabolism.

Chapter II is presented in manuscript form and contains the results and discussion of the specific study carried out for this thesis in which the effect of malnutrition versus linoleic acid deficiency on the tissue levels of essential fatty acids is determined.

Chapter III is a general discussion and conclusion. It includes a broader look at some of the findings in the study, and suggests directions for further study.

C. REVIEW OF THE LITERATURE

Fatty Acids---What are they?
Dietary intake patterns

Fatty acids are aliphatic chains of carbon atoms that are bound by a terminal methyl ($-CH_3$) group and a carboxyl (-COOH) group. Fatty acids can be short chain (2-4 carbons), medium chain (6-12 carbons), or long chain (>12 carbons). They can be saturated (no double bonds), monounsaturated (one double bond), or polyunsaturated (more than one double bond). Most naturally occurring unsaturated fatty acids are of the cis-type configuration i.e. the two hydrogens are on the same side of the carbon chain as opposed to the trans-type configuration in which the hydrogens are on opposite sides of the carbon chain. The length of the carbon chain and the degree and type of unsaturation confer the specific physical and chemical properties on each individual fatty acid.

Fatty acid nomenclature is based on an X:Y system where X represents the number of carbons in the molecule and Y the number of double bonds. All saturated fatty acids will be designated X:Ø. For example, butyric acid, found in butter fat, has four carbons and no double bonds. It is expressed as 4:Ø. Capric acid (milk fat) is 1Ø:Ø, palmitic acid 16:Ø and stearic acid 18:Ø. When naming unsaturated fatty acids the position of the double bond(s)

must be expressed for it is the position of the double bond that differentiates between the polyunsaturated fatty acids isomers. The former convention was to designate the carboxyl end carbon as number one and determine the position of the double bond(s) from there. 18:1Δ12 describes a monounsaturated fatty acid with eighteen carbons and a double bond between carbons twelve and thirteen counting from the carboxyl end. The delta nomenclature system has now been replaced with the omega or 'n' system which sets the methyl carbon as number one. 18:1Δ12 thus becomes 18:1w6 or 18:1n-6 (linoleic acid).

Over 95% of the dietary fat ingested by humans is in the form of triglyceride. The triglyceride molecule comprises a glycerol backbone to which are esterified three fatty acids. The remaining 5% is comprised of phospholipids, cholesterol esters, sterols, fatty alcohols, aldehydes, tepenoids, wax esters, sphingolipids, galactolipids, carotenoids and hydrocarbons. Approximately 40% of the total calories of the average North American diet is derived from fat. Total fat intake can be broken down to its components: 41% saturated fat, 48% monounsaturated fat and 11% polyunsaturated fats.

Fat consumption in North America has exhibited two major trends since 1960 (9). Firstly, total per capita dietary fat consumption has increased from 119 pounds in

196Ø to 13Ø pounds in 1982. Dietary fat here is an all inclusive term for fats and oils, fats in meat and dairy products and the fat used in food as an ingredient (8). Secondly, the sources of dietary fat have changed. Fats of animal origin have, to a large extent, been replaced by fats of vegetable origin reflecting both consumer reaction to public health admonitions to reduce saturated fat consumption in favour of a diet richer in polyunsaturated fat and the expansion of the oilseed industry. Fats and oils from animal sources represented 7Ø% of the total fat consumption in 196Ø. That percentage had dropped to 57% by 1982 (9). Changes in the fatty acid content of the food supply have occurred in keeping with shifts in the source of vegetable oils. From 1913 to 198Ø, the consumption of saturated fatty acids decreased from 4Ø to 34%, whilst that of linoleic acid increased from 9 to 15%, with the consumption of oleic acid remaining essentially unchanged (1Ø). Thus the increased utilization of vegetable oil fat sources has had a positive effect upon the level of essential fatty acids in the diet. Although the fat in the food supply cannot be directly related to the fat consumed, the nature of the available fat indicates the limits of the normal variations. In this regard, food supply data should not be used interchangeably with food consumption as has been the case in several recent reviews (11,12).

DEFINITIONS OF 1. an ESSENTIAL FATTY ACID
2. ESSENTIAL FATTY ACID DEFICIENCY

Delta 9 desaturase introduces a double bond at the ninth carbon, counting from the carboxyl end, of stearic and palmitic acids to form oleic and palmitoleic acids respectively. The other desaturases, delta 6, delta 5, delta 4 and delta 8 are capable of introducing double bonds at positions 6,5,4,8 of a fatty acid molecule, providing a double bond already exists at the delta 9 position. No desaturase enzyme can introduce a double bond between an existing double bond and the methyl end of a fatty acid molecule. This restriction renders the in vivo synthesis of linoleate impossible. It also means that certain interconversions cannot occur i.e. oleic to linoleic, linoleic to linolenic. When a nutrient cannot be synthesized in the body, but is necessary to sustain life supporting functions, it is deemed "essential". Linoleic acid then (and probably alpha linolenic acid) is an essential fatty acid (2). Linoleic acid is the principal dietary form of essential fatty acid and arachidonic acid, a metabolite of linoleic acid, the principal functional form in mammalian tissues (1).

The original research on the nature and function of essential fatty acids began in 1929 when Burr and Burr conducted their classical study (3). The definition

derived from the early research stated: "...properly, the term essential fatty acid should include only those substrates which are active both for growth and for maintenance of dermal integrity, limiting the term to linoleic acid and its metabolite arachidonic acid and to other acids as may be derived metabolically from them (3)".

Burr and Burr, in 1929, created a 'new disease' by feeding a completely fat free diet to rats (3). The total absence of fat from the diet led to the rapid development of scaly skin, caudal necrosis, growth retardation, kidney lesions and hematuria, reproductive failure, increased transdermal water loss, increased metabolic rate and premature death (3). These are the classical, clinical symptoms of essential fatty acid deficiency. When drops of lard were systematically introduced to the diet, even animals close to death miraculously recovered. Burr and Burr determined that it was the fatty acids in the lard that were responsible for this cure. The fatty acid composition of lard was known at the time and Burr and Burr wrote, "...if these well known fatty acids are responsible for the cures described, then we must assign a function far more subtle than the production of 9 calories of energy per gram (3)". Burr and Burr then proceeded to demonstrate that it was, in fact, linoleic acid alone that, when added to the fat deficient diet, reversed the deficiency syndrome (3).

The clinical symptoms that manifest in essential fatty acid deficiency are the result of profound changes at the biochemical level. These biochemical changes can be detected in liver microsomes, the site of essential fatty acid metabolism, within days after fat is excluded from the diet (13). Existing linoleic acid levels fall rapidly. Fat depots are depleted of linoleate as stores are mobilized. All the n-6 metabolites of linoleic acid decrease. Delta 6 desaturase and delta 9 desaturase activity increases, resulting in an increase of n-9 fatty acids. The appearance of the oleic acid metabolite, 20:3n-9, is the hallmark of an essential fatty acid deficiency (14). This n-9 fatty acid is not normally produced since linoleic acid is preferentially desaturated over oleate by the delta 6 desaturase enzyme.

ESSENTIAL FATTY ACID REQUIREMENTS

Burr and Burr found the symptoms manifested by the rigid exclusion of fat from the diet could be reversed completely with as little as 10 drops of lard added to the diet. These 10 drops of lard amounted to approximately 2% of total calories ingested and was just as beneficial as 20% of total calories as lard (3).

Under normal dietary conditions, i.e. a diet in which linoleic acid is present, linoleic acid and oleic acid

compete for the same desaturase enzyme. The enzyme has a greater affinity for linoleate thus the desaturation of oleate is usually blocked (2). This inhibition is the rate limiting step in the biosynthesis of 20:3n-9--a metabolite derived from the desaturation and elongation of oleic acid. When a diet is deficient in linoleate, not only is its own important metabolite, arachidonic acid, decreased but the synthesis of 20:3n-9 is no longer competitively inhibited and thus serum and tissue concentrations of this abnormal fatty acid rise and become detectable (2,14). Holman first observed this peculiar polyunsaturated fatty acids pattern in response to essential fatty acid deficiency in the plasma, hearts and red blood cells of rats and he applied it as a means of evaluating the essential fatty acid status of an animal, and later humans (14).

Eicosatrienoic acid and arachidonic acid are both endogenous polyunsaturated fatty acids, the former synthesized from oleic acid and the latter from linoleic acid (14). The ratio of the triene to the tetraene has proven to be a convenient expression of essential fatty acid status. If the ratios of the triene to the tetraene are plotted against the linoleic acid content (in calories) of a particular diet, a hyperbola can be graphed for which the maximum rate of change of slope lies near a value of 1% of calories. One section of the curve represents the deficient state, the other the sufficient state. The value

of the ratio when linoleate is present at 1% of total calories is approximately Ø.4 (14).

The triene/tetraene ratio for plasma and liver lipids has become the most widely used biochemical index for determining essential fatty acid status and for all species studied so far, a value of < Ø.4 (in humans a value of Ø.2 is used) is considered indicative that essential fatty acid requirements are being met (14). However, Holman's triene/tetraene ratio is only valid when linoleic acid is the major polyunsaturated fatty acids in the diet. Linolenic acid and its metabolites, if present in the diet at high enough levels, depress the synthesis of eicosatrienoic acid and may therefore yield a low triene/tetraene ratio even if the diet is deficient in linoleate (15). Monounsaturated fatty acids, if present at high dietary levels, can partially substitute for essential fatty acid thereby suppressing essential fatty acid utilization and inducing an elevated triene/tetraene ratio (15). Saturated fat may also increase essential fatty acid requirements as manifested by decreased growth, dermal symptoms and elevated triene/tetraene ratios when excess saturated fat is included in the diet (11,15,16,17).

The initial work in establishing the essential fatty acid requirements for humans was done with infants (18,19,2Ø,21). Blood serum levels for dienoic, trienoic

and tetraenoic acids in healthy, well nourished children, were established (19). Healthy children maintained high dienoic and tetraenoic acid levels but low trienoic acid levels. Dienoic and tetraenoic acid levels were significantly reduced and trienoic acid levels significantly elevated in poorly nourished children (20).

Infants fed a skim milk diet developed dryness and thickening of the skin with fine desquamation (21,22). Changing the diet to a milk mixture that provided 2% of total calories as linoleic acid restored skin to normal. Serum lipids of the skim milk fed infants showed significantly decreased dienoic and tetraenoic acids with significantly increased trienoic acids. When the skim milk was supplemented with linoleic acid at 1% of total calories, serum values returned to normal. Supplements of tripalmitin had no effect (21,22).

Minimal normal serum values were maintained when diets provided linoleic acid at 1-2% of total calories (22). Optimum values i.e. optimum caloric efficiency were maintained when linoleic acid was present at 4-5% of total calories (18).

It is difficult to create essential fatty acid deficiency in adult animals and humans. Growing animals and children are more susceptible to essential fatty acid deficiency due to low body stores of essential fatty acid in the adipose tissue and requirement of essential fatty

acid for growth but there are cases of adult human essential fatty acid deficiency resulting from the use of fat free, eucaloric total parenteral nutrition (TPN) diets (23,24).

There are two requirements for essential fatty acid deficiency to develop under such circumstances. First, the infusate must be fat free (and this was the case in the early days of TPN use) and secondly, there must be continuous infusion of glucose to prevent mobilization of the large stores of linoleic acid present in the adipose tissue of adult humans (24). Under these conditions, biochemical essential fatty acid deficiency begins to develop within twenty four hours and is fully manifest in three days (24). Complete recovery can be elicited with the reinstitution of linoleic acid at 2% of total calories (24). Similar patterns are found in infants fed fat free TPN solutions (25).

FUNCTIONS OF ESSENTIAL FATTY ACIDS

LINOLEIC ACID

Two basic functions have been ascribed to linoleic acid that account for its essentiality: the maintenance of cell membrane integrity and the formation of eicosanoids.

CELL MEMBRANES

Animal cell membranes are composed of a phospholipid bilayer interspersed with proteins and free cholesterol

(2). Essential fatty acids have a role to play in maintaining the structural and functional integrity of the cell membrane. They are an integral part of the phospholipid molecule itself, normally esterified to the second carbon of the glycerol backbone (2,26,27). Because of the low melting point of essential fatty acid, their presence in the phospholipid molecules of cell membranes imparts a certain degree of fluidity to the membrane. This fluidity is essential for the processes of cellular transport, permeability to nutrients and the activity of membrane bound enzymes (2,27,28).

EICOSANOID SYNTHESIS

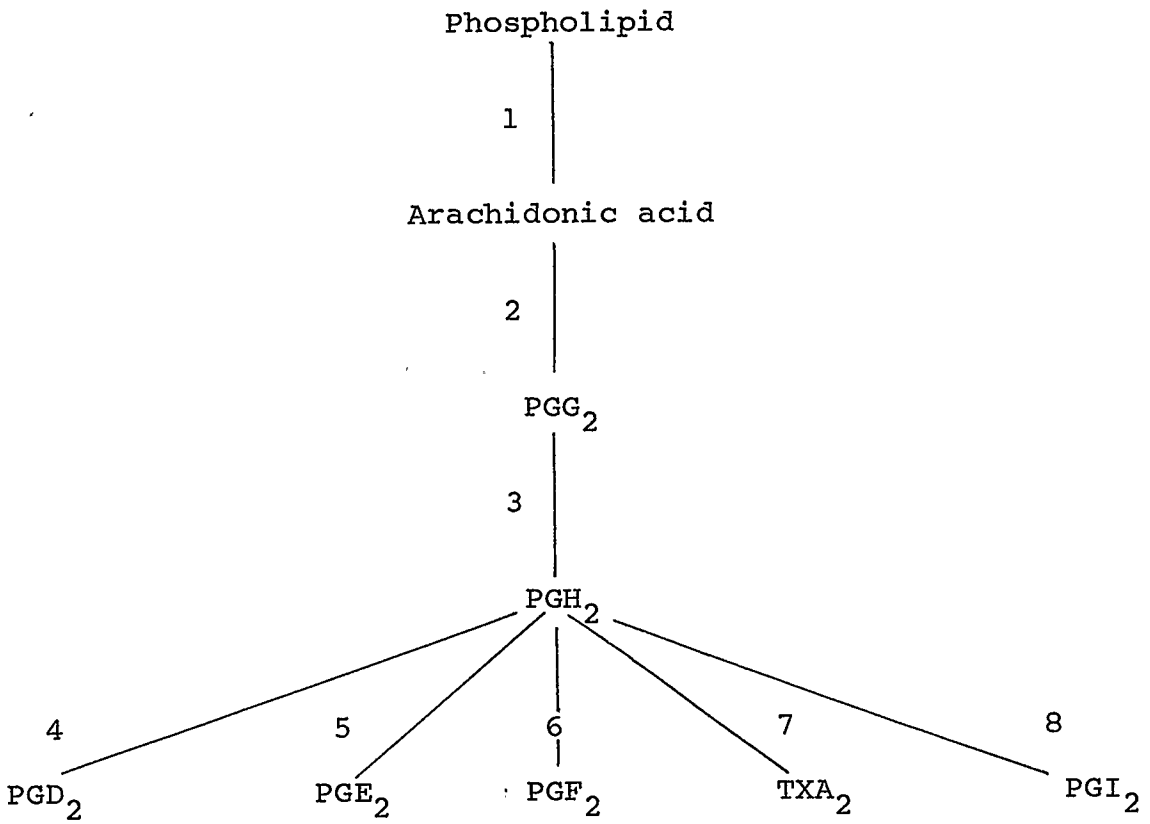
Under normal circumstances, polyunsaturated fatty acids such as arachidonic acid, are bound as integral components of phospholipids, triglyceride and cholesterol esters (2,27). Damage or stimulation to a cell causes their release and rapid oxidation to biologically active prostaglandins and related compounds (27).

The principal precursor fatty acids for eicosanoid synthesis are: dihomogammalinolenic acid (2Ø:3n-6), arachidonic acid (2Ø:4n-6), and eicosapentaenoic acid (2Ø:5n-3). The prostaglandins synthesized from arachidonic acid are shown in FIGURE 1.

Free arachidonic acid is released from tissue lipids by phospholipase A_2 and converted by prostaglandin synthase

FIGURE 1

PROSTANOIDS SYNTHESIZED FROM ARACHIDONIC ACID



1.=phosholipase A_2; 2=prostaglandin endoperoxidase synthetase; 3.=prostaglandin peroxidase; 4.=prostaglandin D-isomerase; 5.=prostaglandin E-isomerase; 6.=prostaglandin F-reductase; 7.=thromboxane synthetase; 8.=prostacyclin synthetase

PGD_2, PGE_2, PGF_2=prostaglandins; TXA_2=thromboxane; PGI_2=prostacyclin

(cyclooxygenase) into cylic endoperoxides. In platelets, these endoperoxides are converted into thromboxanes and in endothelial cells, into prostacyclins. Other cells produce prostaglandins. The principal biologically active products of the prostaglandin synthase enzyme are shown in FIGURE 1. Thromboxane A_2 (TXA_2), produced from the endoperoxide PGH_2, is an unstable compound that is a potent platelet aggregator and vasoconstricting agent. Prostacyclins (PGI) very strongly disaggregate platelets (27). Oxidation of arachidonic acid by the lipoxygenase enzyme is the first step in the formation of leukotrienes, compounds that exert biological effects on white blood cells and smooth muscle.

The current information on eicosanoids is still being assimilated into an understanding of the role of polyunsaturated fatty acids, and their metabolites in human health and disease (29).

ALPHA-LINOLENIC ACID

In the early experiments by Burr and Burr, linolenic acid was found to be 'inferior' to linoleic acid and arachidonic acid because it failed to cure the symptoms of deficiency (3). It did, however, improve growth somewhat (3). There is a growing body of evidence to support the essentiality of linolenic acid and its metabolites (3,4,29,3Ø,31,32).

In mammals, 22:6n-3 is the most abundant member of the

n-3 fatty acid series. It is usually most concentrated in the ethanolamine phosphoglycerides (EPG) and the serine phosphoglycerides (SPG) of any given tissue. The total fatty acids and phospholipids of most mammalian tissues contain less than 5% 22:6n-3 except for the marine mammals and birds whose fish based diets contain a rich supply of n-3 fatty acids. However, certain tissues in the body have very high concentrations of 22:6n-3: brain (cerebral cortex), retina, sperm and testes, suggesting the possibility of a functional role for linolenic acid in these tissues (30). Indeed, documented case studies of human linolenic acid deficicency report a variety of neuropathic symptoms which disappear when the linolenic acid deficiency is corrected (4,33).

In rats, linolenic acid deficient diets lower 22:6n-3 in all body tissues, however, retina, brain and muscle appear to retain 22:6n-3 with greater tenacity (30). The 22:6n-3 content of brain phospholipids in rats fed low linolenate diets will decrease dramatically over time and this has been shown to significantly impair performance in maze-discrimination tests perhaps reflecting impaired visual acuity, or even impaired cerebral function (31).

Severe dermatitis and fatty livers, symptoms associated with linoleic acid deficiency, have also been reported in conjunction with linolenic acid deficiency (32). Restoring linolenic acid to the diet cures the

symptoms.

The n-3 fatty acid series also gives rise to prostaglandin derivatives that are demonstrably active in mammalian tissues and have profound effects upon the n-6 fatty acid derived prostaglandins (29). Understanding the complex interrelationship between the n-6 and n-3 fatty acid derived prostaglandins may help to shed more light on the nature of the essentiality of both alpha-linolenic acid and linoleic acid (29).

THE REGULATION OF ESSENTIAL FATTY ACID METABOLISM

DESATURATION/ELONGATION

The desaturation/elongation of unsaturated fatty acids is a key regulatory mechanism in the metabolism of essential fatty acids. The mechanism itself is important because it leads directly to the formation of the particular polyunsaturated fatty acids that serve as the precursors to the phospholipids, prostaglandins, leukotrienes and thromboxanes necessary for membrane and tissue function and integrity (27). Understanding how various dietary and hormonal factors affect the enzymes involved in the formation of these polyunsaturated fatty acids is important in determining the impact of malnutrition and essential fatty acid deficiency on essential fatty acid metabolism. (see TABLE 1)

The desaturation/elongation of fatty acids occurs in

TABLE 1

THE EFFECT OF VARIOUS MODIFIERS ON DELTA 9, 6 AND 5 DESATURASE ACTIVITY
(ref 37,38,39,40,41)

MODIFIER	DESATURASE ENZYME delta 9	delta 6	delta 5
Starvation/Fasting	D	D	D
High Fat Diet	D	D	D
Fat Free/Low Fat Diet	I	I	D
EFA Deficient Diet	I	I	D
Diabetes (low insulin)	D	D	D
Epinephrine	D	D	D
Hypothyroidism (low T_3)	N/D	N/D	N/D
Cis-PUFA (n-6 series)[3]	D	D	I

D=Decreased; Increased; N=Normal

the cell microsomes of several tissues (see TABLE 2 and FIGURE 2) and is a sequential process of inserting double bonds and lengthening unsaturated fatty acid molecules (see FIGURE 3) (34).

Four, possibly five, desaturase enzymes have been identified which insert double bonds at positions 9,6,5,4, and possibly 8 of a fatty acid molecule. Delta 9 desaturase synthesizes oleic acid from stearic acid. There must be a double bond in the delta 9 position of a fatty acid molecule for the other desaturases to be active, therefore linoleic and linolenic acids cannot be synthesized de novo but must be obtained from dietary sources. Neither do any desaturases exist which are capable of inserting double bonds between an existing one and the methyl end of the fatty acid molecule. For this reason, oleic acid cannot be transformed into linoleate, nor linoleate into linolenate (2).

There are two ways in which one can measure the desaturation/elongation of polyunsaturated fatty acids. Indirectly one can assess enzyme activity by comparing fatty acid ratios. For example the ratio of 18:2/20:4 indicates how well linoleate is being converted to arachidonic acid. A high ratio suggests that delta 6 desaturation is decreased. Enzyme activity can also be directly assessed with the use of enzyme assays. Labelled

TABLE 2

RATE OF MICROSOMAL PROTEIN DESATURATION/ELONGATION IN VARIOUS TISSUES (ref 37)

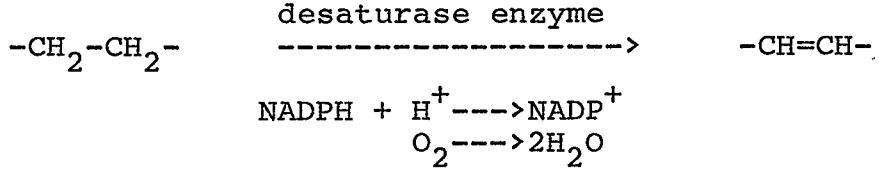
TISSUE	% CONVERSION OF LINOLEATE TO GAMMA-LINOLENATE *
Liver	13.9
Adrenals	19.8
Testicles	6.3
Heart	1.1
Kidney	1.6
Brain	2.Ø
Lung	Ø
Epididymal Fat	Ø

*5 mg microsomal protein + 1Ø nmol C-14 labelled linoleate at 35^{o}C for 2Ø minutes

FIGURE 2

MICROSMAL DESATURATION/ELONGATION (ref 37)

DESATURATION



ELONGATION

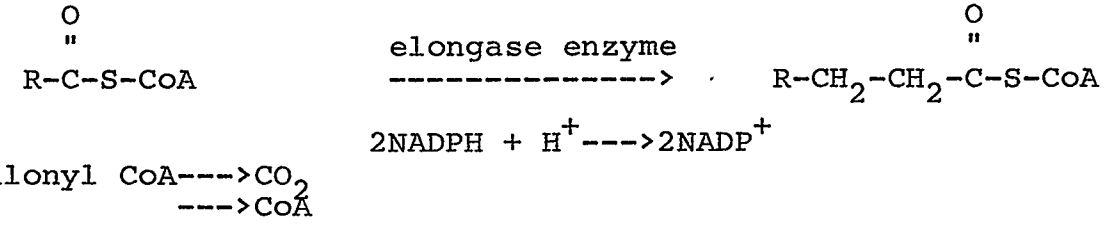
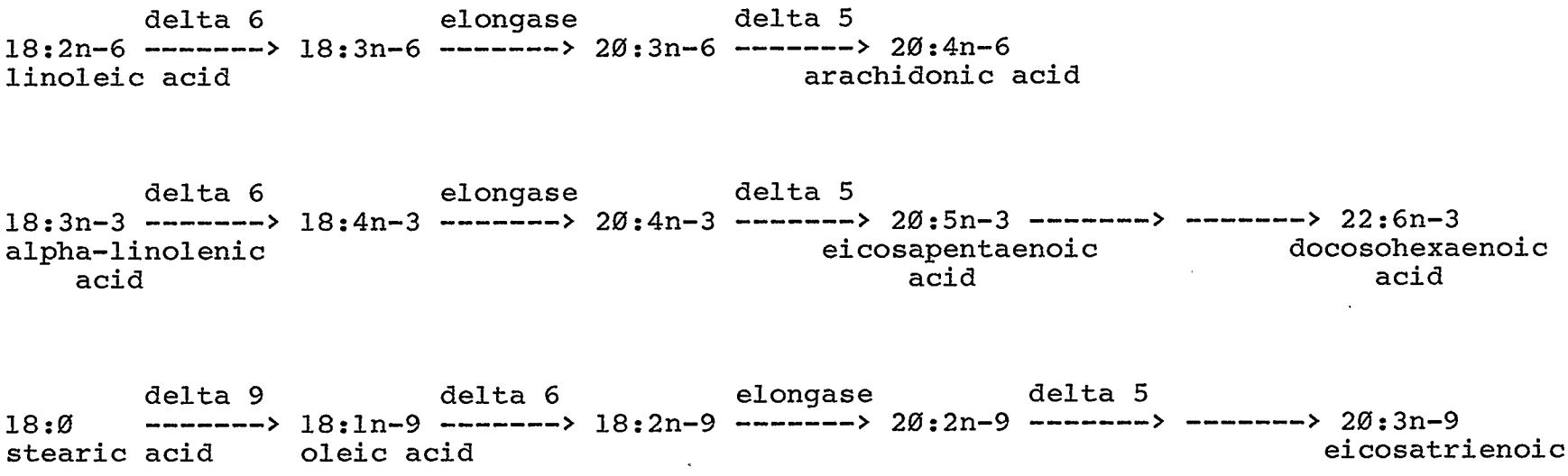


FIGURE 3

DESATURATION AND ELONGATION OF n-6 (18:2n-6), n-3 (18:3n-3), AND n-9 (18:1n-9) FATTY ACID SERIES
(ref 29)



fatty acids can be incubated with microsomal protein and the percent conversion to the next fatty acid along the metabolic pathway can be determined.

The enzymes involved in the desaturation and elongation of polyunsaturated fatty acids are subject to dietary and hormonal factors that can influence the activity of any enzyme system. Of particular interest in this study will be how malnutrition affects the desaturation/elongation system. The importance of distinguishing between the effects of different factors is important and necessitates the use of proper pair fed control groups. The role of zinc in the desaturation/elongation of polyunsaturated fatty acids is discussed to illustrate this point.

A physiological interaction between zinc and essential fatty acid metabolism has been proposed by several researchers (39,40,41,42). This interaction is believed to be that of an essential cofactor for the delta 6 desaturase enzyme (43,44). However, many of the studies are flawed in that they fail to include the pair fed controls necessary to distinguish between the separate roles of zinc and other nutrients (41,42,44).

It is well known that one of the effects of zinc deficiency is anorexia (44). When zinc-adequate control rats are pair fed with zinc-deficient rats in studies examining the role of zinc in essential fatty acid

metabolism, the zinc-deficient and the zinc-adequate pair fed controls show the same alterations in fatty acid metabolism compared with ad lib zinc-adequate controls. This suggests that the changes in essential fatty acid metabolism observed in zinc deficiency are more attributable to the anorexia than to the lack of zinc itself (44). If zinc were an essential cofactor in the desaturation/elongation of essential fatty acid, then a block of the delta 6 desaturase enzyme should occur in zinc deficient animals but not in pair fed controls (44). This reiterates the importance of using pair fed controls to delineate effects of the nutrient deficiency itself from effects caused by the anorexia that often accompanies nutrient deficiency syndromes.

PURE VERSUS RELATIVE ESSENTIAL FATTY ACID DEFICIENCY

In simple essential fatty acid deficiency there are specific and characteristic fatty acid profiles that reflect the specific effect that the dietary lack of essential fatty acid has on the metabolism of the polyunsaturated fatty acids. The levels of linoleic acid and arachidonic acid decrease. Oleic acid and eicosatrienoic acid, n-9 series fatty acids that are usually present in barely detectable amounts, increase significantly. The ratio of eicosatrienoic acid to

arachidonic acid increases and is a useful parameter in establishing essential fatty acid deficiency.

Malnutrition is frequently accompanied with essential fatty acid deficiency. However, the fatty acid profiles do not mimic those of simple essential fatty acid deficiency. This reflects the specific effect that malnutrition exerts on the metabolism of the polyunsaturated fatty acids. Discussed below are three disease states that all involve malnutrition and compromised nutritional status. All three show similarities to simple essential fatty acid deficiency yet all three are unique to simple essential fatty acid deficiency.

PROTEIN-CALORIE MALNUTRITION

Protein-calorie malnutrition (PCM) encompasses three clinical syndromes: kwashiorkor, marasmus and marasmic-kwashiorkor (MK) (5). Kwashiorkor is characterized by edema, dermatitis, enlarged fatty liver, changes in hair pigment and texture and decreased serum albumin in the presence of normal subcutaneous fat. Infants with marasmus present with muscle wasting and decreased subcutaneous fat. Many infants with marasmus exhibit some, but not all, of the signs of kwashiorkor and are diagnosed MK (5). A scoring system has been developed as a means of differentiating between the three syndromes (6).

The etiology and pathogenesis of the types of PCM have not been clarified (5). Animals fed a high carbohydrate,

low protein diet exhibit kwashiorkor and animals fed a low carbohydrate, low protein diet exhibit marasmus. Diet history studies in infants suffering from PCM, however, often fail to show similar dietary correlations (7). The lack of fat in the diets that contribute to the development of PCM, although occasionally noted, was not considered as an important factor in the etiology of the disease until the 197Ø's when it was noted that although the breast milk linoleic acid content was more than adequate in many third world populations, the main food staple of infants and young children, a maize product, provided no more than Ø.74% of linoleic acid as total calories (7).

Changes in the proportions of the major fatty acids, consistent with essential fatty acid deficiency, are observed in patients with kwashiorkor. The concentration of linoleate in the fatty acids of the total plasma lipids is significantly lower than in healthy controls. The concentration of arachidonic acid is less than half that of controls. The decline in essential fatty acid levels is compensated for by an elevation in eicosatrienoic acid, palmitoleic acid and oleic acid. The triene/tetraene ratio is elevated, with a mean value of 1.Ø8 (7). These changes are not as severe as those seen in the simple essential fatty acid deficiency produced by prolonged feeding of fat free TPN but the diets usually provide some linoleic acid

albeit in amounts well below the recommended minimum of 1%.

The essential fatty acid status of children with PCM has been evaluated and the three individual syndromes, kwashiorkor, marasmus, and MK compared. Percent plasma linoleic acid is one third that of control values in all PCM children but percent arachidonic acid is decreased only in cases of kwashiorkor. Only occasionally is eicosatrienoic acid detectable but the triene/tetraene ratio always remains normal. Red blood cell fatty acid profiles yield similar results with no eicosatrienoic acid detected, decreased linoleic acid in all patients, but arachidonic acid decreased only in patients with kwashiorkor (5). The absence of any detectable eicosatrienoic acid is contrary to other findings that show elevated eicosatrienoate levels (7).

Decreased linoleate levels in the absence of elevated eicosatrienoate levels have been reported in acrodermatitis enteropathica an inherited form of zinc deficiency involving abnormal fatty acid metabolism and also in biotin deficiency (45,46,47). Zinc deficiency probably exerts its effects through appetite suppression and not through inability to perform as an enzyme cofactor (5) (see section on desaturation). Biotin is a necessary cofactor for the production of the malonyl-CoA that is required for the microsomal elongation of fatty acids (47).

A simple linoleic acid deficiency is always

accompanied by elevated eicosatrienoic acid levels. In the non deficient state, linoleate competitively inhibits conversion of oleate to eicosatrienoate because of linoleates's greater affinity for the shared desaturation/elongation enzymes. When linoleic acid is depressed the enzymes become available to convert oleic acid to eicosatrienoic acid. The different enzyme systems of desaturation and elongation that exist for the polyunsaturated fatty acid series are affected differently by different metabolic disturbances thus producing the different fatty acid profiles (5,48). Because desaturation is decreased and not increased during fasting, the fatty acid pattern of kwashiorkor, for example, is best explained by decreased elongation as opposed to increased desaturation (48). Perhaps the kwashiorkor children have some form of biotin deficiency that is responsible for the unique fatty acid profile (5,48).

CYSTIC FIBROSIS

Another example of a disease characterized by low serum levels of essential fatty acid but with a fatty acid profile not completely resembling that of a simple dietary essential fatty acid deficiency is cystic fibrosis (49,50,51,52,53,54,55).

In cystic fibrosis the phospholipid levels of essential fatty acid are decreased to values comparable to

simple essential fatty acid deficiency and the 18:1/18:Ø ratio is elevated. However, the 2Ø:4n-6/18:2n-6 ratio does not significantly alter whereas there is an 8 fold change in simple essential fatty acid deficiency. Eicosatrienoic acid is reported absent by some or, when detected, has no effect on the triene/tetraene ratio (49,51,52). Tissue arachidonic acid levels remain normal (49,5Ø,51). The low linoleic acid levels were, for a long time, specifically associated with the fat malabsorption that afflicts some cystic fibrosis patients (51). Cystic fibrosis patients with presumably satisfactory pancreatic function had linoleic acid levels equivalent to normal controls (51). However, other cystic fibrosis patients with good pancreatic function show biochemical evidence of essential fatty acid deficiency even though linoleate intake is above the recommended daily allowance of 3% of total calories (54). The low linoleate levels in some cystic fibrosis patients may be due to increased utilization of polyunsaturated fatty acids for energy production. This observation may be especially valid for patients whose caloric intake is inadequate (51,54). Indeed, one recent study, that included cystic fibrosis patients with biochemical essential fatty acid deficiency, despite pancreatic sufficiency, has shown that increasing the energy intake of cystic fibrosis patients definitely improves their essential fatty acid status (54).

ANOREXIA NERVOSA

Patients with severe anorexia nervosa are in a condition resembling a near, but not total starvation state (56). Very little is known about the nutritional status of such patients with respect to vitamins, minerals, protein, fat and carbohydrate. The essential fatty acid status in anorexia nervosa patients was first documented in 1985 (56). Linoleic acid was found to be depressed but not significantly different from normal. Only 18:Ø was significantly decreased. The arachidonate/linoleate ratio was significantly altered but oleic acid and eicosatrienoic acid were not elevated. Oleic acid was significantly increased in a subgroup comprised of patients who had lost greater than 25% of ideal body weight but eicosatrienoic acid levels remained normal in this group too. The fatty acid profile of anorexia nervosa may be attributable to the presence of adequate protein in the diet (56). Studies in rats show that by increasing the protein content of an essential fatty acid deficient diet the amount of eicosatrienoic synthesis was decreased and some of the clinical essential fatty acid deficiency symptoms were alleviated (57,58).

ENERGY

Energy, in the form of ATP, must be provided for life sustaining metabolic processes at all costs. When faced with a potential energy deficit through food restriction, the body adapts with several energy conserving strategies. In growing animals and children, growth retardation is the primary response to inadequate food energy intake (59). In adults, there is weight loss, reduced basal metabolic rate (BMR) and decreased activity (60). Restricted energy intake in adults generally results in less loss of weight and body energy stores than would be predicted if rates of energy metabolism before energy intake restriction were maintained.

The capacity to turn down BMR is an important factor to consider. If the capacity to do so exists, then food reduction may have to be considerably greater than anticipated in order to override the conservation effect of the decreased BMR.

A decrease in BMR is observed in food restricted adult humans and animals due to changes in body composition and loss of metabolizing tissue (59,60,61,62,63). Some controversy exists as to whether growing animals decrease BMR as a compensatory mechanism or whether they decrease total basal metabolism, without loss of metabolizing tissue, by retarding growth and reducing body size

(59,6Ø,61). In growing animals, the reduction in body tissue deposition may be a more important adaptation to underfeeding than reducing BMR (59).

To provide energy, fatty acids must be catabolized. This occurs via the beta-oxidation pathway in the matrix of the mitochondria. As well as beta-oxidation, free fatty acids can meet several other fates: desaturation/elongation, retroconversion, and incorporation into complex lipids (37). The preferred route that a free fatty acid takes depends upon the structure of the fatty acid molecule in relation to the specificity of a particular participating enzyme and the dietary conditions at the time i.e. have energy needs been met (37)?

Once directed to beta-oxidation, must a fatty acid enter the cycle and be oxidized to completion, or can it be redirected elsewhere at any point? Conversely, can a fatty acid directed to any other metabolic pathway be used for B-oxidation?

Under normal dietary conditions, there is evidence that certain species have the capacity to conserve linoleate by limiting its oxidation. Sheep liver mitochondria exhibit decreased beta-oxidation of linoleate compared to non-ruminant liver mitochondria (63). The acetate derived from rumen fermentation is converted to malonyl-CoA and the malonyl-CoA may prevent beta-oxidation of linoleate by acting at the carnitine acyl transferase

site (CATI) (64,65,66). This ability of ruminants to spare linoleic acid from oxidation would appear to be an important adaptation to a compromised dietary supply of essential fatty acid due to biohydrogenation in the rumen.

Under normal dietary conditions in the rat, there appears to be no evidence for an essential fatty acid sparing mechanism in the beta-oxidation pathway. Long chain polyunsaturated fatty acids may, in fact, be preferentially oxidized over saturated fatty acids. However, when the diet is deficient in essential fatty acid, there is evidence for a capacity to conserve linoleate (67,68). There are differences in the oxidation and tissue uptake of linoleic acid between essential fatty acid deficient and normal rats (8,67,68). Less ^{14}C-linoleate is oxidized to $^{14}CO_2$ and a greater part of the radioactive label is retained specifically in the phospholipids of various tissues when essential fatty acid deficiency exists (8,67,68). Thus n-6 fatty acids can be preferentially directed to incorporation into phospholipids.

One of the primary nutritional deficits in malnutrition is a low dietary energy intake. It therefore becomes very important when testing the hypothesis put forth in this study (that when malnutrition exists, linoleic acid is oxidized to meet basic energy demands

rather than to fulfill its other biological roles) to be aware of the potential existence of conservation mechanisms and the threshold point at which they can be overridden.

CHAPTER II

THE EFFECT OF MALNUTRITION ON ESSENTIAL FATTY ACID STATUS

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A. INTRODUCTION

Simple essential fatty acid deficiency, i.e. linoleic acid being the only deficient nutrient in the diet, was first described by Burr and Burr in 1929 when, by feeding rats a completely fat free diet, they observed the development of scaly skin, caudal necrosis, growth retardation, kidney lesions and hematuria, reproductive failure, increased transdermal water loss and, eventually, premature death (3).

Later, at the biochemical level, a specific, abnormal fatty acid profile was identified as characterizing simple essential fatty acid deficiency (1). Linoleic acid levels are, not surprisingly, depressed. Arachidonic acid and the other linoleate metabolites are also reduced. Because linoleic acid is depressed, it can no longer competitively inhibit the desaturation/elongation of oleic acid and thus the metabolites of this n-9 fatty acid rise. Notably eicosatrienoic acid (2Ø:3n-9) levels become detectable (1). The ratio of triene (2Ø:3n-9) to tetraene (2Ø:4n-6) is always elevated in simple essential fatty acid deficiency and is the most widely used biochemical index for determining essential fatty acid status (1).

Diseases involving malnutrition, such as kwashiorkor,

marasmus, anorexia nervosa and cystic fibrosis are frequently complicated by a relative essential fatty acid deficiency (5,7,49,56). Although linoleate levels are depressed in all cases, the fatty acid profiles are unique to each disease and different from simple essential fatty acid deficiency. Such discrepancies lead one to question the differential effects that malnutrition may or may not be exerting on the desaturation/elongation systems of polyunsaturated fatty acids.

Kwashiorkor manifests as the result of a low protein but high carbohydrate diet. The fatty acid profile of kwashiorkor is one of low linoleate and low arachidonate levels which are compensated for by a rise in eicosatrienoic acid and the triene/tetraene ratio (2). Anorexia nervosa patients, although severely restricted in food intake, tend to consume adequate amounts of protein (56). Linoleic acid is depressed and the linoleate/arachidonate ratio is abnormal but oleic acid and eicosatrienoic acid levels remain normal (4). The presence of adequate protein in the diet of anorexic patients may alleviate or mask more severe symptoms of essential fatty acid deficiency. Low protein intakes have been shown to exacerbate essential fatty acid deficiency (57).

Marasmus and cystic fibrosis involve a malnutrition that results from a reduction in total food intake, rather

than a reduction of either protein or energy although protein requirements may well still be met. Linoleic acid is depressed, but the triene/tetraene ratio tends to remain normal because eicosatrienoic acid is not produced in detectable amounts and linoleate metabolites (arachidonic acid in particular) may remain within normal ranges (5,6,49). It is possible that, in this malnourished state, the fatty acid desaturase enzyme system for linoleic acid is not affected and, therefore, the metabolites of linoleic acid will not be suppressed as they are in simple essential fatty acid deficiency. However, linoleic acid may conceivably be oxidized to meet energy demands (thus preventing it from fulfilling its normal biological functions of cell membrane integrity, prostaglandin and leukotriene synthesis and growth) when total nutritional status is compromised. This would explain the relative essential fatty acid deficiency of malnutrition in cases where total food intake is restricted.

There is evidence that linoleate is conserved when the diet is deficient in essential fatty acid (8,67,68). It is also known that animals have the capacity to conserve energy by turning down basal metabolic rate (BMR) or restricting growth when faced with starvation (59,60,61). Presumably, however, if food restriction is severe enough, any such conservation mechanisms would be overridden and linoleic acid would have to be used to meet energy demands.

It is the objective of this study to determine the effects of malnutrition induced by severe restricted food intake on essential fatty acid status. Kramer et al have demonstrated the importance of distinguishing between the effects of a specific nutrient deficiency, in their case zinc deficiency, and the effects of the reduced food intake that often accompanies deficiency diseases as a result of the animal becoming ill (44). This distinction is made possible by the use of proper pair fed controls. In this study, with the use of such pair fed controls, the differential effects of simple essential fatty acid deficiency and malnutrition resulting from restricted food intake upon essential fatty acid profiles will be examined.

B. MATERIALS AND METHODS

ANIMALS AND DIETS

Five groups of ten, weanling, male Sprague-Dawley rats were assigned to one of five diets-see TABLE 1. There were two ad libitum control groups: one group on Purina rat chow (RC), and the other on a nutritionally complete, semi synthetic diet (1ØØ)-see TABLE 2 and TABLE 3 (15). The two test diets were: an ad libitum semi-synthetic, essential fatty acid deficient diet (EFAD)-see TABLE 2 and TABLE 3- and one in which rats were malnourished by pair feeding them 5Ø% of the amount consumed by the rats in group 1ØØ

TABLE 1

DESCRIPTION OF DIETARY GROUPS

CONTROLS

RC	- ad libitum Purina rat chow
1ØØ	- ad libitum, nutritionally complete, semi synthetic diet
ES	- pair fed to EFAD group the nutritionally complete, semi synthetic diet

TEST

EFAD	- ad libitum essential fatty acid deficient, semi synthetic diet
5Ø	- pair fed 5Ø% of the nutritionally complete, semi synthetic diet consumed by group 1ØØ

TABLE 2

COMPOSITION OF THE NUTRITIONALLY COMPLETE AND THE ESSENTIAL FATTY ACID DEFICIENT SEMI-SYNTHETIC DIETS*
(in gm/100gm)

NUTRITIONALLY COMPLETE		ESSENTIAL FATTY ACID DEFICIENT	
Vitamin free casein	20.0	Vitamin free casein	20.0
Beef tallow	5.0	Beef tallow	6.3
Safflower oil	1.3	D-(+)-sucrose	61.3
D-(+)-sucrose	61.3	Cellulose	5.0
Cellulose	5.0	AIN mineral mix 76 **	5.0
AIN mineral mix 76 **	5.0	AIN vitamin mix 76 **	2.0
AIN vitamin mix 76 **	2.0	Choline bitartrate	0.2
Choline bitartrate	0.2	L-methionine	0.2
L-methionine	0.2		100.0gm
	100.0gm		

*all diet ingredients were obtained from ICN Biochemicals
**see appendix III for composition

TABLE 3

THE FATTY ACID COMPOSITION OF THE NUTRITIONALLY COMPLETE AND ESSENTIAL FATTY ACID DEFICIENT SEMI-SYNTHETIC DIETS (mol%)

Fatty Acid	NUTRITIONALLY COMPLETE DIET (safflower oil+beef tallow)	ESSENTIAL FATTY ACID DEFICIENT DIET (beef tallow)
16:Ø	26.19	44.26
16:1	2.39	Ø.12
18:Ø	16.85	46.58
18:1	35.52	8.27
18:2n-6	18.45	Ø.4Ø
18:3n-3	Ø.25	Ø.11
2Ø:4n-6	Ø.16	Ø.12
22:6n-3	Ø.19	Ø.14

(5Ø). A final control group were pair fed to the EFAD group the nutritionally complete diet (ES). Including both ad libitum controls (RC and 1ØØ) and a pair fed control (ES), ensured that changes due to malnourishment and changes attributable to simple essential fatty acid deficiency could be distinguished.

Weekly weights of each rat were recorded and the daily food consumption measured by weighing feed cups and collecting and subtracting spilled feed. The rats were individually caged and water was supplied ad libitum. Dietary treatment lasted forty two days.

SACRIFICE;COLLECTION OF RED BLOOD CELLS, PLASMA AND LIVER

Rats were weighed immediately prior to sacrifice. The first group of rats (RC) were decapitated to obtain the blood but this resulted in premature coagulation. All subsequent groups were lethally anesthetized with ether and the blood subsequently removed by cardiac puncture. The blood was added to a centrifuge tube containing the anti-coagulant EDTA. The blood was then centrifuged at 1ØØØ rpm for 1Ø minutes and the plasma removed and a 1 ml sample reserved for lipid extraction. Livers were excised, washed in normal saline, blotted dry and weighed. A tissue sample weighing approximately 1 gm was removed and homogenized with chloroform using a pestle and mortar.

EXTRACTION OF LIPIDS FROM RED BLOOD CELLS, PLASMA AND LIVER

RED BLOOD CELLS

To remove residual plasma, white blood cells and platelets, the red blood cells were washed three times in normal saline. A 1 ml aliquot of red blood cells was subject to lipid extraction according to the method of Rose and Oklander (69). This method was chosen because the two solvents used, isopropanol and chloroform (11:7 v/v), yield good lipid recovery (95%) and prevent the red blood cells from clumping during the extraction procedure.

To extract the lipid material from red blood cells, 11 ml of isopropanol was added to the 1 ml sample and the mixture allowed to stand overnight. Chloroform in the amount of 7 ml was added to the tube the next day and left to stand for an hour. The lipid containing solvent layer was removed with a Gomco vacuum pump and ten ml of chloroform/methanol in a 2:1 v/v ratio added to dissolve the lipid material. It was then transferred to a clean 15 ml test tube. Distilled water (2 ml) was added to the tube and the mixture shaken and allowed to stand for 15 minutes. The tube was centrifuged and the upper, water containing phase discarded. The lower phase was dried over anhydrous sodium sulfate, centrifuged for 10 minutes at 1000 rpm, and transferred to a clean test tube. It was then evaporated to dryness using nitrogen and the lipid material dissolved

in chloroform and transferred to a small vial for storage prior to thin layer chromatography.

PLASMA AND LIVER

Folch extractions were performed on both the 1 ml plasma and 1 gm liver samples (7Ø). 2Ø ml of 2:1 v/v chloroform-methanol and 4.2 ml of water were added to the sample. This mixture was allowed to stand overnight. The solvent phase was collected, dried with anhydrous sodium sulfate and centrifuged for 1Ø minutes at 1ØØØ rpm. The solvent was transferred to a clean test tube and evaporated to dryness under nitrogen. The lipid material was dissolved in chloroform and transferred to a small vial for storage prior to thin layer chromatography.

THIN LAYER CHROMATOGRAPHY (TLC)

Analtech TLC plates (2Øx2Øcm and 25Ø microns thickness) were activated overnight at 35Ø^{o}C in a Despatch oven. TLC tanks were lined with Whatman No.1 filter paper and allowed to equilibrate for at least one hour with the developing solvent before the plates were inserted.

Total phospholipids were separated from neutral lipids on Analtech Silica Gel G-plates using hexane/diethylether/acetic acid in a ratio of 8Ø:3Ø:1 by volume as the developing solvent (71). The extraction of the phospholipid classes from the silica gel was done

according to Skipski (71). The silca gel phospholipid band, as visualized under ultra violet light, was scraped from the TLC plate and then the lipid material was extracted four times using the following solvents: 3 ml chloroform/methanol/acetic acid/water (25:15:4:2 v/v) for two extractions, 3 ml of methanol for one extraction and 3 ml of methanol/acetic acid/water (94:1:5 v/v) for one extraction. The recovered phospholipids were dried under nitrogen, then re-dissolved in chloroform.

METHYLATION OF LIPIDS

The phospholipid fatty acids were transmethylated with boron trifluoride and methanol (72). The lipid extract was dried under nitrogen and Ø.5 ml of benzene added. Boron trifluoride methanol in the amount of 3 ml was added and the glass tubes, with their teflon lined caps securely on, were placed in a block heater at 8Ø^{o}C for 9Ø minutes. After the heating period, 5 ml of water was added to each tube. Petroleum ether (3 ml/extraction) was used to extract the methylated phospholipid fatty acids three times. The upper layer was collected after each extraction. Anhydrous sodium sulphate was added to this upper layer and the tubes centrifuged at 1ØØØ rpm for 1Ø minutes. The upper layer was transferred to a new test tube and dried under nitrogen. Finally, the methylated phospholipid fatty acids were re-dissolved in hexane.

GAS LIQUID CHROMATOGRAPHY ANALYSIS

The gas liquid chromatography of the fatty acid methyl esters was performed on a Varian Vista gas chromatograph equipped with a flame ionization detector and a 30 M Supelco Wax 10 fused silica column of mid polarity. The internal diameter of the column was 0.2 mm. The column oven was programmed from 120°C at 5°C per minute to a final temperature of 240°C. The injector and detector temperatures were both set at 300°C. The carrier gas was nitrogen. The fatty acid methyl esters were identified by comparison with a standard mixture of fatty acid methyl esters of known composition. Peak areas were computed by electronic integration and the fatty acid concentration expressed on a mole percent basis.

STATISTICAL ANALYSIS

The mean and standard deviation of the fatty acids in each group were determined. Analysis of variance (ANOVA) of the means was performed. Comparison between the five diets was made using the Neuman-Keuls multiple range test after ANOVA showed an effect of diet treatment. All analysis was done on a micro computer using Systat software.

C. RESULTS

The six week weight record (means +/- SD) for each group of rats is shown in TABLE 4. Only a final weight was available for the group fed rat chow. The ad libitum semi-synthetic diet group (1ØØ) was run a second time, independently (1ØØ-2) and closely paralleled the weight gain pattern of group 1ØØ rats. Rats in groups 5Ø, EFAD, and ES gained less weight than groups RC and 1ØØ and between weeks 5 and 6 there was a weight loss in groups 5Ø and EFAD. The EFAD group and its pair fed counterpart, group ES, had very similar weight gain patterns.

At the end of six weeks of dietary treatment, the rats fed rat chow (group RC) were significantly heavier than all the other groups. The rats fed ad libitum on the semi synthetic diet (group 1ØØ) weighed significantly less than the RC rats but significantly more than groups 5Ø, EFAD and ES. There were no significant differences between groups 5Ø, EFAD and ES. The EFAD rats, however, had marked clinical symptoms of essential fatty acid deficiency. Their faces, feet, ears and tails were extremely scaly, the hair coat rough, and the animals were lethargic.

When weight gain is expressed as a ratio to food intake i.e. feed efficiency (TABLE 5) there are no significant differences between the groups.

TABLE 4

WEIGHT GAIN RECORD (gm) OF RATS FOR SIX WEEKS OF DIETARY TREATMENT
(MEAN +/- SD)

DIETARY GROUP	N	WEEK 1	2	3	4	5	6	SACRIFICE
RC	1Ø							[j]291+/-2Ø
1ØØ	1Ø	[a]7Ø+/-4	[b]84+/-15	[e]11Ø+/-27	[f]147+/-34	[g]187+/-33	[i]22Ø+/-34	[k]23Ø+/-31
1ØØ-2	8	55+/-7	[c]66+/- 8	[d]92+/-22	124+/-35	[h]166+/-41	[i]2Ø7+/-43	[k]235+/-44
5Ø	1Ø	56+/-9	[c]73+/- 8	[d]129+/-14	129+/-1Ø	156+/- 9	161+/- 8	148+/- 7
EFAD	8	55+/-7	57+/- 6	92+/-17	119+/-23	145+/-19	174+/-18	153+/-16
ES	1Ø	5Ø+/-4	58+/- 6	81+/- 9	1Ø6+/-13	128+/-13	155+/-12	162+/-1Ø

p<.Ø5

RC=RAT CHOW; 1ØØ, 1ØØ-2=AD LIBITUM SEMI-SYNTHETIC; 5Ø=PAIR FED 5Ø% OF THE AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; N=NUMBER OF RATS/GROUP; p=LEVEL OF SIGNIFICANCE

[a]significantly heavier [b]significantly heavier
[c]significantly heavier than 1ØØ-2, EFAD, ES [d]significantly heavier
[e]significantly heavier than 1ØØ-2, EFAD, ES [f]significantly heavier than ES
[g]significantly heavier [h]significantly heavier than ES
[i]significantly heavier [j]significantly heavier
[k]significantly heavier than 5Ø, EFAD, ES

TABLE 5

WEIGHT GAIN, DAILY FOOD INTAKE AND FEED EFFICIENCY

GROUP	WEIGHT GAIN (gm) (mean +/- SD)	DAILY FOOD INTAKE (gm) (mean +/- SD)	FEED EFFICIENCY
*1ØØ	159.Ø +/- 1Ø.5	14.2 +/- 2.1	Ø.27 +/- .Ø2
5Ø	92.Ø +/- 1Ø.1	7.1 +/- 1.Ø	Ø.32 +/- .Ø1
EFAD	98.Ø +/- 9.7	8.7 +/- 1.4	Ø.28 +/- .Ø2
ES	112.2 +/- 7.4	8.7 +/- 1.4	Ø.32 +/- .Ø3

p<.Ø5

FEED EFFICIENCY=WEIGHT GAIN DIVIDED BY (DAILY FOOD INTAKE TIMES NUMBER OF DAYS ON DIET)

1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF THE AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT GROUP; ES=PAIR FED TO EFAD GROUP

*group 1ØØ gained significantly more weight than groups 5Ø, EFAD and ES and group 1ØØ consumed significantly more food than groups 5Ø, EFAD and ES; there were no significant differences in weight gain and amount of food consumed between groups 5Ø, EFAD and ES.

TABLE 6 shows the liver weights (gm) at sacrifice of the five different dietary groups. The absolute weights of the livers were significantly different for all comparisons except groups ES and EFAD. However, when liver weight is expressed as a percentage of body weight the relative weights are not significantly different.

TABLE 7 and TABLE 8 show the effect of the five different dietary treatments [rat chow (RC); 100% semi-synthetic (100); 50% semi-synthetic (50); essential fatty acid deficient (EFAD); pair fed to EFAD (ES)] on rat liver phospholipid fatty acids. TABLE 7 presents the means and standard deviations of the fatty acids from each dietary treatment. TABLE 8 compares the results from the different dietary treatments, showing where significant differences occur.

The significant differences in liver phospholipid fatty acids, as identified for each dietary treatment through analysis of variance and a Neuman-Keuls multiple range test, see TABLE 8, are described below. Group RC is first compared to group 100, then to group 50, ES and EFAD. Group 100 is then compared to groups 50, ES and EFAD. Group 50 is compared to groups ES and EFAD and then groups ES and EFAD are compared.

The liver phospholipid fatty acids of rats fed rat chow had significantly higher 16:0, 18:0, 18:2n-6, 20:3n-6, 20:5n-3 and 22:6n-3 than group 100. 16:1n-7, 18:1n-9,

TABLE 6

ABSOLUTE AND RELATIVE RAT LIVER WEIGHTS

DIETARY GROUPS	N	LIVER WEIGHTS(gm) (mean +/- SD)	LIVER WEIGHT/ FINAL BODY WEIGHT
RC	10	9.27 +/- 0.37*	0.032**
100	10	7.25 +/- 0.89	0.032
50	10	4.78 +/- 0.36	0.032
EFAD	8	5.69 +/- 0.30	0.037
ES	10	5.69 +/- 0.33	0.035

P<.05

RC=RAT CHOW; 100=AD LIBITUM SEMI SYNTHETIC; 50=PAIR FED 50% OF THE AMOUNT OF DIET CONSUMED BY GROUP 100; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; N=NUMBER OF RATS/GROUP,P=LEVEL OF SIGNIFICANCE

*RC and 100 are not significantly different; 50, EFAD and ES are not significantly different; RC and 100 are significantly heavier than 50, EFAD and ES
** no significant differences between groups

TABLE 7

EFFECT OF DIET ON RAT LIVER PHOSPHOLIPID FATTY ACIDS
(MEAN +/- SD IN MOL%)

FATTY ACID	DIETARY TREATMENT				
	RC	1ØØ	5Ø	EFAD	ES
	N=9	N=5	N=9	N=8	N=8
16:Ø	2Ø.87 +/- Ø.58	18.74 +/- 1.49	21.76 +/- 1.36	21.76 +/- Ø.74	23.69 +/- Ø.62
16:1	Ø.44 +/- Ø.11	1.37 +/- Ø.54	Ø.98 +/- Ø.65	2.82 +/- 1.14	1.94 +/- Ø.21
18:Ø	24.45 +/- Ø.85	17.85 +/- 1.24	23.12 +/- Ø.58	21.93 +/- Ø.5Ø	21.22 +/- 1.39
18:1(c)	3.13 +/- Ø.25	11.ØØ +/- 3.68	1Ø.41 +/- Ø.61	15.36 +/- 1.12	9.76 +/- 1.ØØ
18:1(t)	2.Ø6 +/- Ø.21	5.38 +/- 2.Ø5	2.11 +/- Ø.12	2.81 +/- Ø.35	3.22 +/- Ø.36
18:2n-6	18.24 +/- 1.32	7.13 +/- 1.2Ø	8.25 +/- Ø.52	3.5Ø +/- Ø.87	7.63 +/- Ø.55
18:3n-6	Ø.Ø2 +/- Ø.Ø3	Ø.19 +/- Ø.1Ø	Ø.11 +/- Ø.Ø6	Ø.16 +/- Ø.Ø6	Ø.11 +/- Ø.12
18:3n-3	Ø.37 +/- Ø.8Ø	4.Ø8 +/- 3.61	Ø.31 +/- Ø.38	Ø.45 +/- Ø.32	Ø.32 +/- Ø.16
2Ø:3n-9	Ø.32 +/- Ø.Ø9	Ø.99 +/- Ø.49	Ø.65 +/- Ø.13	16.81 +/- 1.66	Ø.77 +/- Ø.12
2Ø:3n-6	Ø.73 +/- Ø.11	Ø.54 +/- Ø.14	Ø.53 +/- Ø.Ø5	Ø.45 +/- Ø.11	Ø.84 +/- Ø.Ø9
2Ø:4n-6	22.28 +/- Ø.95	26.56 +/- 4.45	27.Ø4 +/- Ø.87	9.11 +/- 1.5Ø	25.37 +/- Ø.5Ø
2Ø:5n-3	Ø.7Ø +/- Ø.1Ø	Ø.31 +/- Ø.23	Ø.1Ø +/- Ø.15	Ø.36 +/- Ø.Ø3	Ø.37 +/- Ø.39
22:4n-6	Ø.Ø7 +/- Ø.1Ø	Ø.48 +/- Ø.16	Ø.26 +/- Ø.17	Ø	Ø.42 +/- Ø.Ø4
22:5n-6	Ø.Ø1 +/- Ø.Ø3	2.65 +/- Ø.9Ø	1.76 +/- Ø.25	1.11 +/- Ø.17	2.34 +/- Ø.34
22:5n-3	Ø.85 +/- Ø.13	Ø.11 +/- Ø.11	Ø.12 +/- Ø.Ø7	Ø	Ø.Ø7 +/- Ø.12
22:6n-3	5.46 +/- Ø.83	2.6Ø +/- Ø.55	2.5Ø +/- Ø.22	3.35 +/- Ø.77	1.93 +/- Ø.4Ø

RC=RAT CHOW CONTROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; N=NUMBER OF RATS/GROUP; c=CIS; t=TRANS

TABLE 8

EFFECT OF DIET ON RAT LIVER PHOSPHOLIPID FATTY ACIDS SHOWING DIFFERENCES BETWEEN DIETS

ANOVA/NEUMAN-KEULS MULTIPLE RANGE TEST

DIET COMPARISONS

FATTY ACID	RCv1ØØ	RCv5Ø	RCvES	RCv EFAD	1ØØv5Ø	1ØØvES	1ØØv EFAD	5ØvES	5Øv EFAD	ESv EFAD
16:Ø	^S	n	S^	n	S^	S^	S^	S^	n	^S
16:1	S^	n	S^	S^	^S	n	S^	S^	S^	S^
18:Ø	^S	^S	^S	^S	S^	S^	S^	^S	^S	n
18:1(c)	S^	S^	S^	S^	n	S^	S^	n	S^	S^
18:1(t)	S^	n	S^	n	^S	^S	^S	S^	n	n
18:2n-6	^S	^S	^S	^S	n	n	^S	n	^S	^S
18:3n-6	S^	S^	n	S^	n	n	n	n	n	n
18:3n-3	S^	n	n	n	^S	^S	^S	n	n	n
2Ø:3n-9	n	n	n	S^	n	n	S^	n	S^	S^
2Ø:3n-6	^S	^S	S^	^S	n	S^	n	S^	n	^S
2Ø:4n-6	S^	S^	S^	^S	n	n	^S	n	^S	^S
2Ø:5n-3	^S	n	^S	^S	n	n	n	n	n	n
22:4n-6	n	n	n	n	n	n	n	n	n	n
22:5n-6	S^	S^	S^	^S	^S	n	^S	S^	^S	^S
22:5n-3	n	n	n	n	n	n	n	n	n	n
22:6n-3	^S	^S	^S	^ S	n	n	S^	n	S^	S^

p<.Ø5

RC=RAT CHOW CONTROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP S=SIGNIFICANT; n=NOT SIGNIFICANT; c=CIS; t=TRANS; p=LEVEL OF SIGNIFICANCE

^=designates a significant fatty acid increase in the indicated dietary group

18:3n-6, 18:3n-3, 20:4n-6 and 22:5n-6, however, were significantly lower.

The RC group had significantly higher 18:0, 18:2n-6, 20:3n-6 and 22:6n-3 than group 50 but significantly lower 18:1n-9(c), 18:3n-6, 20:4n-6 and 22:5n-6.

RC rats had significantly more 18:0, 18:2n-6 and 20:5n-3 compared to group ES but significantly less 16:0, 16:1n-7, 18:1n-9, 20:3n-6, 20:4n-6 and 22:5n-6 than group ES.

Group RC, in comparison to essential fatty acid deficient rats, had significantly higher 18:0, 18:2n-6, 20:3n-6, 20:4n-6, 20:5n-3, 22:5n-6 and 22:6n-3 but significantly lower 16:1n-7, 18:1n-9(c), 18:3n-6 and 20:3n-9.

In group 100, 16:1n-7, 18:1n-9(t), 18:3n-3 and 22:5n-6 were significantly higher than they were in group 50 and 16:0 and 18:0 were present in significantly lower amounts.

Group 100 had significantly higher 18:1n-9(t) and 18:3n-3 than group ES but significantly lower 16:0, 18:0, 18:1n-9(c) and 20:3n-6.

Group 100 had significantly more 18:1n-9(t), 18:2n-6, 18:3n-3, 20:4n-6 and 22:5n-6 than group EFAD. 16:0, 16:1n-7, 18:0, 18:1n-9(c), 20:3n-9 and 22:6n-3 were significantly lower in group 100.

Group 50 had significantly more 18:0 compared to group

ES and significantly less 16:Ø, 16:1n-7, 18:1n-9(t), 2Ø:3n-6 and 22:5n-6.

Compared to group EFAD, group 5Ø rats had significantly higher amounts of 18:Ø, 18:2n-6, 2Ø:4n-6 and 22:5n-6. 16:1n-7, 18:1n-9(t), 2Ø:3n-9 and 22:6n-3 were significantly lower.

Comparing group ES to group EFAD, ES had significantly more 16:Ø, 18:2n-6, 2Ø:3n-6, 2Ø:4n-6 and 22:5n-6 and significantly lower 16:1n-7, 18:1n-9(c), 2Ø:3n-9 and 22:6n-3.

TABLES 9 and 1Ø show the effect of the five different dietary treatments on the sums and ratios of liver phospholipid fatty acids. TABLE 9 presents the means and standard deviations. TABLE 1Ø compares the results from the different dietary treatments, showing where significant differences occur.

The significant differences, as identified in TABLE 1Ø, are described below in the same format as the description of TABLE 8.

For group RC, the sum of the saturates and the 18:2/2Ø:4 ratio were significantly higher compared to group 1ØØ but the sum of the n-6 fatty acids and the 18:1/18:2 ratio were significantly lower.

Comparing group RC to group 5Ø, the sum of the n-3 fatty acids and 18:2/2Ø:4 were significantly higher in RC. The n-6 fatty acids and 18:1/18:2 ratio were significantly

TABLE 9

EFFECT OF DIET ON RATIOS AND SUMS OF RAT LIVER PHOSPHOLIPID FATTY ACIDS
(MEAN +/- SD)

	DIETARY TREATMENT				
FATTY ACID	RC	1ØØ	5Ø	EFAD	ES
	N=9	N=5	N=9	N=8	N=8
tri/tetra	Ø.Ø1 +/- Ø.ØØ	Ø.Ø4 +/- Ø.Ø2	Ø.Ø2 +/- Ø.Ø1	1.9Ø +/- Ø.39	Ø.Ø3 +/- Ø.Ø1
sum n-3	7.39 +/- 1.16	7.1Ø +/- 3.27	3.Ø3 +/- Ø.47	4.17 +/- Ø.82	2.69 +/- Ø.8Ø
sum n-6	23.12 +/- Ø.92	3Ø.41 +/- 4.98	29.7Ø +/- Ø.94	1Ø.84 +/- 1.6Ø	29.Ø7 +/- Ø.57
sum sat	45.32 +/- Ø.98	36.6Ø +/- 1.5Ø	44.87 +/- Ø.99	43.68 +/- 1.Ø7	44.92 +/- Ø.98
18:2/2Ø:4	Ø.82 +/- Ø.Ø9	Ø.27 +/- Ø.Ø7	Ø.3Ø +/- Ø.Ø2	Ø.38 +/- Ø.Ø6	Ø.3Ø +/- Ø.Ø2
18:1/18:2	Ø.28 +/- Ø.Ø2	2.37 +/- Ø.51	1.52 +/- Ø.12	5.48 +/- 1.36	1.7Ø +/- Ø.16

RC=RAT CHOW CONTROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF THE AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; N=NUMBER OF RATS/GROUP; tri/tetra=2Ø:3n-9/2Ø:4n-6; sum n-3=SUM OF THE N-3 FATTY ACID SERIES; sum n-6=SUM OF THE N-6 FATTY ACID SERIES; sum sat=SUM OF THE SATURATED FATTY ACIDS

TABLE 1Ø

EFFECT OF DIET ON RATIOS AND SUMS OF RAT LIVER PHOSPHOLIPID FATTY ACIDS SHOWING DIFFERNCES BETWEEN DIETS

ANOVA/NEUMAN KEULS MULTIPLE RANGE TEST

DIET COMPARISONS

FATTY ACID	RCv1ØØ	RCv5Ø	RCvES	RCv EFAD	1ØØv5Ø	1ØØvES	1ØØv EFAD	5ØvES	5Øv EFAD	ESv EFAD
tri/tetra	n	n	n	S^	n	n	S^	n	S^	S^
sum n-3	n	^S	^S	^S	^S	^S	^S	n	n	n
sum n-6	S^	S^	S^	^S	n	n	^S	n	^S	^S
sum sat	^S	n	n	^S	S^	S^	S^	n	^S	n
18:2/2Ø:4	^S	^S	^S	^S	n	n	S^	n	S^	S^
18:1/18:2	S^	S^	S^	S^	^S	n	S^	n	S^	S^

p<.Ø5

RC=RAT CHOW CONTROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF THE AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; S=SIGNIFICANT; n=NOT SIGNIFICANT; tri/tetra=2Ø:3n-9/2Ø:4n-6; sum n-3=SUM OF THE N-3 FATTY ACID SERIES; sum n-6=SUM OF THE N-6 FATTY ACID SERIES; sum sat=SUM OF THE SATURATED FATTY ACIDS; p=LEVEL OF SIGNIFICANCE

^=designates a significant fatty acid increase in the indicated dietary group

lower.

Comparing group RC to group ES, the n-3 fatty acids and 18:2/20:4 ratio were significantly increased and the n-6 fatty acids and 18:1/18:2 ratio significantly lower.

Group RC had significantly higher sums of the n-3, n-6 and saturated fatty acids and the 18:2/20:4 ratio and significantly lower triene/tetraene and 18:1/18:2 ratios compared to group EFAD.

Group 100, compared to group 50, had a significantly higher sum of the n-3 fatty acids and 18:1/18:2 ratio and a significantly lower sum of the saturated fatty acids.

The sum of the n-3 fatty acids was significantly elevated and the sum of the saturated fatty acids significantly decreased in group 100 compared to group ES.

Group 100 showed significantly higher sums of the n-6 and n-3 fatty acids and significantly lower triene/tetraene, 18:2/20:4 and 18:1/18:2 ratios than group EFAD.

Comparing group 50 to group EFAD, group 50 had significantly higher sums of n-6 and saturated fatty acids and significantly lower triene/tetraene, 18:2/20:4 and 18:1/18:2 ratios.

Group ES had a significantly higher sum of the n-6 fatty acids and significantly lower triene/tetraene, 18:2/20:4, and 18:1/18:2 ratios than group EFAD.

TABLE 11 and TABLE 12 show the effect of the five different dietary treatments on rat plasma phospholipid fatty acids. TABLE 11 presents the means and standard deviations of the fatty acids from each dietary treatment. TABLE 12 compares the results from the different dietary treatments, showing where significant differences occur.

The significant differences, as identified in TABLE 12, are described below in the same format as the description of TABLE 8.

Group RC had significantly higher 18:Ø, 18:2n-6 and 22:6n-3 compared to group 1ØØ and significantly lower 18:1n-9(c) and 18:3n-3.

Rats fed rat chow had significantly higher 18:2n-6 and 22:6n-3 compared to group 5Ø.

Group RC had significantly higher 18:2n-6 and 22:6n-3 and significantly lower 2Ø:3n-9 compared to group ES. Group RC had significantly higher amounts of 18:2n-6, 2Ø:4n-6 and 22:6n-3 and significantly less 16:1n-7, 18:1n-9(c) and 2Ø:3n-9 than group EFAD.

Comparing group 1ØØ and group 5Ø, group 1ØØ had significantly more 18:1n-9(c) and 18:3n-3 and significantly less 16:Ø, 18:Ø, 2Ø:4n-6 and 22:6n-3 than group 5Ø.

A significantly higher amount of 18:3n-3 in group 1ØØ was the only significant difference between group 1ØØ and group ES.

Group 1ØØ had significantly more 18:2n-6 and 18:3n-3

TABLE 11

EFFECT OF DIET ON RAT PLASMA PHOSPHOLIPID FATTY ACIDS
(MEAN +/- SD in mol%)

FATTY ACID	DIETARY TREATMENT				
	RC	1ØØ	5Ø	EFAD	ES
	N=8	N=9	N=1Ø	N=5	N=6
16:Ø	26.ØØ +/- 4.58	19.71 +/- 3.88	27.Ø2 +/- 4.84	25.4Ø +/- 1.87	19.93 +/- 7.38
16:1	Ø.49 +/- Ø.35	Ø.38 +/- Ø.16	Ø.83 +/- Ø.51	2.4Ø +/- 1.11	Ø.75 +/- Ø.4Ø
18:Ø	18.62 +/- 4.71	11.72 +/- 4.32	18.5Ø +/- 2.51	17.25 +/- 1.57	14.41 +/- 6.62
18:1(c)	9.44 +/- 7.26	22.Ø3 +/-1Ø.75	9.57 +/- 3.17	12.65 +/- 6.22	15.39 +/- 6.Ø8
18:1(t)	2.26 +/- Ø.53	4.35 +/- 2.46	2.93 +/- 2.58	7.15 +/- 6.97	3.4Ø +/- 2.38
18:2n-6	18.48 +/- 3.56	9.76 +/- 1.66	9.62 +/- 2.98	5.Ø3 +/- 1.7Ø	1Ø.11 +/- 2.81
18:3n-6	Ø.Ø5 +/- Ø.11	Ø.29 +/- Ø.15	Ø.1Ø +/- Ø.24	Ø.84 +/- 1.89	Ø.17 +/- Ø.22
18:3n-3	4.98 +/- 7.78	17.37 +/- 6.79	4.39 +/- 3.93	7.28 +/- 3.Ø2	9.77 +/- 6.81
2Ø:3n-9	1.68 +/- 1.77	3.Ø4 +/- Ø.71	2.67 +/- 2.68	11.1Ø +/- 2.61	5.61 +/- 3.55
2Ø:3n-6	Ø.55 +/- Ø.25	Ø.25 +/- Ø.19	Ø.31 +/- Ø.23	Ø.15 +/- Ø.33	Ø.39 +/- Ø.35
2Ø:4n-6	12.98 +/- 3.47	8.85 +/- 5.58	18.84 +/- 4.Ø8	6.65 +/- Ø.97	14.ØØ +/- 6.38
2Ø:5n-3	Ø.63 +/- Ø.81	Ø.71 +/- Ø.19	Ø.86 +/- 1.34	Ø.22 +/- Ø.48	2.32 +/- 2.13
22:4n-6	1.3Ø +/- 1.72	Ø.57 +/- Ø.21	2.49 +/- 2.87	2.33 +/- 2.66	1.97 +/- 1.Ø8
22:5n-6	Ø.Ø8 +/- Ø.19	Ø.52 +/- Ø.53	Ø.87 +/- Ø.66	Ø	Ø.84 +/- Ø.7Ø
22:5n-3	Ø.4Ø +/- Ø.27	Ø.Ø2 +/- Ø.Ø6	Ø.Ø2 +/- Ø.Ø6	Ø.33 +/- Ø.75	Ø.21 +/- Ø.51
22:6n-3	2.Ø5 +/- Ø.93	Ø.42 +/- Ø.18	Ø.98 +/- Ø.35	1.21 +/- Ø.45	Ø.73 +/- Ø.32

RC=RAT CHOW CONTROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø%OF THE AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; N=NUMBER OF RATS/GROUP; c=CIS; t=TRANS

TABLE 12

EFFECT OF DIET ON RAT PLASMA PHOSPHOLIPID FATTY ACIDS SHOWING DIFFERENCES BETWEEN DIETS

ANOVA/NEUMAN-KEULS MULTIPLE RANGE TEST

DIET COMPARISONS

FATTY ACID	RCv1ØØ	RCv5Ø	RCvES	RCv EFAD	1ØØv5Ø	1ØØvES	1ØØv EFAD	5ØvES	5Øv EFAD	ESv EFAD
16:Ø	n	n	n	n	S^	n	n	^S	n	n
16:1	n	n	n	S^	n	n	S^	n	S^	S^
18:Ø	^S	n	n	n	S^	n	S^	n	n	n
18:1(c)	S^	n	n	n	^S	n	n	n	n	n
18:1(t)	n	n	n	S^	n	n	S^	n	n	n
18:2n-6	^S	^S	^S	^S	n	n	^S	S^	n	^S
18:3n-6	n	n	n	n	n	n	n	n	n	n
18:3n-3	S^	n	n	n	^S	^S	^S	n	n	n
2Ø:3n-9	n	n	S^	S^	n	n	S^	n	S^	S^
2Ø:3n-6	n	n	n	n	n	n	n	n	n	n
2Ø:4n-6	n	n	n	^S	S^	n	n	n	^S	^S
2Ø:5n-3	n	n	n	n	n	n	n	n	n	^S
22:4n-6	n	n	n	n	n	n	n	n	n	n
22:5n-6	n	n	n	n	n	n	n	n	n	n
22:5n-3	n	n	n	n	n	n	n	n	n	n
22:6n-3	^S	^S	^S	^S	S^	n	S^	n	n	n

p<.Ø5

RC=RAT CHOW CONTROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF THE AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; S=SIGNIFICANT; n=NOT SIGNIFICANT; c=CIS; t=TRANS; p=LEVEL OF SIGNIFICANCE

^=designates a significant fatty acid increase in the indicated dietary group

than group EFAD but significantly less 16:1n-9, 18:Ø, 18:1n-9(c), 2Ø:3n-9 and 22:6n-3.

16:Ø was significantly increased and 18:2n-6 significantly decreased in group 5Ø compared to group ES.

Group 5Ø had a significantly greater amount of 2Ø:4n-6 than group EFAD and significantly less 16:1n-7 and 2Ø:3n-9.

18:2n-6 and 2Ø:4n-6 were significantly elevated in group ES compared to group EFAD. 16:1n-7 and 2Ø:3n-9 were significantly lower.

TABLE 13 and TABLE 14 show the effect of the five different dietary treatments on the sums and ratios of plasma phospholipid fatty acids. TABLE 13 presents the means and standard deviations of the fatty acids from each dietary treatment. TABLE 14 compares the results from the different dietary treatments, showing where significant differences occur.

The significant differences, as identified in TABLE 14, are described below in the format used to describe TABLE 8.

The rats fed rat chow had a significantly higher sum of the saturated fatty acids than group 1ØØ but a significantly lower sum of n-3 fatty acids and 18:1/18:2 ratio.

In group RC, the 18:2/2Ø:4 ratio was significantly higher than it was in group 5Ø but the sum of the n-6 fatty

TABLE 13

EFFECT OF DIET ON RATIOS AND SUMS OF RAT PLASMA PHOSPHOLIPID FATTY ACIDS
(MEAN +/- SD)

FATTY ACID	DIETARY TREATMENT				
	RC	1ØØ	5Ø	EFAD	ES
	N=8	N=9	N=1Ø	N=5	N=6
tri/tetra	Ø.16 +/- Ø.21	Ø.5Ø +/- Ø.31	Ø.18 +/- Ø.23	1.71 +/- Ø.51	Ø.6Ø +/- Ø.53
sum n-3	8.Ø6 +/- 7.34	18.51 +/- 6.64	6.26 +/- 4.89	9.Ø5 +/- 3.99	13.Ø3 +/- 8.43
sum n-6	14.96 +/- 4.39	1Ø.48 +/- 5.71	22.61 +/- 2.26	9.96 +/- 3.19	17.36 +/- 6.43
sum sat	44.62 +/- 9.Ø2	31.44 +/- 7.96	45.53 +/- 6.81	42.65 +/- 1.12	34.34 +/-12.64
18:2/2Ø:4	1.45 +/- Ø.24	1.49 +/- Ø.76	Ø.57 +/- Ø.36	Ø.78 +/- Ø.36	Ø.94 +/- Ø.59
18:1/18:2	Ø.73 +/- Ø.62	2.88 +/- 1.45	1.48 +/- Ø.75	4.13 +/- Ø.83	1.88 +/- Ø.44

RC=RAT CHOW CONTROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF THE AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; N=NUMBER OF RATS/GROUP; tri/tetra=2Ø:3n-9/2Ø:4n-6; sum n-3=SUM OF THE N-3 FATTY ACIDS; sum n-6=SUM OF THE N-6 FATTY ACID; sum sat=SUM OF THE SATURATED FATTY ACIDS

TABLE 14

EFFECT OF DIET ON RATIOS AND SUMS OF RAT PLASMA PHOSPHOLIPID FATTY ACIDS SHOWING DIFFERENCES BETWEEN DIETS

ANOVA/NEUMAN-KEULS MULTIPLE RANGE TEST

	DIET COMPARISONS									
FATTY ACID	RCv1ØØ	RCv5Ø	RCvES	RCv EFAD	1ØØv5Ø	1ØØvES	1ØØv EFAD	5ØvES	5Øv EFAD	ESv EFAD
tri/tetra	n	n	n	S^	n	n	S^	n	S^	S^
sum n-3	S^	n	n	n	^S	n	^S	n	n	n
sum n-6	n	S^	n	n	S^	S^	n	^S	^S	^S
sum sat	^S	n	n	n	S^	n	S^	n	n	n
18:2/2Ø:4	n	^S	n	^S	^S	n	^S	n	n	n
18:1/18:2	S^	n	n	S^	^S	^S	S^	n	S^	S^

p<.Ø5

RC=RAT CHOW CONTROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF THE AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; S=SIGNIFICANT; n=NOT SIGNIFICANT; tri/tetra=2Ø:3n-9/2Ø:4n-6; sum n-3=SUM OF THE N-3 FATTY ACIDS; sum n-6=SUM OF THE N-6 FATTY ACIDS; sum sat=SUM OF THE SATURATED FATTY ACIDS p=LEVEL OF SIGNIFICANCE

^=designates a significant fatty acid increase in the indicated dietary group

acids was significantly lower.

Comparing group RC and group EFAD, the 18:2/20:4 ratio was again significantly higher in group RC. The triene/tetraene and 18:1/18:2 ratios were significantly lower.

The sum of the n-3 fatty acids and the 18:2/20:4 and 18:1/18:2 ratios were significantly increased in group 100 compared to group 50 but the sum of the n-6 and the sum of the saturated fatty acids were significantly lower.

The 18:1/18:2 ratio was significantly higher in group 100 than in group ES. The sum of the n-6 fatty acids was significantly lower in group 100.

Comparing group 100 to group EFAD, the sum of the n-3 fatty acids and the 18:2/20:4 ratio were significantly higher in group 100 and the triene/tetraene and 18:1/18:2 ratios along with the sum of the saturated fatty acids were significantly lower.

The sum of the n-6 fatty acids was significantly elevated in group 50 compared to group ES.

Comparing group 50 to group EFAD, the n-6 fatty acids were significantly higher and the triene/tetraene and 18:1/18:2 ratios significantly lower in group 50.

The sum of the n-6 fatty acids was significantly higher in group ES compared to group EFAD. The triene/tetraene and 18:1/18:2 ratios were significantly lower.

TABLE 15 and TABLE 16 show the effect of the five different dietary treatments on rat red blood cell phospholipid fatty acids. TABLE 15 presents the means and standard deviations of the fatty acids from each dietary treatment. TABLE 16 compares the results from the different dietary treatments, showing where significant differences occur.

The significant differences, as identified in TABLE 16, are described below in the same format used to describe TABLE 8.

Group RC had significantly higher 16:0, 18:0, 20:4n-6 and 22:6n-3 and significantly lower 18:1n-9(c), 18:3n-3 and 20:3n-9 than group 100.

Group RC had significantly more 18:2n-6 and 22:6n-3 and significantly less 18:1n-9(c) than group 50.

Comparing group RC to group ES, the RC group had significantly more 18:0, 18:2n-6, 20:4n-6 and 22:6n-3 and significantly less 18:1n-9(c) and 20:3n-9.

Group RC had significantly more 18:0, 18:2n-6, 20:4n-6 and 22:6n-3 in comparison to group EFAD but significantly less 18:1n-9(c) and 20:3n-9.

Group 100 had significantly more 16:0, 18:1n-9(c), 18:3n-3 and 20:3n-9 than did group 50 but had significantly lower 18:0, 20:4n-6 and 22:6n-3.

Group 100 had significantly more 16:0, 18:1n-9(c) and

TABLE 15

EFFECT OF DIET ON RAT RED BLOOD CELL PHOSPHOLIPID FATTY ACIDS
(MEAN +/- SD in mol%)

FATTY ACID	DIETARY TREATMENT			
	RC	100	50	EFAD	ES
	N=6	N=9	N=9	N=9	N=6
16:0	35.09 +/- 4.45	19.65 +/- 5.88	39.21 +/- 2.50	33.42 +/- 4.07	33.75 +/- 7.75
16:1	0.81 +/- 0.57	1.41 +/- 1.04	0.99 +/- 0.31	1.30 +/- 0.32	1.03 +/- 0.46
18:0	14.81 +/- 1.88	9.55 +/- 3.79	13.63 +/- 0.53	10.88 +/- 1.28	11.10 +/- 2.36
18:1(c)	4.47 +/- 2.97	20.37 +/- 8.06	13.89 +/- 1.30	19.07 +/- 1.51	13.54 +/- 1.62
18:1(t)	3.53 +/- 1.48	5.33 +/- 2.63	3.47 +/- 0.25	4.90 +/- 2.91	4.93 +/- 2.44
18:2n-6	14.46 +/- 0.95	9.06 +/- 3.03	7.65 +/- 0.61	3.87 +/- 2.14	8.49 +/- 2.66
18:3n-6	0	0.54 +/- 0.36	0	0	0.77 +/- 1.31
18:3n-3	1.48 +/- 1.54	16.90 +/- 6.90	0.76 +/- 0.41	2.50 +/- 2.14	3.00 +/- 3.14
20:3n-9	0.93 +/- 0.39	3.27 +/- 1.80	0.60 +/- 0.26	12.46 +/- 0.71	2.58 +/- 2.30
20:3n-6	0.79 +/- 0.49	0.32 +/- 0.20	0.56 +/- 0.21	0	0.16 +/- 0.25
20:4n-6	16.79 +/- 1.72	10.67 +/- 6.41	17.19 +/- 3.50	8.74 +/- 1.27	15.98 +/- 2.47
20:5n-3	0.79 +/- 0.43	0.98 +/- 0.55	0	0.62 +/- 0.69	1.34 +/- 1.30
22:4n-6	1.20 +/- 0.94	0.61 +/- 0.50	0.39 +/- 0.26	1.21 +/- 0.92	1.71 +/- 1.74
22:5n-6	0.57 +/- 0.51	0.78 +/- 0.38	0.78 +/- 0.19	0	0.74 +/- 0.21
22:5n-3	1.59 +/- 0.33	0.07 +/- 0.14	0.03 +/- 0.10	0	0.18 +/- 0.44
22:6n-3	2.68 +/- 0.43	0.49 +/- 0.33	0.84 +/- 0.08	1.04 +/- 0.21	0.69 +/- 0.21

RC=RAT CHOW CONTROLS; 100=AD LIBITUM SEMI SYNTHETIC CONTROLS; 50=PAIR FED 50% OF THE AMOUNT OF DIET CONSUMED BY GROUP 100; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; N=NUMBER OF RATS/GROUP; c=CIS; t=TRANS

TABLE 16

EFFECT OF DIET ON RAT RED BLOOD CELL PHOSPHOLIPID FATTY ACIDS SHOWING DIFFERENCES BETWEEN DIETS

ANOVA/NEUMAN-KEULS MULTIPLE RANGE TEST

	DIET COMPARISONS									
FATTY ACID	RCvlØØ	RCv5Ø	RCvES	RCv EFAD	1ØØv5Ø	1ØØvES	1ØØv EFAD	5ØvES	5Øv EFAD	ESv EFAD
16:Ø	^S	n	n	n	^S	^S	^S	n	n	n
16:1	n	n	n	n	n	n	n	n	n	n
18:Ø	^S	n	^S	^S	S^	n	n	^S	n	n
18:1(c)	S^	S^	S^	S^	^S	^S	n	n	S^	S^
18:1(t)	n	n	n	n	n	n	n	n	n	n
18:2n-6	n	^S	^S	^S	n	n	^S	n	^S	^S
18:3n-6	n	n	n	n	n	n	n	n	n	n
18:3n-3	S^	n	n	n	^S	^S	^S	n	n	n
2Ø:3n-9	S^	n	S^	S^	^S	n	S^	S^	S^	S^
2Ø:3n-6	n	n	n	n	n	n	n	n	n	n
2Ø:4n-6	^S	n	^S	^S	S^	n	n	^S	^S	^S
2Ø:5n-3	n	n	n	n	n	n	n	n	n	n
22:4n-6	n	n	n	n	n	n	n	n	n	n
22:5n-6	n	n	n	n	n	n	n	n	n	n
22:5n-3	n	n	n	n	n	n	n	n	n	n
22:6n-3	^S	^S	^S	^S	S^	n	S^	^S	n	S^

p<.Ø5

RC=RAT CHOW CONTROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF THE AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; S=SIGNIFICANT; n=NOT SIGNIFICANT; c=CIS; t=TRANS; p=LEVEL OF SIGNIFICANCE

^=designates a significant fatty acid increase in the indicated dietary group

18:3n-3 than group ES.

16:Ø, 18:2n-6 and 18:3n-3 were significantly higher and 2Ø:3n-9 and 22:6n-3 significantly lower in group 1ØØ compared to group EFAD.

Comparing group 5Ø to group ES, 18:Ø, 2Ø:4n-6 and 22:6n-3 were significantly higher and 2Ø:3n-9 was significantly lower in group 5Ø.

Group 5Ø had significantly higher amounts of 18:2n-6 and 2Ø:4n-6 and significantly lesser amounts of 18:1n-9(c) and 2Ø:3n-9 than group EFAD.

18:2n-6 and 2Ø:4n-6 were significantly greater in group ES in comparison to group EFAD. 18:1n-9(c), 2Ø:3n-9 and 22:6n-3 were all significantly lower in group ES.

TABLE 17 and TABLE 18 show the effect of the five diffferent dietary treatments on the sums and ratios of rat red blood cell phospholipid fatty acids. TABLE 17 presents the means and standard deviations for each dietary treatment. TABLE 18 compares the results from the different dietary treatments, showing where significant differences occur.

The significant differences, as identified in TABLE 18, are described below in the same format as the other tables.

Significantly increased sums of n-6 and saturated fatty acids and a significantly lower sum of the n-3 fatty acids, 18:1/18:2 and triene/tetraene ratios were found in

TABLE 17

EFFECT OF DIET ON RATIOS AND SUMS OF RAT RED BLOOD CELL PHOSPHOLIPID FATTY ACIDS
(MEAN +/- SD)

FATTY ACID	DIETARY TREATMENT RC N=6	1ØØ N=9	5Ø N=9	EFAD N=9	ES N=6
tri/tetra	Ø.Ø6 +/- Ø.Ø3	Ø.48 +/- Ø.44	Ø.Ø3 +/- Ø.Ø1	1.45 +/- Ø.21	Ø.18 +/- Ø.18
sum n-3	6.54 +/- 1.33	18.44 +/- 7.11	1.64 +/- Ø.38	4.16 +/- 2.56	5.22 +/- 4.21
sum n-6	19.35 +/- 1.74	12.93 +/- 6.56	18.92 +/- 3.87	9.95 +/- 1.29	19.36 +/- 2.83
sum sat	49.9Ø +/- 4.21	29.19 +/- 8.75	52.85 +/- 2.77	44.3Ø +/- 5.23	44.85 +/-1Ø.Ø9
18:2/2Ø:4	Ø.86 +/- Ø.Ø9	1.22 +/- Ø.97	Ø.48 +/- Ø.2Ø	Ø.48 +/- Ø.38	Ø.55 +/- Ø.24
18:1/18:2	Ø.56 +/- Ø.27	3.23 +/- 1.8Ø	2.28 +/- Ø.21	7.39 +/- 2.94	2.24 +/- Ø.3Ø

RC=RAT CHOW CONTROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF THE AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; N=NUMBER OF RATS/GROUP; tri/tetra=2Ø:3n-9/2Ø:4n-6; sum n-3=SUM OF THE N-3 FATTY ACIDS; sum n-6=SUM OF THE N-6 FATTY ACIDS; sum sat=SUM OF THE SATURATED FATTY ACIDS

TABLE 18

EFFECT OF DIET ON RATIOS AND SUMS OF RAT RED BLOOD CELL PHOSPHOLIPID FATTY ACIDS

ANOVA/NEUMAN-KEULS MULTIPLE RANGE TEST

DIET COMPARISONS

FATTY ACID	RCv1ØØ	RCv5Ø	RCvES	RCv EFAD	1ØØv5Ø	1ØØvES	1ØØv EFAD	5ØvES	5Øv EFAD	ESv EFAD
tri/tetra	S^	n	n	S^	^S	^S	S^	n	S^	S^
sum n-3	S^	n	n	n	^S	^S	^S	n	n	n
sum n-6	^S	n	n	^S	S^	S^	n	n	^S	^S
sum sat	^S	n	n	n	S^	S^	^S	n	n	n
18:2/2Ø:4	n	n	n	n	n	n	n	n	n	n
18:1/18:2	S^	n	n	S^	n	n	S^	n	S^	S^

p<.Ø5

RC=RAT CHOW CONTROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF THE AMOUNT OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; S=SIGNIFICANT; n=NOT SIGNIFICANT; tri/tetra=2Ø:3n-9/2Ø:4n-6; sum n-3=SUM OF THE N-3 FATTY ACID SERIES; sum n-6=SUM OF THE N-6 FATTY ACID SERIES; sum sat=SUM OF THE SATURATED FATTY ACIDS; p=LEVEL OF SIGNIFICANCE

^=designates a significant fatty acid increase in the indicated dietary group

group RC compared to group 1ØØ.

Group RC had a significantly higher sum of the n-6 fatty acids and significantly lower triene/tetraene and 18:1/18:2 ratios than group EFAD.

Group 1ØØ had a significantly higher triene/tetraene ratio and sum of the n-3 fatty acids compared to group 5Ø. The sums of the n-6 and saturated fatty acids were significantly lower in group 1ØØ.

Compared to group ES the triene/tetraene ratio and the sum of the n-3 fatty acids were significantly higher and the sums of the n-6 and saturated fatty acids significantly lower in group 1ØØ..

Group 1ØØ had a significantly higher sum of the n-3 fatty acids and sum of the saturated fatty aids than group EFAD but had significantly lower triene/tetraene and 18:1/18:2 ratios.

Group 5Ø had a significantly increased sum of the n-6 fatty acids and significantly lower triene/tetraene and 18:1/18:2 ratios compared to group EFAD.

Group ES had a significantly higher sum of the n-6 fatty acids than group EFAD but significantly lower triene/tetraene and 18:1/18:2 ratios.

TABLE 19 summarizes the data presented and described in TABLES 7 through 18. The effect of different diets on liver, plasma and red blood cell fatty acid profiles are

TABLE 19

COMPARISON OF THE EFFECT OF DIET ON LIVER, PLASMA AND RED BLOOD CELL FATTY ACID PROFILES

	RC v 1ØØ			1ØØv5Ø,ES,EFAD			5Ø v 1ØØ			ES v 1ØØ			EFAD v all		
	L	P	R	L	P	R	L	P	R	L	P	R	L	P	R
FATTY ACIDS															
16:Ø	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16:1	-	-	-	i	-	-	-	-	-	i	-	-	i	i	i
18:Ø	i	i	i	d	d	d	i	i	i	i	i	i	d	d	d
18:1	d	d	d	i	i	i	i	i	i	i	i	i	i	i	i
18:2n-6	i	i	i	-	-	-	-	-	-	-	-	-	d	d	d
18:3n-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18:3n-3	-	-	-	i	i	i	-	-	-	-	-	-	-	-	-
2Ø:3n-9	-	-	-	-	-	i	-	-	-	-	-	i	i	i	i
2Ø:3n-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2Ø:4n-6	-	-	-	-	-	-	-	-	-	-	-	-	d	d	d
2Ø:5n-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:4n-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:5n-6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:6n-3	i	i	i	-	-	-	-	-	-	-	-	-	i	i	i
RATIOS															
tri/tetr	-	-	-	-	-	i	-	-	-	-	-	-	i	i	i
18:2/2Ø:4	-	-	-	-	-	-	d	d	d	d	d	d	d	d	d
18:1/18:2	d	d	d	i	i	i	i	i	i	i	i	i	i	i	i
SUMS															
sum n-3	-	-	-	i	i	i	d	d	d	d	d	d	-	-	-
sum n-6	-	-	-	-	-	-	-	-	-	-	-	-	d	d	d
sum sat	-	-	-	-	-	-	i	i	i	i	i	i	-	-	-

RC=RAT CHOW CONROLS; 1ØØ=AD LIBITUM SEMI SYNTHETIC CONTROLS; 5Ø=PAIR FED 5Ø% OF DIET CONSUMED BY GROUP 1ØØ; EFAD=ESSENTIAL FATTY ACID DEFICIENT; ES=PAIR FED TO EFAD GROUP; L=LIVER; P=PLASMA; R=RED BLOOD CELLS

-=indicates no significant difference; i=indicates a significant increase; d=indicates a significant decrease

compared and the similar trends identified.

D. DISCUSSION

The weight record for the independently run, nutritionally complete, semi-synthetic ad libitum control group (1ØØ-2) closely paralleled that of the other such control group (1ØØ) thus acting as a check on the reproducibility of group 1ØØ results and showing that although group 1ØØ rats were significantly heavier at the start of the experiment, this did not affect the final outcome of the weight gain pattern.

The pre-sacrifice weight for rats fed the rat chow was significantly higher than all other groups, including those rats feeding ad libitum on the nutritionally complete semi-synthetic diet (group 1ØØ). There are three possible explanations for this. First, the semi-synthetic diet was deficient in one or more essential nutrients and thus impeded maximal growth. This is, however, unlikely since the diet was prepared in strict accordance with the nutrient requirements of rats and even included supplemental choline and L-methionine (15). Second, the rats preferred the hard, pelleted form of the rat chow to the powdery semi-synthetic diet and this difference in palatability between the two diets resulted in rat chow rats consuming more food and growing bigger. Third, the

rats may have eaten the same amount of rat chow as semi-synthetic diet, but because the caloric density of rat chow is higher than that of the semi-synthetic diet, due to a higher proportion of fat, the rat chow rats received more calories per gram of food. Recording the amount of food consumed by rat chow rats would clarify this.

The significantly reduced weights for the malnourished rats (groups 5Ø and ES) and the essential fatty acid deficient rats (group EFAD) are indicative that the specific dietary treatments did induce malnutrition, but only essential fatty acid deficient rats developed physical signs of a specific nutrient deficit. This indicates that essential fatty acid deficiency was successfully induced since the absence of linoleic acid from the diet was the only difference between these three groups. The similar weight gain pattern between essential fatty acid deficient and pair fed groups and the similar feed efficiencies (total weight gain/total food intake-TABLE 5) strongly suggests that, initially, the decreased growth seen in essential fatty acid deficiency may be a result of decreased food intake and not a function of the essential fatty acid deficiency itself i.e. an effect of general malnutrition, not a specific nutrient deficiency.

Between weeks five and six of the experiment the weights of group 5Ø and essential fatty acid deficient rats dropped. The pair fed group, ES, did not exhibit this

weight loss. This group was not essential fatty acid deficient and was consuming 11.3% more food than the group 5Ø rats. The length of time that the study was carried out was not long enough to establish whether the pair fed group, ES, would also have followed the same pattern. It was also not long enough to determine whether or not the weight gain pattern of the essential fatty acid deficient rats would continue to parallel that of group 5Ø and group ES rats or whether it would diverge.

FATTY ACID PROFILES

Total phospholipids (TPL) were measured because they are a well accepted biochemical means to determine essential fatty acid status; although the phosphatidylcholine fraction may be preferable to use in that it more closely reflects changes in essential fatty acid status (13).

The liver, plasma and red blood cell fatty acid profiles of the essential fatty acid deficient rats are consistent with the literature on the biochemical indices of essential fatty acid deficiency. The depressed 18:2n-6 and 2Ø:4n-6, the elevated 18:1n-9 and 2Ø:3n-9 and the high triene/tetraene ratio along with the marked physical appearance of these rats clearly demonstrate that these rats developed an essential fatty acid deficiency.

The two ad libitum control groups, RC and 1ØØ, were

less similar in fatty acid profiles than might be expected, reflecting a difference between the semi-synthetic and rat chow diet compositions. It has already been suggested that rat chow rats have a more calorically dense diet due to a greater proportion of fat and this higher fat content may be responsible for the differences in the fatty acid profiles.

In view of the differences in the fatty acid profiles between the two control groups, the discussion of the results will use the profiles of the ad libitum semi synthetic diet group (group 1ØØ) as the control comparison.

It was an interesting discovery that the pair fed group ate similar amounts of the same diet as the malnourished group. In comparison to the ad libitum semi synthetic controls this amounted to 61.3% and 5Ø% respectively. Not surprisingly malnourished and pair fed rats have closely matched liver, plasma and red blood cell profiles reflecting their similar level of food restriction. Despite quite severe food restriction these rats did not develop a biochemical essential fatty acid deficiency as assessed by depressed levels of linoleic acid, elevated levels of eicosatrienoic acid and an increased triene/tetraene ratio. The diet must have been supplying enough linoleic acid to meet nutritional needs. Many studies have shown that rats have the capacity to turn down

their basal metabolic rate when faced with food restriction meaning that less nutrients are required for growth and/or maintenance (60,61). It has been suggested that growing rats may lack this inherent capability, relying more on growth retardation as a conservation mechanism (59). Even if growing rats cannot turn down their basal metabolic rate, the growth retardation would result in a much smaller animal and in feeding this smaller animal 50% of the amount that a control group ate, one would in reality be supplying much more than 50% of the animal's nutritional requirements.

Another factor to consider when examining why the food restricted rats did not develop essential fatty acid deficiency is the level of protein that they were receiving at the restricted level. At 50% restriction the rats would still be receiving 10% casein and this is enough to meet growth requirements even if basal metabolic rate is not turned down (15). Low protein diets have also been shown to reduce the enzyme activities of the delta 5, delta six and delta nine desaturase enzymes involved in the desaturation/elongation metabolism of polyunsaturated fatty acids (43). The protein deprivation in this study does not appear to have been enough to induce these enzymatic changes and the corresponding changes in polyunsaturated fatty acid metabolism. The linoleate/arachidonate ratio (18:2/20:4) is decreased in the food restricted rats but

since arachidonic acid levels remain normal in these rats as compared to the control groups, this would support that desaturation/elongation of linoleic acid is not affected by the reduced level of dietary protein. It also means that the reduced linoleate/arachidonate ratio is caused by reduced linoleate levels, supporting the hypothesis of this study that linoleic acid is oxidized to meet energy demands in the face of an energy deficit.

It must be noted, however, that although an overt biochemical essential fatty acid deficiency did not manifest in food restricted rats, there were indications that a trend towards essential fatty acid deficiency was developing. The 18:1/18:2 ratio was elevated in the liver, plasma and red blood cell tissues of food restricted rats, suggesting that oleic acid was being synthesized to offset the commencement of reduced levels of linoleate. This trend was, in part, masked by the fact that the ad libitum semi-synthetic control rats had decreased levels of linoleate in liver, plasma and red blood cell tissues as compared to rat chow controls.

There is evidence that a low protein diet, such as is found in chronic malnutrition, will exacerbate an existing essential fatty acid deficiency or increase the essential fatty acid requirement so as to precipitate marginal deficiency (57). Essential fatty acid deficient rats show

a more severe biochemical deficiency at low protein levels than they do at normal and high levels (57). The food restricted rats in this study were restricted in protein intake to 50% and 61.3% of control intake respectively and their essential fatty acid intake was similarly compromised. Had the study been conducted over a longer period of time, the low protein levels combined with marginal linoleate intake would have in all probability culminated in the full development of essential fatty acid deficiency.

Eicosatrienoic acid is elevated in the red blood cells of pair fed rats (ES) but since it is not also elevated in the red blood cells of malnourished rats (50) and these two groups were so similar, perhaps some factor other than essential fatty acid deficiency is at work. An anomaly present in the red blood cell fatty acid profiles of the test and control groups is the high level of 20:3n-9 in the 100 control group (3.2%) compared to the low level (0.6%) in the malnourished group. The triene/tetraene ratio in the red blood cells of ad libitum semi-synthetic control rats is also elevated above that of malnourished and pair fed rats although it is still significantly less than in essential fatty acid deficient rats. The reason for this is not clear.

The effect of malnutrition on essential fatty acid status is to compromise it. Although overt essential fatty

acid deficiency did not develop during the time frame of this study, the fatty acid profiles from liver, plasma and red blood cells reflect a tendency towards developing a relative essential fatty acid deficiency when food intake is severely restricted and support the hypothesis that in the malnourished state there is increased oxidation of tissue linoleic acid in order to meet energy demands. With continuance of the study or a greater food restriction so that energy conservation mechanisms are overridden, this compromise would be made more apparent.

In conclusion, one must question the validity of the current custom of expressing essential fatty acid requirement as a percentage of total calories when in a malnourished state. If the hypothesis of this study holds true, that in a sufficiently malnourished state linoleic acid is preferentially oxidized to meet energy demands, then the essential fatty acid requirement when caloric intake is depressed will be greater than 1.3% of total calories. It would appear to be more prudent to express essential fatty acid requirement on a gram/kilogram of body weight.

CHAPTER III

CONCLUSION AND DISCUSSION

CHAPTER III

CONCLUSION AND DISCUSSION

The purpose of this study was to examine the effect of malnutrition versus linoleic acid deficiency on the body tissue levels of essential fatty acids and to determine the influence that malnutrition has on linoleic acid metabolism and requirements. This was accomplished by inducing malnutrition in laboratory rats by restricting their total food intake over a six week period. Upon sacrifice, liver, plasma and red blood cells were analyzed for their fatty acid profiles. To ensure that the differences observed in fatty acid profiles could be attributed to the correct experimental variable, great care was taken with the use of pair fed controls. Thus it was possible to discern between the effects of essential fatty acid deficiency alone and the effects of total reduced food intake on essential fatty acid status.

The fatty acid profiles of essential fatty acid deficient rats in this study were in accordance with findings from similar studies in the literature. There is one important observation to be made here with regard to simple essential fatty acid deficiency. Through the use of a pair fed control group, that ate the same amount as the essential fatty acid deficient rats but received an

essential fatty acid sufficient diet, it became apparent that, at least for the initial stages of essential fatty acid deficiency, the growth impairment that is observed in essential fatty acid deficiency is attributable to anorexia and not directly to the lack of essential fatty acid since the pair fed rats exhibited exactly the same weight gain pattern as their essential fatty acid deficient counterparts. This is not to say, however, that had the experiment been carried out over a longer period of time the essential fatty acid deficient and pair fed control patterns may have diverged considerably and a distinct growth impairment linked specifically to essential fatty acid deficiency become apparent.

The latter comment concerning the length of the study warrants further discussion from another aspect. The fatty acid profiles that were obtained after six weeks of dietary treatment clearly established biochemical essential fatty acid deficiency (accompanied by severe clinical signs of deficiency) in the essential fatty acid deficient group. However, the fatty acid profiles of the rats that were receiving restricted food intake were only just beginning to develop identifiable trends at the end of the six weeks. In the interest of better establishing these trends and in determining the outcome of the growth patterns an extension of the feeding period by two or more weeks would be desirable.

Malnutrition, i.e. severely restricted food intake, does affect essential fatty acid status. The changes observed in fatty acid profiles suggested the commencement of the development of a relative essential fatty acid deficiency that would have become more pronounced over time. Although linoleic acid levels were not depressed, the 18:2/2Ø:4 ratio was decreased. This particular finding provides support to the hypothesis of this study, that linoleic acid is oxidized to meet energy demands when the body is faced with an energy deficit. The arachidonic acid (2Ø:4n-6) levels remained normal in food restricted rats suggesting that desaturation/elongation was not affected. In order for the 18:2/2Ø:4 ratio to be decreased then, linoleic acid levels had to be compromised and the trend towards linoleic acid deficiency established. Oleic acid (18:1) was elevated as was the 18:1/18:2 ratio; both compensatory changes in response to a reduced supply of linoleic acid. To further examine these changes, aside from increasing the length of the experiment, there are some interesting directions for further study.

The main contention of this study was that in the malnourished state there is increased oxidation of tissue linoleic acid. That is to say, under conditions of malnutrition, the body is unable to conserve linoleic acid and will oxidize it like any other fatty acid in order to

provide the body with the energy it needs to live. This of course would lead to reduced levels of essential fatty acid and a relative essential fatty acid deficiency would ensue. But animals have the ability to conserve energy by reducing basal metabolic rate (BMR) and/or reducing growth. The degree to which an animal is able to conserve energy is a very important factor if one wishes to test the hypothesis put forth in this study. Linoleate levels were not reduced in the malnourished animals. One must suspect that the degree of food restriction was not enough to override the animals' energy conserving mechanisms over the length of time that the study was carried out. To take this study one step further then, the actual changes in BMR could be monitored with the use of metabolic chambers and food could be restricted to the point that energy conserving mechanisms would be overridden.

To create malnutrition in the rats in this study total food intake was restricted. This meant that the animals were receiving inadequate energy, protein, fat, vitamins and minerals. Although one could attribute any changes in the fatty acid profiles of these animals to 'malnutrition' it is not possible to distinguish between the effects of any of the specific deficiencies that the malnutrition encompassed. The hypothesis of this study was that linoleic acid is oxidized in the malnourished state to meet energy demands, an energy deficit being a prime feature of

malnutrition. Rather than inducing a general malnutrition, it would be of value to induce only an energy deficiency by decreasing the sucrose content of the diet and adjusting the other components of the diet so that they remain the same. In this way, the specific effect of energy deficiency on linoleate metabolism could be distinguished from the effects of other nutrient deficiencies.

It is well documented in the literature that certain aspects of malnutrition such as protein deficiency affect the desaturation/elongation enzyme systems of polyunsaturated fatty acid metabolism. This can be determined indirectly by examining ratios of various fatty acids from fatty acid profiles, or it can be determined directly with enzyme assays that can measure the activity of each enzyme involved. What is not known, is whether energy deficiency alone compromises the desaturation/elongation enzyme system. It would certainly be advantageous in future studies of this nature to determine this by obtaining direct measurements of enzyme activities in conjunction with the information fatty acid profiles provide.

Chronic malnutrition is frequently complicated by essential fatty acid deficiency. Protein and energy requirements are not usually met. The lack of protein alters the functioning of the desaturase enzymes and the

lack of energy diverts linoleic acid into pathways that utilize it as an energy source rather than for its other biological functions. This means that in a malnourished state, the requirement for essential fatty acid may actually be increased above that of normal needs. The conventional method of expressing essential fatty acid requirement as a percentage of total calories perhaps needs to be re-evaluated in light of this with consideration given to determining essential fatty acid needs on a gram/kilogram body weight basis in keeping with all other nutrient requirements.

CHAPTER IV

REFERENCES

CHAPTER IV

REFERENCES

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APPENDIX I

FEEDING RECORD FOR RATS CONSUMING THE AD LIBITUM SEMI SYNTHETIC DIET (1ØØ)
Average Daily Consumption (gm) *

DAY	AMOUNT CONSUMED	DAY	AMOUNT CONSUMED	DAY	AMOUNT CONSUMED
1	9.5	15	13.5	29	15.7
2	11.3	16	16.4	3Ø	17.3
3	12.Ø	17	16.Ø	31	16.5
4	1Ø.2	18	15.7	32	16.8
5	11.9	19	16.9	33	15.5
6	1Ø.9	2Ø	13.8	34	15.Ø
7	11.7	21	14.5	35	14.5
8	1Ø.5	22	16.8	36	16.7
9	12.4	23	15.3	37	15.4
1Ø	11.7	24	13.3	38	14.4
11	14.9	25	15.1	39	14.8
12	12.4	26	15.Ø	4Ø	15.7
13	13.Ø	27	14.6	41	16.8
14	13.4	28	15.4	42	FAST

TOTAL FOOD CONSUMED = 583.2 gm
AVERAGE DAILY INTAKE= 14.22 gm +/- 2.1Ø gm

* Rat test group 5Ø was fed 5Ø% of the average daily food intake consumed by group 1ØØ

APPENDIX II

FEEDING RECORD FOR RATS CONSUMING THE ESSENTIAL FATTY ACID DEFICIENT DIET
Average Daily Consumption (gm) *

DAY	AMOUNT CONSUMED	DAY	AMOUNT CONSUMED	DAY	AMOUNT CONSUMED
1	6.7	15	7.7	29	9.7
2	7.1	16	7.8	3Ø	8.Ø
3	7.1	17	8.2	31	8.1
4	7.Ø	18	8.6	32	8.3
5	7.Ø	19	9.2	33	8.9
6	7.1	2Ø	7.5	34	9.9
7	6.9	21	9.Ø	35	1Ø.6
8	7.Ø	22	9.4	36	11.1
9	7.3	23	8.8	37	11.2
1Ø	8.4	24	9.1	38	11.5
11	7.8	25	9.1	39	11.7
12	7.8	26	9.9	4Ø	11.5
13	8.Ø	27	9.4	41	FAST
14	8.Ø	28	9.8	42	

TOTAL FOOD CONSUMED = 347.2 gm
AVERAGE DAILY INTAKE= 8.68 gm +/- 1.43 gm

* Pair fed control group ES consumed the same amount of food as essential fatty acid deficient rats (EFAD) but their diet was EFA sufficient

APPENDIX III

COMPOSITION OF AIN MINERAL MIXTURE 76 AND VITAMIN MIXTURE 76

AIN MINERAL MIXTURE 76 composition: g/kg mixture	
calcium phosphate	5ØØ
sodium chloride	74
potassium citrate monohydrate	22Ø
potassium sulfate	52
magnesium oxide	24
manganous oxide	3.5
ferric citrate	6.Ø
zinc carbonate	1.6
cupric carbonate	Ø.3
potassium iodate	Ø.Ø1
sodium selenite	Ø.Ø1
chromium potassium sulfate	Ø.55
sucrose	118

AIN VITAMIN MIXTURE 76 composition: g/kg mixture	
thiamin hydrochloride	6ØØ
riboflavin	6ØØ
pyridoxine hydrochloride	7ØØ
nicotinic acid	3
D-calcium pantothenate	1.6
folic acid	2ØØ
D-biotin	2Ø
cyanocobalamin	1
dl-alapha-tocopherol acetate	2Ø
cholecalciferol	2.5
menoquinone	5
sucrose	927.9