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EFFECTS OF DIETARY CARBOHYDRATE AND FAT ON PANTOTHENIC ACID STATUS IN RATS

By

Debbie E Gould

A THESIS

Submitted to
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ABSTRACT

EFFECTS OF DIETARY CARBOHYDRATE AND FAT ON PANTOTHENIC ACID STATUS IN RATS

By

Debbie E. Gould

Effects of diet on pantothenic acid (PA) status in weanling rats were examined to determine if a diet high in fat results in higher PA requirements. In the first of two studies, 24-hour urine samples were collected daily from three groups of rats fed a high-fat (67.5% kcal from fat) or a high-carbohydrate (67.5% kcal from carbohydrate) diet deficient in PA, or a high-carbohydrate diet supplemented with 312 ug PA/ 100 kcal (12 mg PA/ kg diet) and analysed for PA content. The second experiment examined PA content of tissues, whole blood and plasma, and plasma triglycerides of rats fed high-fat or high-carbohydrate diets deficient or supplemented in PA. No differences were seen between high-fat and high-carbohydrate PA deficient groups with the exception of plasma triglycerides which were significantly elevated in the high-fat PA deficient diet group. In this study, a diet high in fat did not result in a higher PA requirement then a high-carbohydrate diet.

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INTRODUCTION

Pantothenic acid (PA), one of the B-vitamins, is the vitamin moiety of coenzyme A and the phosphopantetheine of acyl carrier protein. As a part of these coenzymes, PA is involved in carbohydrate, lipid and amino acid metabolism. While distinct deficiency signs are not as apparent in a PA deficiency as they are in some of the other B-vitamins, it is known that PA is essential in humans and some animals for normal growth, reproduction, and physiological functioning. Although it is generally assumed that PA is adequate due to the wide variety of foods in which it can be found, subclinical deficiencies may in fact exist (Chipponi et al., 1982). These deficiencies and their resultant signs may be difficult to relate to low levels of PA in the diet because of the lack of overt clinical signs as well as due to a lack of information on the metabolic pathways affected by a PA deficiency. Although PA deficiency has been induced in rats, the clinical signs are far from specific. Deficiency signs reported in the literature include growth retardation, a rough hair coat, muscle weakness, and eventual death (Reibel et al., 1982).

While no Recommended Dietary Allowance has yet been established for PA, it is possible that as a result of the extensive biochemical functions of the coenzyme form of PA (coenzyme A and acyl carrier protein) in lipid metabolism, a high fat diet might increase the PA requirement compared to the amount needed when a high carbohydrate diet is consumed. No studies to date have looked at such indices as tissue or blood PA content along with growth rate and clinical observations to support or disprove the theory of a high fat diet lacking in PA more rapidly inducing a vitamin deficiency than a similar diet high in carbohydrate. It is this theory upon which we based our hypothesis that weanling rats fed a high fat diet deficient in PA would become depleted of this vitamin (as measured in urine, tissue and blood) more rapidly than would rats fed a high carbohydrate diet lacking in PA The goal of this study was to determine whether the PA requirement in rats is altered by the composition of fat to carbohydrate in the diet.

Literature Review

1) Dietary Effects of a High Fat Diet vs a High Carbohydrate Diet

Examining the dietary effects of a high-fat diet versus a high-carbohydrate diet on PA status in rats, may help to determine some of the underlying metabolic processes adversely affected during a PA deficiency. It has been repeatedly shown that the energy from dietary fat is utilized more efficiently than is energy from dietary carbohydrate during growth (Leveille and Cloutier, 1987; Donato, 1987). Regardless of sex or strain, rats fed a high-fat ration (40% - 87% kcal from fat) have been reported to gain weight more rapidly and to reach a higher maximum weight than those on a high-carbohydrate ration (Mickelsen et al., 1955; Schemmel et al., 1969; Schemmel et al.,1970; Oscai et al., 1984; Siedler et al., 1962; Jen et al., 1987;). Rats fed a high-fat diet had a higher percentage of their total body weight as fat than rats on the grain ration (Schemmel et al., 1969; Schemmel et al., 1970; Donato and Hegsted, 1985).

Sprague-Dawley rats fed a high-fat diet were observed to consume 20% more calories as compared to rats fed a high-carbohydrate ration (Schemmel et al., 1970). Neither the high fat nor the high carbohydrate diet had any effect on body protein through the 100th

day of life, after which time the animals fed the high-fat ration had 10% more body protein. This increase may be explained by the increased blood flow and organ size of the rats on the high-fat diet (Schemmel et al., 1969). Champigny and Hitier (1987), feeding a 55% fat diet vs a 12% fat diet found that at equal consumption (kJ/day) of both diets, the high-fat group gained more weight than the high-carbohydrate group.

2) Pantothenic Acid Requirements and Deficiency

Early studies suggested that rats require 80 ug to 100 ug of calcium pantothenate per day for maximum growth. The rate of growth paralleled the level of PA intake up to 80 ug per day. Studies on the urinary excretion of this vitamin indicated a marked increase in the urinary excretion of PA as the level of intake was raised from 80 to 150 ug. Up to 80 ug the excretion was very low, and at 150 ug about 50 ug of PA was excreted suggesting that 100 ug is approximately the requirement for the growing rat (Henderson et al., 1941). Weanling rats consuming 5.3 g food daily required 80 ug to 100 ug of PA for optimal growth as compared with a food consumption of 15.5 g and a daily requirement of about 25 ug of PA in 10 week old rats. This suggests that the pantothenate requirement is reduced with age (Unna and Richards, 1942). It has therefore been suggested that other parameters in addition to growth be used in determining the PA status of adult rats (Henderson et

al., 1942). Brown and Sturtevant (1949) reviewed the literature concerning PA requirements of the growing rat and concluded that the optimal concentration lies between 0.8 to 1.0 mg PA per 100 g of diet. A lower level of vitamin supply tended to slow down the growth, while higher vitamin concentrations did not result in greater weight gains. Barboriak et al., (1956) confirmed 0.8 mg to 1.0 mg PA per 100 g diet as the requirement for growing and adult rats. The accepted PA requirement for growth of young rats is based on a purified diet containing 5% corn oil and 385 kcal/ 100 g diet (Barboriak et al., 1956; NRC, Committee on Animal Nutrition, 1962).

Mature rats, fed a diet deficient in PA, stop growing in about one month (Reibel et al., 1982) and die later, displaying typical PA deficiency signs prior to their death. These signs include retarded growth rate in young animals, achromotrichia, scaly dermatitis, rusty fur coloration and alopecia (loss of hair) (Henderson et al., 1942; Williams, 1943). Neuromuscular disorders, gastrointestinal malfunction, adrenal cortical failure and sudden death are also commonly seen signs of PA deficiency (Nelson et al., 1947). Anorexia, myelin nerve degeneration, sciatic nerve and spinal cord damage are a few of the numerous other common signs observed during a PA deficiency (Williams, 1943).

Voris et al. (1942) concluded that PA has a specific growth promoting effect unrelated to appetite, even though they observed appetite depression in rats fed a diet deficient in PA. Pantothenic acid deficient rats have a depletion of lipids and impaired carbohydrate metabolism as demonstrated by decreased glucose tolerance and increased insulin sensitivity (Baker and Frank, 1968). Weanling rats, after 2-3 weeks on a PA deficient diet had a marked drop in urinary PA excretion, loss of weight and rusty coloration of the fur. After 5 weeks on the same diet, marked weight loss was observed, and urine levels of PA remained low, similar to the values seen at weeks 2-3 (Hatano, 1962). While PA deficiency signs are well documented, the underlying metabolic processes which result in these signs have yet to be elucidated.

There is evidence indicating that PA is synthesized by intestinal microorganisms in rats and other animals (Giovannetti, 1982; Mameesh et al., 1959; Henderson et al., 1941). It is not known whether this PA is available for absorption and use by the body, and if it is, how much is actually available. By allowing coprophagy in the rats, another variable is added to the experiment, in that it is possible that this presents an alternate or additional source of PA in the diet. Treatment of PA deficient rats with oxytetracycline caused a reduction in the deficiency signs (Mameesh et

al., 1959) in the presence and absence of coprophagy. Oxytetracycline stimulates growth in animals fed diets limiting in PA (1 mg PA/kg diet; 360 kcal/100 gm). As growth was seen with and without copraphagy it is not known whether the sparing effects of oxytetracycline are as a result of increased production of PA in the intestinal flora or due to improved absorption of the PA produced.

3)Dietary Effects of a High-Fat vs a High-Carbohydrate Diet with or without Pantothenic Acid Supplementation.

It has been suggested that a high-fat diet without PA supplementation would result in the more rapid development of a PA deficiency than would a high-carbohydrate diet without PA supplementation. Hatano et al. (1966) theorized that the increased dependence on fat for energy may alter PA metabolism in rats.

Myszkowska (1964) determined that male rats fed a diet containing 12% fat (by weight) without PA supplementation displayed signs of PA avitaminosis more rapidly than did rats consuming a similar diet containing only 6% fat. (The protein content of both diets was fairly constant.) The avitaminosis was determined by changes in hair gloss, bald areas behind the ears, on the neck and around the eyes, and the average body weight gain which fell to zero. When calcium pantothenate was orally administered in therapeutic doses of 0.5 mg per day, improved conditions

of the rats were noted after two weeks time. No data were provided in this study concerning the food intake of the rats on the two different dietary regimens. Without this information, it is difficult to ascertain whether the resultant signs were in fact due solely to different levels of fat in the diet or if level of consumption may have been a factor. Similar effects of a high-fat diet on pantothenate status have been reported in pigs (Sewell et al., 1962) where a 12% fat diet (corn oil, by weight) was compared with a 2% fat diet.

Williams et al. (1968), comparing the effects of a 6% fat (cottonseed oil, by weight) diet with an 18% fat semi-purified diet with or without PA supplementation, found lower body weights in rats on the high-fat (18%) PA deficient diet than in rats on the 6% fat diet deficient in PA after 6 weeks. On diets supplemented with graded levels of PA (200 ug/100 g diet to 3000 ug/100 g diet) these same reseachers found no evidence that a high level of dietary fat reduced the growth response to limiting intakes of PA or increased the PA requirement for maximal growth of young male rats. Again in this study, while the authors indicate that food intake was measured two to three times per week, this information was not reported. It is thus difficult for readers to accurately interpret the data. In both of the previous studies involving rats, the original weight

of the rats was similar (approx. 60 g). Differences in results cannot therefore be dismissed for this reason.

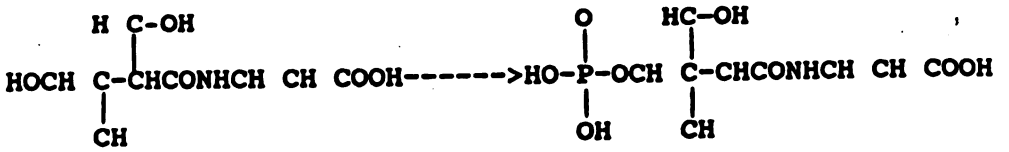
Carter and Hockaday (1962) found a significant decrease in the concentration of total carcass lipids in PA deficient animals as compared to supplemented animals. They further found that this decrease was uninfluenced by the level of fat in the diet (5% fat vs 24 % fat by weight), a theory also suggested elsewhere (Williams et al., 1968). Differences in body weight were also significant between PA deficient and supplemented groups, but relatively unaffected by whether the diet was high or low fat. No conclusive evidence to support or disprove the theory of a high-fat diet more rapidly inducing PA deficiency has yet been produced.

4) Synthesis and Degradation of Coenzyme A and Acyl Carrier Protein

Coenzyme A (CoA), synthesized from PA which must be obtained from dietary sources (Robishaw and Neely, 1985), is an essential cofactor for numerous enzyme reactions. Phosphopantetheine of acyl carrier protein is another metabolically active form of PA. The biosynthesis of CoA from PA also requires ATP and cysteine. PA is phosphorylated to 4'-phosphopantothenic acid by the action of pantothenate kinase. This is the rate limiting step in CoA formation, as pantothenate

kinase is inhibited by 4'-phosphopantetheine, CoA and acetyl CoA (Abiko, 1975; Halvorsen and Skrede, 1982)). 4'-phosphopantothenic acid and cysteine are then converted to 4'-phosphopantothenoyl-cysteine. This reaction is catalyzed by 4'-phosphopantothenoyl-cysteine synthetase. Next, a dicarboxylase enzyme converts 4'-phosphopantothenoyl-cysteine to 4'-phosphopantetheine. These first three enzymes are located exclusively in the cytosol (Skrede and Halvorsen, 1979). In the following two steps in this pathway, 4'-phosphopantetheine is adenylated to form dephospho-coenzyme A which is phosphorylated at the 3' position of ribose to form CoA. These last two reactions mediated by dephospho CoA pyrophosphorylase and kinase respectively, are present in both the cytosol and the mitochondria (Novelli et al.,1949; Brown, 1958; Robishaw and Neely, 1985; Abiko, 1975).

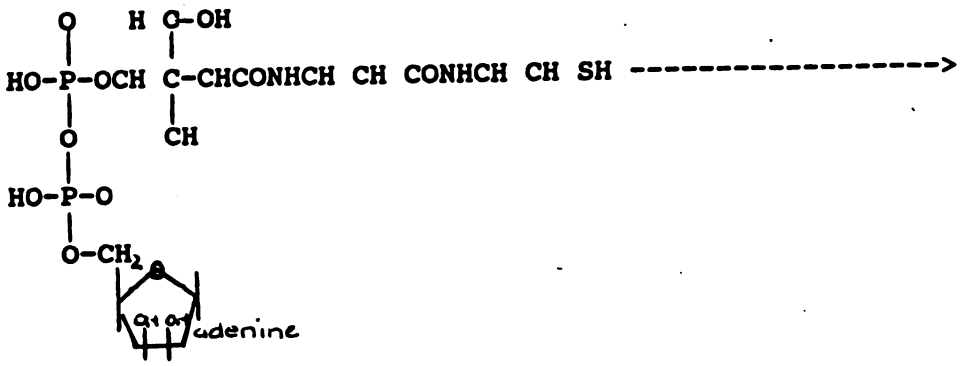
The proposed route of degradation of CoA is the reverse of the sythesis up to the point of 4'phosphopantetheine. 4'-phosphopantetheine is thought to breakdown to pantetheine, and to PA and cysteamine. The cysteamine is further degraded to hypotaurine and taurine. The fate of PA as a result of CoA degradation is unknown. It may reenter the synthetic pathway or be excreted (Abiko, 1975; Robishaw and Neely, 1985). There are some problems with the proposed route of degradation of CoA, one of which is that the majority of CoA is found in the mitochondria, but the first degradative enzyme is lysosomal



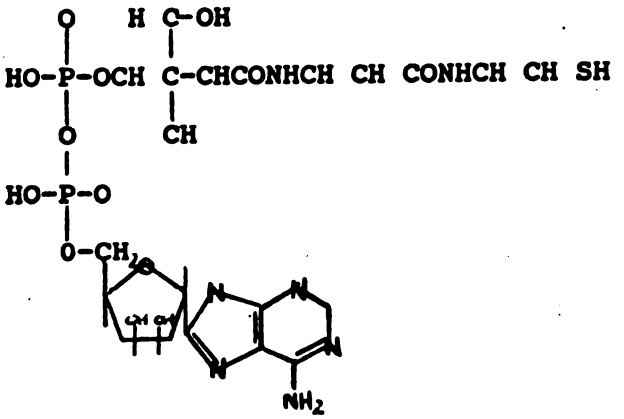
Pantothenic acid 4'-phosphopantothenic acid

4'-phosphopantothenyloyl-l-cysteine

4'-phosphopantetheine



dephosphoCoA



CoA

FIGURE 1: SYNTHETIC PATHWAY OF COENZYME A

(Brown 1958, Abiko,1975).

(Robishaw and Neely, 1985).

Experimentally, PA can be released from CoA by incubation of the CoA with the enzymes pantetheinase and alkaline phosphotase. (A step by step protocol can be found in Wyse et al., 1985.) This enzymatic treatment to release the bound form is essential for PA determination by means of RIA as this procedure can only measure free PA (Wyse et al., 1979). Most PA in biological materials exist as CoA. In acyl carrier protein, phosphopantetheine is linked to serine via a phosphodiester bond (Majerus et al., 1965) and even with enzymatic treatment, the pantothenate in acyl carrier protein will not be released.

5) Biochemical Role of Pantothenic Acid and CoA in Carbohydrate and Fat Metabolism

Dietary deficiency of PA in animals results in a wide spectrum of biochemical defects that eventually manifest themselves as signs described previously. Abiko (1975) has listed more than 70 catalytic reactions that involve CoA or phosphopantetheine as cofactors. The physiological role of PA as a component of CoA, is important in the release of energy from carbohydrates, in gluconeogenesis, in the synthesis and degradation of fatty acids, in the synthesis of ketone bodies, as well as in the synthesis of sterols and steroid hormones, porphyrins and other compounds (Goldman and Vagelos, 1964, Abiko 1975).

The role of PA in carbohydrate metabolism comes from the breakdown of glucose to ATP via glycolysis and the citric acid cycle. At the end of glycolysis, pyruvate combines with CoA to form acetyl CoA which can then be incorporated into the citric acid cycle. In the tricarboxylic acid cycle, CoA functions as an acyl acceptor for the pyruvate and alpha-ketoglutarate dehydrogenase complexes forming acetyl CoA and succinyl CoA respectively. In gluconeogenesis, PA again as a component of CoA, is necessary for the activity of the pyruvate carboxylase enzyme which converts pyruvate to oxaloacetate (OAA), as the first step in synthesizing glucose from pyruvate. Biotin, a needed cofactor in the conversion of pyruvate to OAA, is not carboxylated (a necessary step in the conversion) unless acetyl CoA (or acyl CoA) is bound to the enzyme pyruvate carboxylase. Pyruvate carboxylase also plays a critical (anapleurotic) role in maintaining the level of citric acid cycle intermediates at appropriate levels (Stryer, 1975).

Fatty acid synthesis, which takes place in the cytosol, requires PA as a component of acyl carrier protein (ACP). The fatty acid chain is elongated (starting with malonyl ACP) by the sequential addition of two carbon units derived from acetyl CoA (Stryer, 1975).

Fatty acids are oxidized in the mitochondria. Prior to entry into the mitochondria, the fatty acids

are activated in a reaction involving CoA. These activated acyl CoAs are then transported inside the mitochondria by means of a special transport mechanism involving carnitine. Once inside the mitochondria, the acyl CoA compound is degraded by a recurring sequence of B-oxidation involving CoA. The final product of the degradative pathway is acetyl CoA for even numbered fatty acids or propionyl CoA for odd numbered fatty acids. Both these products can be incorporated in the citric acid cycle and used for energy (Stryer, 1975). If excess fatty acids are broken down to acetyl CoA, ketone bodies will be formed. These ketone bodies also involve CoA in their synthesis. Acetyl CoAs are converted to acetoacetate which is further converted to hydroxybutyrate and acetone. Acetyl CoA is the carbon source for the biosynthesis of prostaglandins, cholesterol, steroid hormones and other compounds. Succinyl CoA, a tricarboxlic acid cycle intermediate, is an essential precursor for porphyrins and hence for hemoglobin and cytochromes.

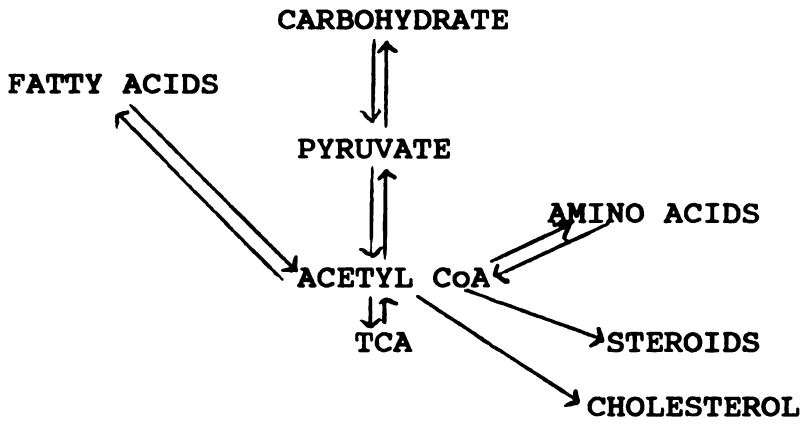


FIGURE 2: PANTOTHENATE DERIVATIVES MEDIATE CARBOHYDRATE, LIPID AND AMINO ACID METABOLISM

6) Pantothenic Acid Status and Plasma Triglyceride Levels

Triglycerides are highly concentrated stores of metabolic energy. For triglycerides to be utilized as energy they must first be broken down into glycerol and free fatty acids. Glycerol can be converted into pyruvate or glucose in the liver, while free fatty acids are degraded by beta-oxidation (which requires CoA) to acetyl CoA which can then be incorporated into the citric acid cycle (Stryer, 1975). Triglycerides from the diet are transported by chylomicra from the intestine to adipose tissue where they are stored until needed, while triglycerides synthesized endogenously are carried by very low density lipoproteins (VLDL) to the adipose tissue. The fate of exogenous fatty acids may in part be regulated by the cytosolic carnitine to CoA ratio (Idell-Wenger et al., 1978; Idell-Wenger and

Neely, 1978; McGarry et al., 1975). Changes in the CoA levels result in a concomitant change in PA metabolism. An inadequate supply of PA has been shown to raise blood triglyceride and free fatty acid levels in rats fed a high fat diet. After two weeks on a high fat diet (30% of kcal) with graded amounts of PA (0 mg/kg diet to 1600 mg/kg diet), Peterson et al. (1987) observed significant differences in rat serum triglycerides and free fatty acids (FFA). Those animals receiving less PA in their diets had the higher serum triglycerides and FFAs. The greatest differences in triglyceride levels were observed to be between the deficient (0 mg/kg) and the normally supplemented (16 mg/kg) groups suggesting a vitamin related effect rather than a pharmacologic one. Triglyceride differences were found to be significant long before weight loss and other conventional signs of deficiency appeared in this study. In the high fat PA deficient group, triglyceride levels were subsequently reduced with supplemental pantothenate (amount not indicated) or pantethine.

Pantethine is a derivative of PA which contains PA and cysteamine. It can be converted biochemically into CoA and is used in some countries as a natural hypolipemic drug to decrease total serum cholesterol and triglycerides. The cholesterol lowering effect has been shown to be due to the cysteamine fragment of pantethine (Wittwer et al., 1985). Although still unproven, it is

widely assumed that the hypotriglyceridemic effect of pantethine is as a result of increased levels of pantothenate derivatives (i.e. as a result of the PA portion of pantethine). Proposed mechanisms for the hypotriglyceridemic effect include fatty acid oxidation stimulation (Kameda and Abiko, 1980), activation of the citric acid cycle (Prisco et al., 1984) and lipoprotein lipase activation (Noma et al., 1984).

Hypertriglyceridemia can be caused by increased secretion of VLDL by the liver, decreased degradation by lipoprotein lipase or both. As PA is incorporated into CoA which is extensively involved in lipid metabolism, certain reactions may be suppressed by an inadequate supply of CoA as a result of insufficient PA in the diet. This could result in elevated serum triglycerides.

7) Tissue Pantothenic Acid and CoA Levels

Levels of PA acid and CoA have been measured extensively in the liver, heart, and kidney of rats for a variety of reasons, using a number of different experimental conditions. In general, it has been concluded that when animals are fed on a diet deficient in PA, tissue levels of the vitamin will drop 70% to 90% (Reibel et al., 1982) while CoA levels in the same tissue remain relatively unchanged from the norm (liver 250-450 nmol/g wet tissue; heart 130-180 nmol/g; kidney 120-300 nmol/g) as seen in tables 1-3 below (Hatano,

1962; Reibel et al., 1981, 1982; Moiseenok et al., 1986).

Although tissue levels of CoA remain fairly constant in a PA deficient state, the distribution of CoA between free and acyl ester forms may vary over a wide range. The ratio of free CoA to acyl CoA is important in determining the rate of a number of key metabolic reactions including those catalyzed by acyl CoA synthetase (activates fatty acids prior to entering the mitochondria) (Oram et al., 1973) pyruvate dehydrogenase (pyruvate to acetyl CoA) and alphaketogluterate dehydrogenase (alphaketogluterate to succinyl CoA) (Rabinowitz and Swift, 1970). One suggested explanation for the observed lack of change in CoA levels in a PA deficiency is that the organs of the body normally contain a large excess of PA (Reibel et al., 1982).

In tables 1-3 below, it can be seen that there is alot of variation in reported values of CoA and PA in the tissues. Explanations for this variability include differences in age and strain of rats, as well as individual differences among animals and differences in methodology between different laboratories.

Livers of rats fed PA deficient diets for 4 weeks, contain about 30% of normal levels of free PA (Reibel et al., 1982). Hatano (1962) measuring 9 different tissues found that liver had the highest value of PA in the

TABLE 1: REPORTED LIVER PANTOTHENIC ACID CONCENTRATIONS: TOTAL AND FREE (nmol PA/g wet tissue)

Treatment	Total	Free	Reference
Control	388 ± 54	11.3 ± 4.4	Smith et al 1978
Control	251 ± 46	27.2 ± 3.3	Smith 1978
Control	264 ± 12		Israel and Smith 1987
Control	445 (97.5 ug/g)	183 (40 ug/g)	Hatano
Deficient	217 (47.5 ug/g)	137 (30 ug/g)	1962
Control	477 ± 15 *	26 ± 3 *	Reibel et
Deficient	400 ± 21 *	7 ± 1 *	al.,1982

all numbers represent mean ± std dev
* values presented as nmol PA/ g dry tissue

TABLE 2: REPORTED HEART PANTOTHENIC ACID CONCENTRATIONS: TOTAL AND FREE (nmol PA/g wet tissue)

Treatment	Total	Free	Reference
Control	130 ± 15	94 ± 21	Smith 1978
Control	138 ± 36	36 ± 6	Smith et al 1978
Control	141 ± 10	58 ± 10	Israel and Smith 1987
Control	180 (39.5ug/g)	73 (16 ug/g)	Hatano
Deficient	85 (18.5 ug/g)	48 (10.5ug/g	1962
Control	617 ± 26 *	284 ±14 *	Reibel et
Deficient	630 ± 15 *	32 ± 2 *	al., 1982

all numbers represent mean ± std dev
* values presented as nmol PA/ g dry tissue

TABLE 3: REPORTED KIDNEY PANTOTHENIC ACID CONCENTRATIONS: TOTAL AND FREE (nmol PA/g wet tissue)

Treatment	Total	Free	Reference
Control	128 ± 4	31 ± 9	Israel and Smith 1987
Control	324 (71 ug/g)	173 (38 ug/g)	Hatano
Deficient	176 (38.5 ug/g)	130 (28.5ug/g)	1962
Control	474 ± 49 *	322 ± 35 *	Reibel et
Deficient	489 ± 52 *	27 ± 11 *	al., 1982

all numbers represent mean ± std dev
* values presented as nmol PA/ g dry tissue

animals on control diet (457 nmol/g). In more recent studies, heart has been found to maintain higher pools of PA and lower total CoA content than liver (Smith, 1978). Further study has quantified this observation, reporting that heart (total organ) contains about ten times as much free PA as liver (Reibel et al., 1981). This relates in part to the different functions of the two organs with respect to fat matabolism. In heart, the major fate of fatty acids is oxidation. This correlates well with the fact that cytosolic total CoA levels are low compared with those of carnitine (Lopaschuk et al., 1986). In liver, cytosolic CoA levels are much higher and fatty acid conversion to complex lipids is a more prominent function of the tissue (McGarry et al., 1975).

The kidney conserves whole body PA through increased reabsorption (Reibel et al., 1981) when dietary intake of PA is low. It is further believed that this reabsorption is an active process at physiological plasma concentrations and, that at higher concentrations of PA, tubular secretion of plasma PA occurs (Karnitz et al., 1984). When rats were fed PA deficient diets for 4 weeks, CoA levels were maintained in the kidney tissue, while free PA content was reportedly reduced from 35-40% (Carter and Hockaday, 1962) to 80% (Reibel et al., 1981). Possible explanations for this phenomenon are binding of PA to

the plasma proteins and/or tubular reabsorption of PA by the kidney. Since no specific plasma protein binding of PA was found, it appears that conservation mechanism for free PA in the rat is through tubular reabsorption (Karnitz et al., 1984). Tubular reabsorption of PA has also been observed in dogs (Taylor et al., 1974). It thus appears that two processs regulate excretion of PA in the kidney, tubular reabsorption and tubular secretion.

Differences were also noted in the PA content of the organs between animals fasted prior to sacrifice versus those fed right up until sacrifice (Table 4). Fasting PA deficient animals resulted in increased PA levels in heart, liver and kidney as compared with non-fasted deficient animals (Reibel et al., 1981, 1982; Smith et al., 1978). These researchers similarly found the levels of pantothenate to rise in fasted versus fed control rats. Smith et al. (1978) postulated that the higher PA acid content in the liver of fasted versus fed rats may reflect an influx of PA from other tissues not being measured. Another possible explanation is that the increase in fasting CoA levels arises from the drastic decrease seen in the level of fatty acid synthase in the liver during fasting. Since the enzyme contains 4′-phosphopantetheine as a prosthetic group, degradation of the enzyme could release 4′-phosphopantetheine which could subsequently be converted

TABLE 4:REPORTED LIVER PANTOTHENIC ACID CONTENT- FASTED vs FED
nmol PA/ g wet weight tissue

Group	PA Supplemented	PA Deficient	Reference
Fed	(26 ± 3) *	(7± 1)*	Reibel et al.
Fasted (48h)	(46 ± 4) *	(18± 3) *	1981, 1982
Fed	4.8 ±.5		Israel and Smith
Fasted (24h)	3.6 ±.6		1987
Fed	6.3 ±.6		"
Fasted (24h)	4.4 ±.6		
Fed	3.3 ±1.1		Smith et al.
Fasted (21h)	5.2 ± 2.9	meal fed	1978
Fasted (21h)	11.3 ±4.4	ad lib	"

* (nmol/g dry wt)
numbers represent mean ± std dev

to PA (Reibel et al., 1982). (The authors (Reibel et al.) did not measure the activity of fatty acid synthtase). Israel and Smith (1987) have shown higher liver PA levels in fed versus fasted control rats. One explanation for this discrepancy is the differences seen in the length of time the animals were fasted prior to sacrifice (24 vs 48 hrs) and in the type of diet they were consuming during this time (meal fed vs ad libitum). Treatment of the tissue samples was similar in all studies.

8) Urinary Excretion of Pantothenic Acid

PA excretion in the urine is generally accepted as an indication of nutritional status with regard to this vitamin (Tao and Fox, 1976). A number of studies (Srinivasan et al., 1981, Fox and Linkswiler, 1961, Oldham et al., 1946; Cohenour and Calloway, 1972; Song et al., 1984) looking at both intake and excretion of PA in humans, suggest that urinary excretion of PA is related to dietary intake. It has been shown that a certain amount of excretion represents minimal daily requirements of PA for tissue maintenance (Fox and Linkswiler, 1961) although no specific quantity has been identified at this time. These studies also suggest that little or no bound PA appears in the urine.

Similar results have been found in rats with respect to urinary excretion correlating well with dietary intake (Hatano, 1962). It has been observed as

well, that urinary excretion of PA increases directly with the age of the rat fed ad libitum (Nelson et al., 1947; Tao and Fox, 1976) PA deficient or supplemented diets. This is consistent with the theory that the PA requirement of the rat decreases with age (Henderson et al., 1942; Unna and Richards, 1942). In these studies, all experimental diets were deficient in PA, therefore increased urine excretion reported with age could not be as a result of increased food intake and thus increased PA intake. Many studies have measured the urinary PA excretion in rats (Hatano et al., 1967; Hatano, 1962; Nelson et al., 1947; Tao and Fox, 1976; Reibel et al., 1981). A marked decrease in urinary PA was observed 11 days after initiation of a PA deficiency experiment in young weanling rats. The excretion rate levelled off at 1 ug/day after 3 weeks on the deficient diet (Hatano, 1962). A summary is found in Table 5.

Although different methods were used to determine urine PA levels (microbiological assay or RIA), good correlation between these techniques has been documented (Wyse et al., 1979; Srinivasan et al., 1981). Urinary PA levels seem to be by far the most indicative parameter when determining short term dietary sufficiency of PA although further information is still needed to relate urinary PA values to blood and tissue PA concentrations.

TABLE 5 :REPORTED PANTOTHENIC ACID VALUES OF RAT URINE

DIET weight	PA IN DIET ug/100g	FOOD INTAKE g/day	URINARY PA ug/24 hrs	REFERENCE
68% sucrose 17% casein 10% fat	0	N/A	1.0	Hatano 1962
basal	N/A	N/A	32.1±6.1	Hatano et al. 1967
24% casein 64% sucrose 8% fat	0	N/A	1.6	Nelson et al. 1947
68.8% sucrose 20% casein 5% fat	0	8.5	2.4	Tao and Fox 1976
"	200	12.2	5.0	"
"	2000	15.6	69.6	"
N/A	N/A	N/A	250 ± 37	Reibel et al. 1981
	20		.67 wk1 1.0 wk2 .60 wk3 1.4 wk4 2.2 wk5 1.7 wk6 1.3 ug/24 hr average	Henderson et al. 1942

N/A = not available

9) Reported Values for Blood Pantothenic Acid

Generally, the vitamin content of blood is influenced by the amount in the diet. In the case of some vitamins, level in the blood is used as a criterion of the adequacy of intake. Many human studies have been done to determine the PA levels of whole blood and serum (or plasma) in hopes of defining such a criterion to distinguish adequate from inadequate PA intake in the diet or PA status in the person in general. The results of these studies have produced a wide range of numbers. In humans, serum is known to contain only small amounts of PA all in the form of free PA (Wyse et al., 1985). Sauberlich et al., (1974) suggested that a total level of PA less than 5 nmol/ml of whole blood may be indicative of low levels of PA in the diet in human subjects. Baker and Frank (1968) also with humans, suggested a level of less than 0.7 nmol/ml whole blood as a hypovitaminosis. This large variation may be due in part to variations in technique between laboratories. Cohenour and Calloway (1972) failed to relate dietary intakes with blood levels of PA in their study of pregnant teenagers. Srinivasan et al., (1981) also found no correlation between PA intake and blood levels of PA in an elderly population. Even when PA supplementation was used, no correlation was seen between PA intake and blood PA concentrations (Srinivasan et al., 1981). This might be the result of

excretion of all excess PA being consumed. Fry et al., (1976) found that although blood PA levels decreased in unsupplemented subjects and remained constant in supplemented subjects, in general, blood PA levels responded less readily to intake than did urinary PA levels.

In animal studies, (rats and chickens) Pearson et al., (1946) reported that the level of PA in the whole blood, the plasma, and the cells is influenced by the amount of PA in the diet. Pearson (1941) reported that from 44 - 62% of the PA in blood occurs in the plasma. Published data on the concentrations of PA in the serum show large variations, even within the same species (Robinson, 1966). These variations may be due to the methods used to assay PA, differences in sample preparation or due to genuine large variations existing within and among species. RIA determination of PA in blood seems to give more consistent results than previously obtained with other methods as shown by the results of Israel and Smith (1987), and Reibel et al. (1981, 1982) in Table 6. Whole blood values range from 2.28 nmol/ml to about 4.57 nmol/ml whereas serum values vary modestly from 1.5 nmol/ml to 1.75 nmol/ml (Table 6). Many of the studies reporting PA levels in the whole blood and serum fail to provide information on PA intake, making interpretation of their findings difficult and creating problems when values from

TABLE 6:REPORTED PANTOTHENIC ACID VALUES OF RAT WHOLE BLOOD AND SERUM

PA/DAY INTAKE	PA in WHOLE BLOOD nmol/ml	PA in SERUM nmol/ml (PLASMA)	REFERENCE
N/A	2.57±.32 *	(1.44± .26)*	Hatano et al.,1967
390ug/day	4.84±1.64 *		Israel and Smith 1986
"	2.46±.32 *		"
" (fasted 24 hr)	4.15± 1.00 *		"
"	4.75± .55 *		"
N/A		1.75± 0.17	Reibel et al. 1981
50 mg PA/ kg diet		1.54±0.30	Reibel et al. 1982
0 mg PA		undetected	"

* units converted from ug/ml
numbers represent mean ± std dev

different studies are compared.

10) Brief Review of Methods

PA concentrations of tissue, blood, or foodstuff can be determined by means of radioimmunoassay (RIA). D-pantothenic acid is conjugated with bovine serum albumin by use of a bromoacetyl derivative of PA, and antibody to this antigen is raised by injecting it into footpads of rabbits. A 1:100 dilution of this resulting antiserum is incubated with radiolabelled PA. The antibodies are precipitated and dissolved, and the radioactivity of the solution measured in a liquid scintillation counter (Wyse et al., 1979). By using known amounts of labelled and unlabelled PA in the RIA, a standard curve can be derived based on the percent binding of the unlabelled PA. This curve can be used in determining the amount of PA in biological samples being analyzed.

METHODS

This study included 2 experiments:

Experiment 1:

This experiment was undertaken to measure daily PA excretion in urine of rats fed one of three diets: high-fat PA deficient (67.5% kcal from fat); high-CHO PA deficient (AIN-76 diet 67.5% kcal from CHO);high-CHO PA supplemented (312 ug PA/ 100 kcal;Appendix A). Fifteen weanling Sprague-Dawley rats (Harlan Sprague-Dawley Inc. Indianapolis, Indiana) were randomly divided into equal groups (5 rats per group), housed individually in metabolism cages, and fed one of the three experimental diets described above. Twenty-four hour urine samples were collected daily in the mid morning hours. Each urine sample was diluted to 50 ml with distilled water and 100 ul of this fresh solution was then used to determine PA content of the urine daily by means of radioimmunoassay (Wyse et al, 1979). Food intake was measured daily and body weights were recorded weekly (Appendix B).

After a one week depletion period, all groups were fed a stock diet for repletion (Wayne Research Animal Diet, Chicago, Illinois). This was done to observe if differences could be detected between fat and CHO PA deficient groups with respect to repletion of PA. Nine days after the beginning of the repletion period, the

rats were again randomly divided into three groups and fed the three experimental diets as above. The redepletion phase of the experiment was planned for a week because the rapid decline in PA in the urine observed in the first depletion period had not previously been reported in the literature.

Experiment 2:

In this experiment, eighty-seven male weanling Sprague-Dawley rats were randomly divided into four treatment groups: high-fat PA deficient; high-CHO PA deficient; high-fat PA supplemented pair fed to high-fat PA deficient; high-fat PA supplemented ad libitum (diet composition as in the first experiment). PA supplemented groups received 312 ug PA/ 100 kcal (17 mg PA/kg high-fat diet; 12 mg PA/kg high-CHO diet). Rats were housed individually in wire bottom cages.

At weeks 0.5, 1.5, 2.5, and 4.0 five rats per group were killed by exsanguination via cardiac puncture and liver, kidneys and heart were removed at this time. Organs were rinsed in 0.9% saline and weighed. Heart was divided into two equal portions approximately 0.3 g each, to measure total and free PA content. One kidney was used for measuring total PA and the other kidney was used to measure free PA. Two liver pieces, weighing approximately 0.5 g each, were used for these two determinations. These tissues were then homogenized using the Brinkmann polytron at speed setting 3 for one

minute. Kidney and heart were homogenized with 0.5 ml of distilled water, while 1.0 ml of distilled water was used for liver.

Tissue samples homogenized for free PA measurements were immediately deproteinized with equimolar saturated $Ba(OH)_2$ and 10% $ZnSO_4$ (approximately 300 ul total volume, the ratio being determined by titration using phenolphthalein as an indicator) and centrifuged at 4,000 x g for 10 minutes in a Sorvall Superspeed RC2-B centrifuge. Supernatants were stored at -20^{o} C. Tissue samples to measure total PA were treated with an enzyme mixture containing 30 units alkaline phosphatase and 15 units pantetheinase in phosphate buffered saline (PBS). The optimum amount of enzyme needed for each sample to release PA bound as CoA had previously been determined in this laboratory. These samples were then incubated overnight (8-10 h) at 37^{o} C.

Whole blood was separated into two portions. One portion was divided into packed cells and plasma by centrifugation at 4,000 x g for 10 minutes in a Sorvall Superspeed RC2-B centrifuge and the other portion was saved as whole blood. Whole blood was hemolyzed by rapidly freezing and thawing the samples three consecutive times. Two 50 ul aliquots of the hemolyzed blood was used for measurement of total and free PA. The aliquot for free PA was immediately deproteinized with equimolar saturated $Ba(OH)_2$ and 10% $ZnSO_4$

(approximately 200 ul total volume). The aliquot for total PA measurement was treated with 10 units of alkaline phosphatase and 20 units of pantetheinase in PBS prior to deproteinization. Plasma was immediately deproteinized and all the supernatants were stored at -20^{o} C.

Fifty ul of tissue sample supernatant and 100 ul of blood supernatant were analyzed for PA content by means of RIA. Plasma triglyceride concentrations were measured by means of colorimetric determination at 410 nm (Fletcher, 1968). Statistical analysis was performed in both studies using ANOVA followed by the Bonferroni t-test (Miller, 1977) which allow any number of contrasts to be compared using the type I error rate based on all the contrasts.

RESULTS

In experiment 1, it was found that PA content of the urine decreased significantly after one day on the experimental diets (Figure 3). At day 8, when all groups were fed stock diet for PA repletion, differences between control and previously deficient groups became significant for the following 2 days. No significant differences were found at any time between the group receiving the high-fat PA deficient diet and the high-CHO PA deficient diet. After switching the two groups on deficient diets onto the stock diet, increased excretion of PA was apparent by the following day. No significant differences were shown between the high-fat and high-CHO deficient groups although excretion was

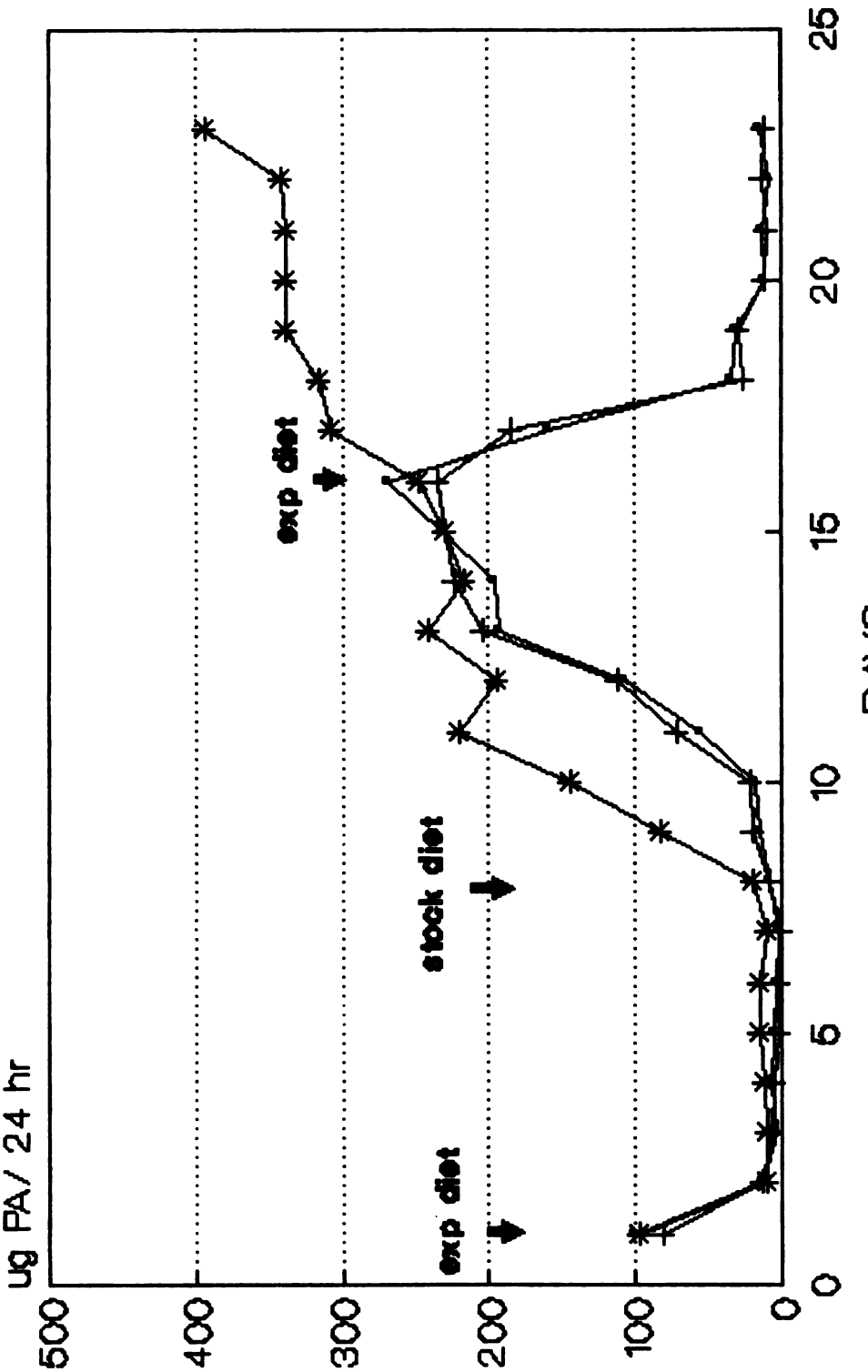


Figure 3: DAILY PANTOTHENIC ACID EXCRETION
Urinary excretion of rats fed a high-fat PA deficient (HF PA-), high-CHO PA deficient (HC PA-), or high-CHO PA supplemented (HC PA+) diet. Supplemented diet contained 312 ug PA/ 100 kcal.

significantly lower in the groups which had been on deficient diets as compared to the group which had been on the control (PA supplemented) diet. Nine days after beginning to consume a stock diet, no significant differences were seen among PA excretion of the three groups (268±45; 234±19; 247±7 ug PA/ 24 hr for high-fat PA deficient, high-CHO PA deficient and high-CHO PA supplemented groups respectively). At day 9, rats were randomly redivided into three treatment groups of 5 rats each, and immediately (the following morning) after receiving a PA deficient high-fat or high-CHO diet, PA excretion began to decrease significantly ($p < .01$) as compared to control values (158±5; 184±27; 307±7 ug PA/ 24 hr). PA excretion stayed significantly lower in the PA deficient groups for the remainder of the study (6 days). Differences in excretion over time were only significant between days 8 and 9 where rats were being switched from experimental diets to stock diet and between days 17 and 18 when rats were being switched from stock diet to experimental diet. In this experiment, weight differed significantly over time, but not among the groups (Appendix B).

As PA excretion in the urine dropped so dramatically in one day in the first experiment, a second experiment was undertaken to measure tissue and blood PA levels and plasma triglyceride concentrations. A dietary deficiency of PA would likely take longer to

affect changes in blood and tissue PA levels. By examining these parameters at frequent intervals beginning 3 days after the initiation of the experiment, any existing differences in PA concentrations as a result of a high-fat or a high-CHO diet would be observed.

Food intake of the high-fat PA supplemented ad libitum fed group was significantly greater ($p<.01$) than all other groups by the third week of the study (Table 7). A difference in food intake between the high-CHO and the high-fat diets was not seen in the PA deficient groups. Body weight increased significantly over time in all four treatment groups: high-fat PA deficient, high-CHO PA deficient, high-fat PA supplemented pair fed to high-fat PA deficient, or high-fat PA supplemented fed ad libiutm (Table 8).

Differences in weight were significant beginning at week 2 in the high-fat PA supplemented ad lib group as compared with high-fat PA deficient group (121 ± 8 vs 111 ± 9 g) and beginning at week 3 in PA supplemented pair fed group as compared with the high-fat PA deficient group (145 ± 12 vs 131 ± 12 g). No significant difference, however, was observed between the two PA deficient groups at any time point measured.

Wet tissue weight of individual organs at time of sacrifice increased significantly over time but did not differ among the groups at any one time (Appendix C).

Table 7 [1]
Weekly Food Intake (kcal) of Rats

Treatment	Week 1	2	3	4
HF PA-	305 ±26	347 ±38	360 ±34	249 ±48
HC PA- [3]	283 ±57	322 ±55	334 ±25	278 ±20
HF PA+ PR FED [4]	305 ±26	347 ±38	360 ±34	249 ±48
HF PA+ AD LIB [5]	304 ±22	373 ±50	471 [2] ±36	473 [2] ±49

1 mean std dev; n=20 at wk 0, 15 at wk 1, 10 at wk 2, 5 at wk 3 & 4

2 $p < .01$ compared to high-fat PA deficient group (HF PA-)

3 HC PA- = high-carbohydrate diet deficient in PA

4 HF PA+ pr fed = high-fat PA supplemented diet (312 ug / 100 kcal) pair fed to HF PA- gp

5 HF PA+ ad lib = high-fat PA supplemented diet (312 ug / 100 kcal) fed ad libitum

Table 8 [1]
Weekly Weights (g) of Rats

Treatment	Week 0	1	2	3	4
HF PA- [3]	40 ±3	80 ±6	111 ±9	131 ±12	131 ±11
HC PA- [4]	39 ±4	76 ±6	103 ±9	127 ±11 [2]	131 ±10 [2]
HF PA+ PR FED [5]	40 ±2	78 ±6	116 ±8	145 ±13 [2]	160 ±23 [2]
HF PA+ AD LIB	39 ±3	77 ±6	121 ±8 *	164 ±8	212 ±13

1
mean std dev; n=20 at wk 0, 15 at wk 1, 10 at wk 2, 5 at wk 3 & 4

2
p < .01 compared to high-fat PA deficient group (HF PA-)

* p <.05 compared to high-fat PA deficient group (HF PA-)

3
HC PA- = high-carbohydrate diet deficient in PA

4
HF PA+ pr fed = high-fat PA supplemented diet (312 ug / 100 kcal) pair fed to HF PA- group.

5
HF PA+ ad lib = high-fat PA supplemented diet (312 ug / 100 kcal) fed ad libitum

By week 1.5, heart and kidney free PA concentration (Figure 4, 5) in both pair fed and ad libitum fed groups consuming diets containing PA had significantly ($p < .01$) greater amounts of free PA than did the high-fat PA deficient group (71±4, 87±11 vs 4±2 nmol PA/ g heart tissue: 135±8, 131±8 vs 27±20 nmol PA/ g kidney). No significant difference was found in free PA content of heart or kidney between high-fat and high-CHO PA deficient groups at any time point measured. No significant differences were seen between pair fed and ad libitum fed PA supplemented groups in heart and kidney free PA content. In liver, only the high-fat PA supplemented ad lib group had significantly greater amounts of free PA at weeks 2.5 (94±20 vs 20±10 nmol PA/ g liver) and 4.0 (49±34 vs 13±5 nmol PA/ g liver) as compared to the high-fat PA deficient group (Figure 6).

Whole blood, total and free PA content (Figure 7), and plasma free PA (Figure 8) showed similar trends to one another. Pair fed and ad libitum fed rats receiving PA in their diets had significantly greater ($p<.01$) amounts of PA than the high-fat PA deficient treatment group by 0.5 week (the first sacrifice time; 2.0±0.5, 1.6±0.9 vs 0.4±0.1 nmol PA/ml blood). No significant differences were seen between the high-fat PA deficient and the high-CHO PA deficient groups in blood or plasma PA content at any time in the study.

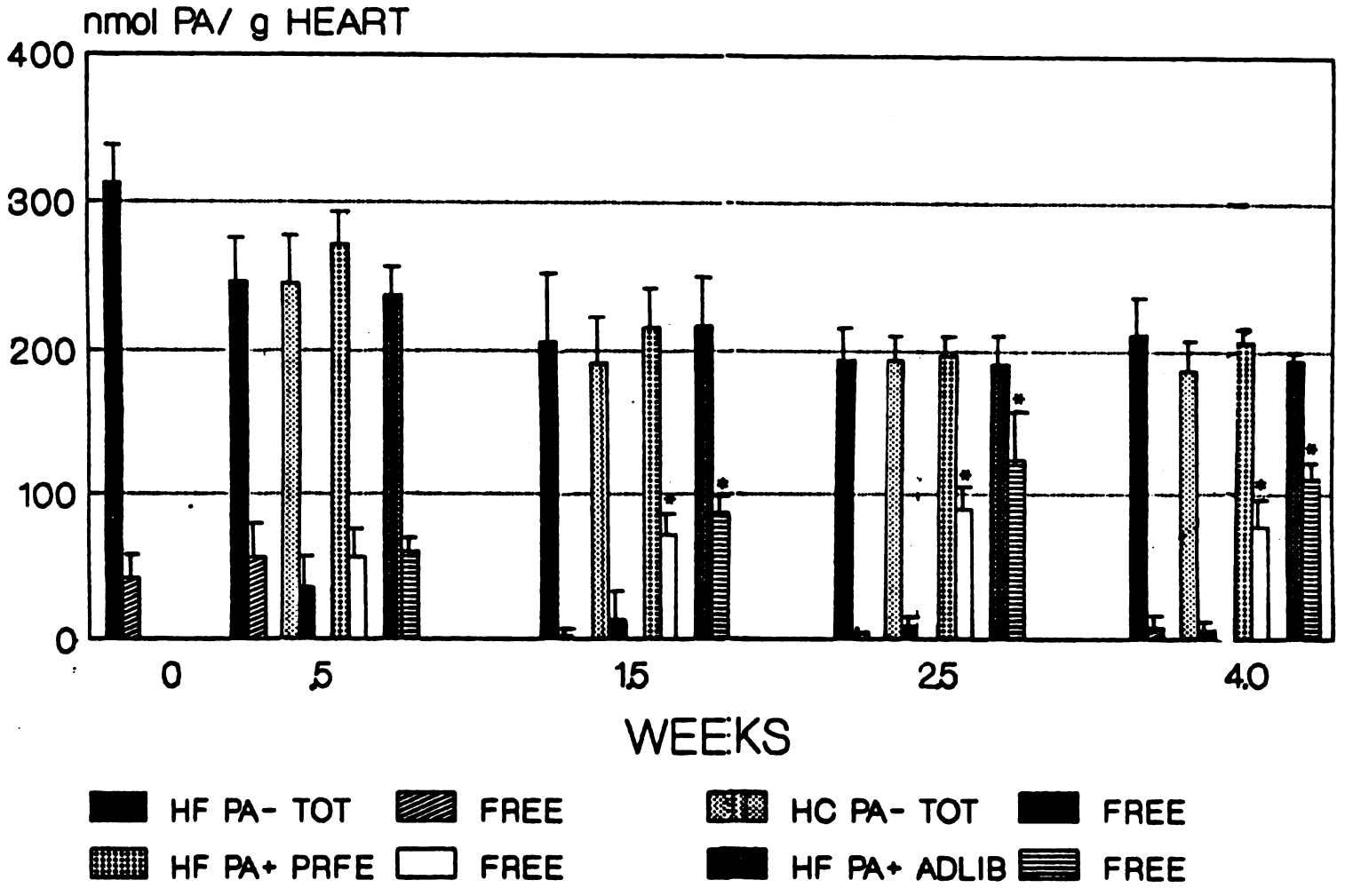


Figure 4: HEART PANTOTHENIC ACID CONTENT: TOTAL AND FREE
Total and free PA content in heart of rats fed high-fat PA deficient (HF PA-), high-CHO PA deficient (HC PA-), high-fat PA supplemented diet pair fed to HF PA- (HF PA+ pr fe), or high-fat PA supplemented diet fed ad libitum (HF PA+ ad lib). Supplemented diets contained 312 ug PA/ 100 kcal. Values represent mean±std dev for 5 animals per group. * p .01 to HF PA- group.

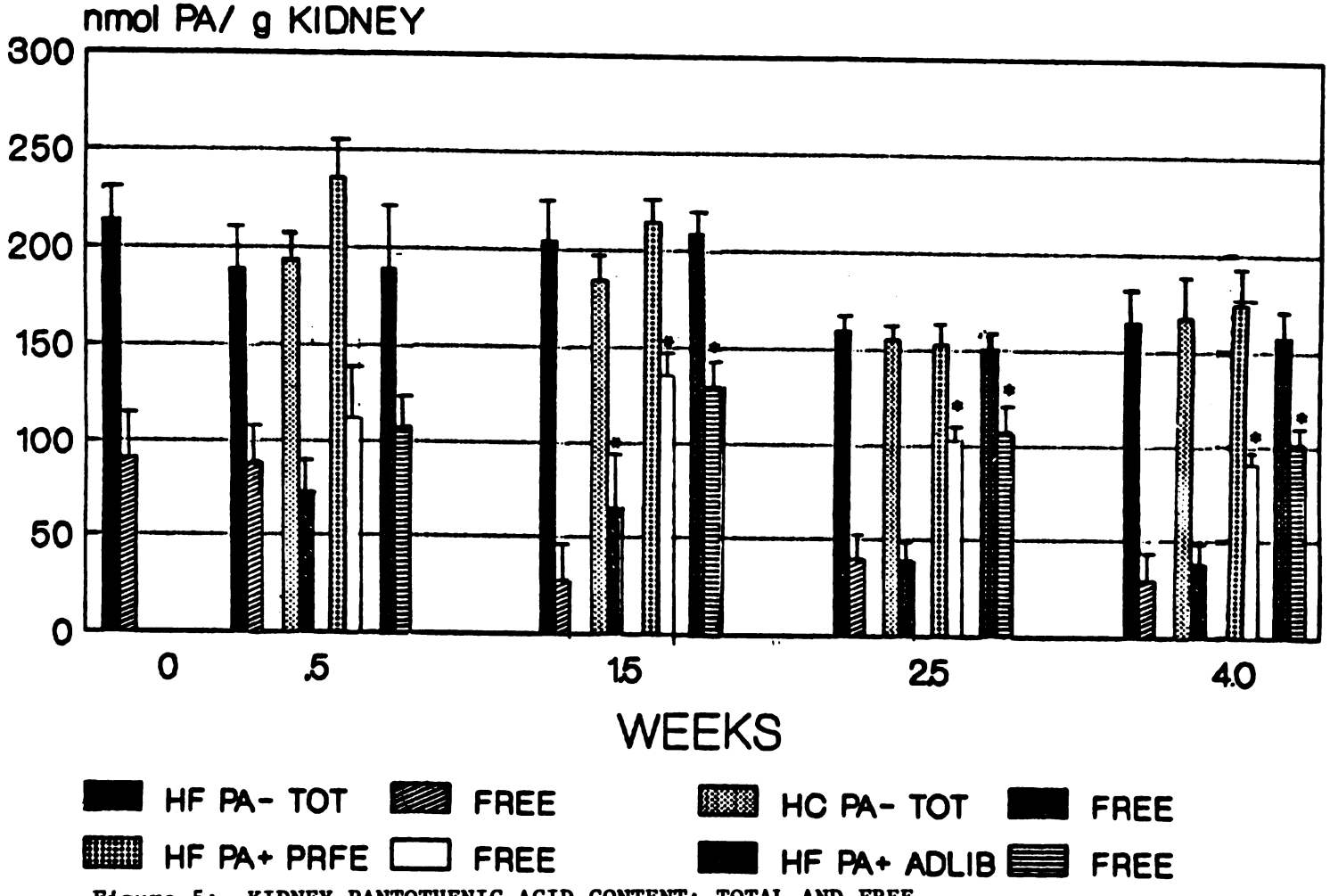


Figure 5: KIDNEY PANTOTHENIC ACID CONTENT: TOTAL AND FREE

Total and fre PA contnet in kidney of rats fed high-fat PA deficient (HF PA-), high-CHO PA deficient (HC PA-), high-fat PA supplemented piar fed to HF PA- (HF PA+ pr fe), or high-fat PA supplemented fed ad lib (HF PA+ ad lib). Supplemented diets contained 312 ug PA/ 100 kcal. Values represent mean±std dev for 5 animals per group. * p .01 to HF PA- group.

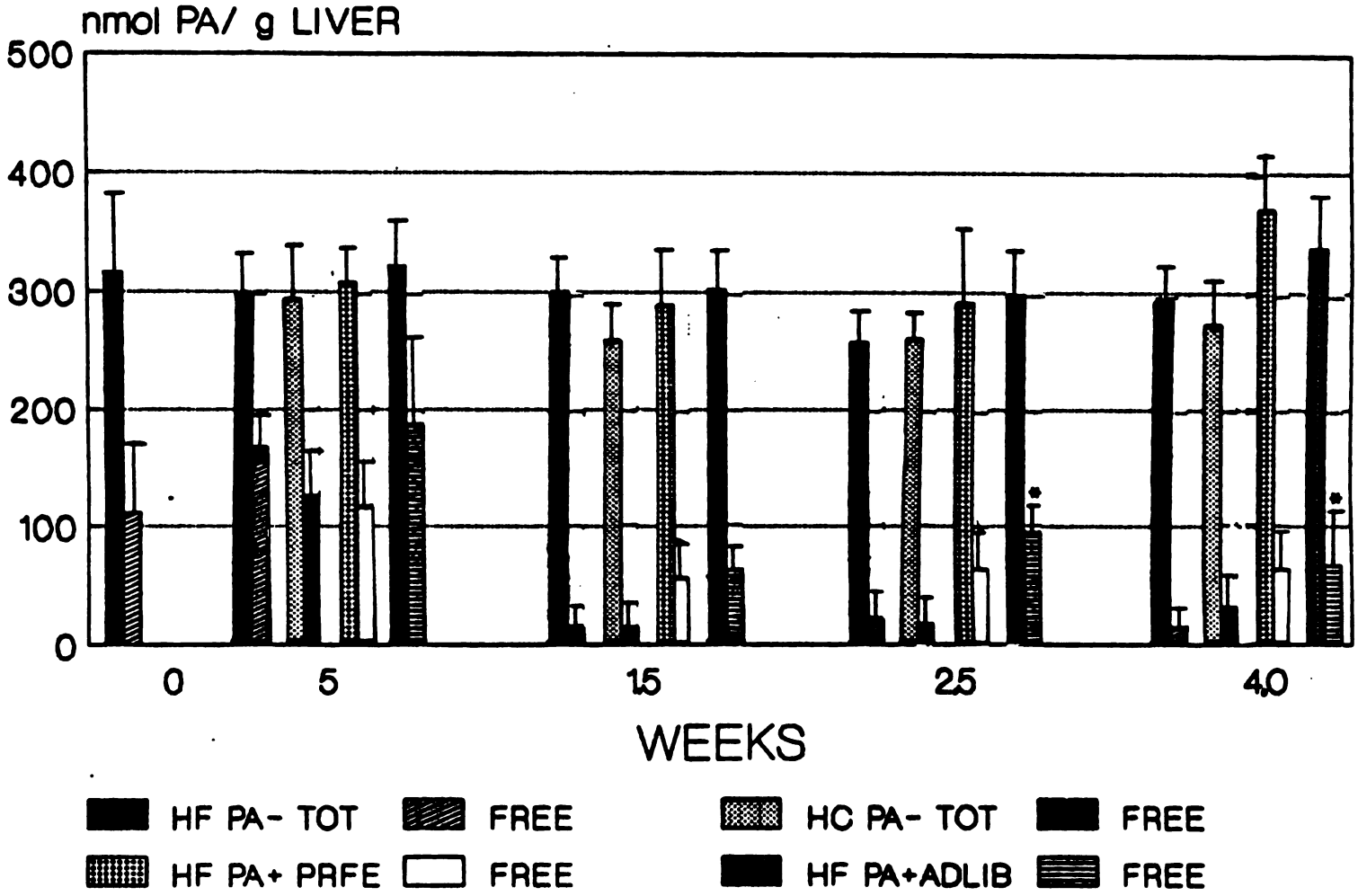


Figure 6: LIVER PANTOTHENIC ACID CONTENT: TOTAL AND FREE
Total and fre PA content in liver of rats fed high-fat PA deficient (HF PA-), high-CHO PA deficient (HC PA-), high-fat PA supplemented pair fed to HF PA- (HF PA+ pr fe), or high-fat PA supplemented ad libitum diet (HF PA+ ad lib). Supplemented diets contained 312 ug PA/ 100 kcal. Values represent mean±std dev for 5 animals per group. * p .01 to HF PA- group.

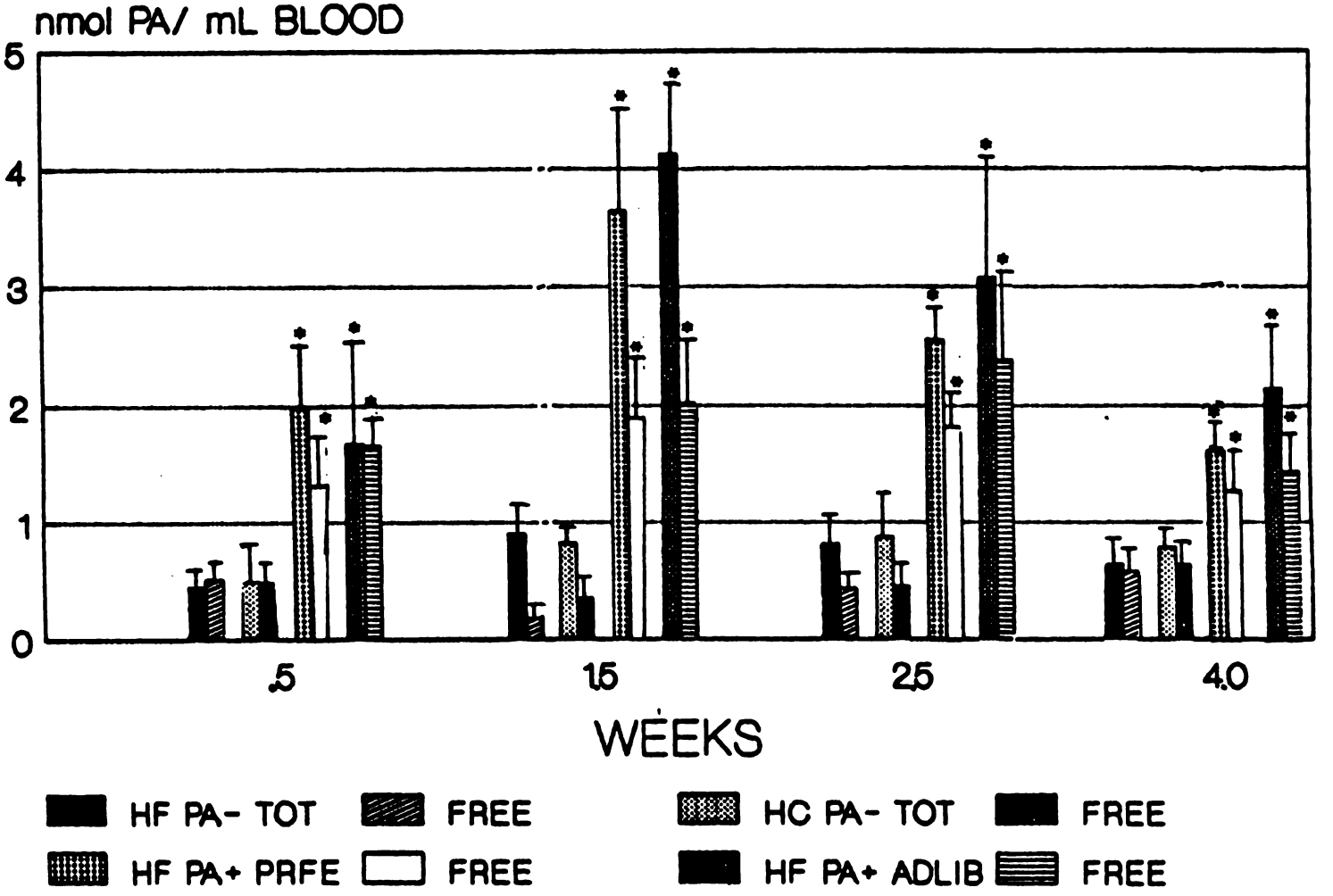


Figure 7: WHOLE BLOOD PANTOTHENIC ACID CONTNET: TOTAL AND FREE
Total and free PA content in whole blood of rats fed high-fat PA deficient (HF PA-), high-CHO PA deficient (HC PA-), high-fat PA supplemented pair fed to HF PA- (HF PA+ pr fe), or high-fat PA supplemented fed ad libitum (HF PA+ ad lib). Supplemented diets contained 312 ug PA/ 100 kcal. VAlues represent mean± std dev for 5 animals per group. * p .01 to HF PA-.

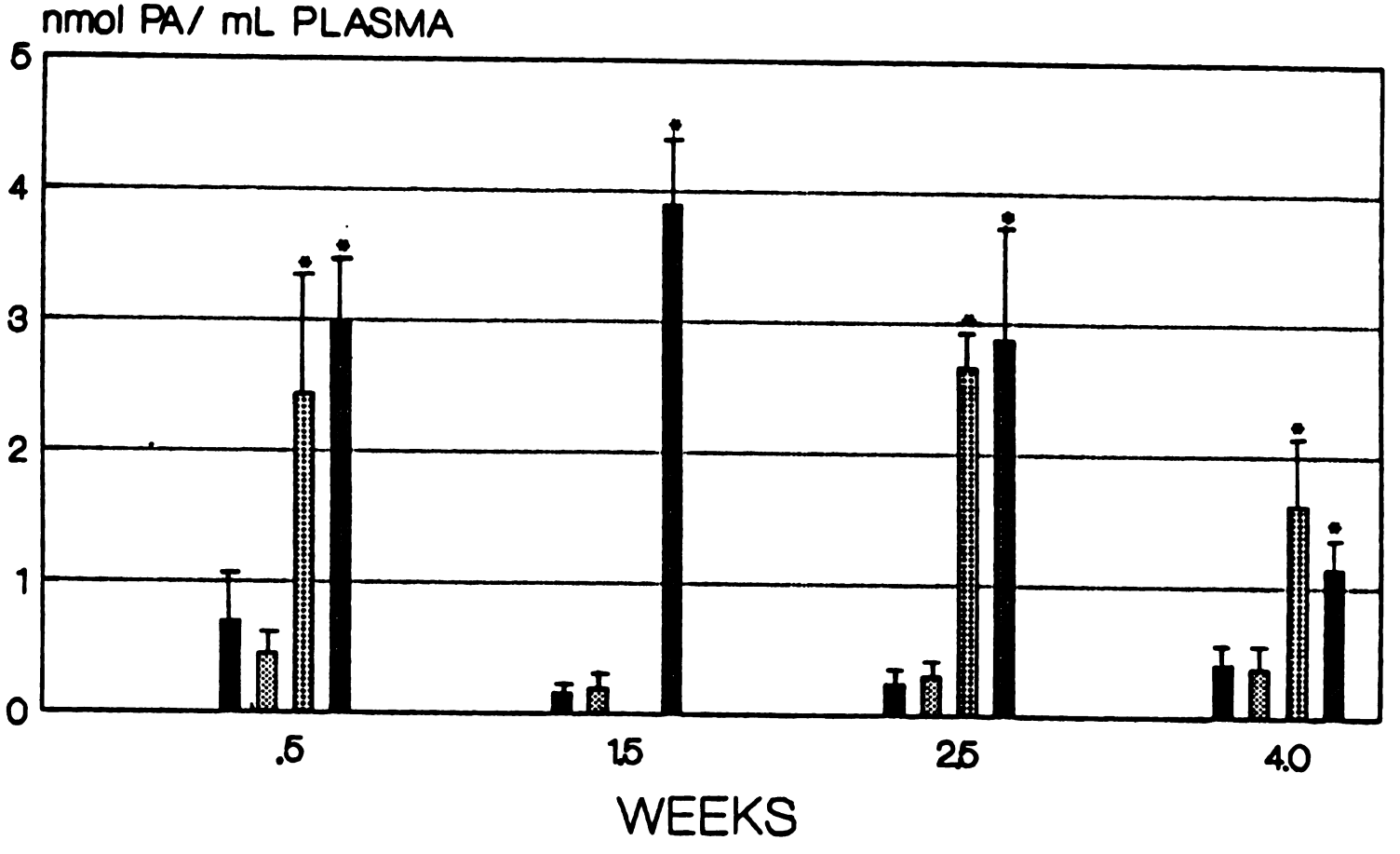


Figure 8: PLASMA PANTOTHENIC ACID CONTENT
Free PA content in plasma of rats fed high-fat PA deficient (HF PA-), high-CHO PA deficient (HC PA-), high-fat PA supplemented pair fed to HF PA- (HF PA= pr fe), or high-fat PA supplemented fed ad libitum (HF PA+ ad lib). Supplemented diets contained 312 ug PA/100 kcal. Values represent mean± std dev for 5 animals per group. * p .01 to HF PA- group.

Previous studies (Beck et al, 1988; Peterson et al, 1987) have reported that plasma triglyceride concentrations are correlated inversely with PA intake at vitamin but not pharmacologic levels when rats were fed a high fat diet. Plasma triglycerides were measured in our study to further examine the relationship between PA intake and its effects on lipid metabolism in rats fed high-fat and high-CHO diets. By week 2.5, high-fat PA supplemented pair fed, high-fat PA supplemented ad lib and high-carbohydrate PA deficient groups all exhibited significantly lower ($p < .01$) plasma triglyceride levels than the high-fat PA deficient treatment group (119±23 in high-fat deficient group vs 59±29, 29±6 and 70±33 mg/dl in the other three respective groups). The plasma triglycerides of both of the high-fat PA supplemented and high-CHO PA deficient groups compared closely to normal plasma triglyceride values in rats (Carlile et al., 1981).

DISCUSSION

Differences in PA status were not found between high-fat and high-CHO PA deficient treatment groups in weanling rats in any parameter measured except plasma triglycerides. Significant differences were found in free PA content of tissue and plasma, and in total and free PA content of whole blood between PA supplemented and PA deficient groups. This suggests that the presence or absence of PA in the diet is more

important with respect to PA status than is the amount of fat or carbohydrate present. These conclusions agree with Williams et al. (1968) and Carter and Hockaday (1962) who concluded that a high fat diet did not increase the PA requirement in rats.

Urinary excretion of PA showed no significant differences at any time in the study between high-fat and high-CHO PA deficient diet groups. Within 1 day, excretion of PA dropped significantly in PA deficient treatment groups, which was a much more rapid response than previously reported by Hatano (1962). This suggests a mechanism in the body sensitive to PA levels. Karnitz et al. (1984) concluded earlier that the only mechanism for conservation of free PA in the rat is tubular reabsorption in the kidney. PA excretion in this study, however, never dropped to a zero level, indicating that a small amount of PA is not reabsorbed. Fox and Linkswiler (1961) observed a similar trend in their humans studies and suggested this as the minimal daily requirement of PA for tissue maintenance.

The lack of significant differences in urinary PA excretion between the control and deficient groups for the first week of the depletion in the study could be the result of low food intake of the semipurified diet by the control group and the rapid growth resulting in a higher PA requirement than that added during this time. As rats age, their requirement for PA decreases (Unna

and Richards, 1943). This can also partially explain the PA excretion pattern of the control group in that as they grow, their requirements decrease and therefore PA excretion increases. While the National Research Council recommends 8 - 10 mg PA /kg diet to be sufficient for growth and maintenance in the rat, it has been previously determined that weanling rats require 80 - 100 ug PA per day for optimal growth (Unna and Richards, 1942). Although the diet supplemented in PA contained 12 mg PA/ kg diet, it is possible that due to the small quantity of food consumed by the weanling rat, not enough PA was ingested for optimum growth. This would result in lower than expected excretion of PA in the urine. It would be desirable to repeat the first part of this study to confirm the results obtained. Hatano et al. (1968) reported a larger increase in PA excretion in the control group versus the PA deficient group when both groups were given a PA load of 4 mg $Ca(PA)_2$ subcutaneously. This pattern was similar to the excretion patterns observed in this study when all groups were fed stock diet. Since urinary excretion of PA fell after 1 day on the experimental diets and remained at low levels until a stock diet was fed, we felt it was not necessary to measure urinary excretion again in the second experiment as the diets fed in both experiments were the same.

It is possible that blood PA concentrations which were significantly lower in PA deficient treatment groups by 0.5 week in experiment 2, had dropped prior to this first measurement and in so doing had triggered the reaborption of PA from the kidney tubules. Urine and blood PA concentrations have previously been found to be indicative of PA levels in the diet (Tao and Fox, 1976; Hatano, 1962; Pearson et al., 1946) and our studies offer further proof of this. We need, however, further examination of the relationship between urine and blood PA, with tissue PA concentrations to be able to elaborate on the urine and blood PA concentrations indicative of depleted stages.

Although total PA levels did not change among the treatment groups in any of the tissues analysed, there was a decrease of 80% to 90% in free PA in heart and of 50% to 60% in kidney in the deficient groups over the 4 weeks of the study (mostly occuring between 0.5 and 1.5 weeks). Reibel et al. (1981) reported decreases of 70% to 90% in free PA in organs in adult rats fed PA deficient diets for 4 weeks. The observed conservation of total PA content might suggest that organs normally contain a large excess of PA (Reibel et al., 1982). Alternative explanations for this lack of change in total PA per gram tissue include increased synthesis or increased absorption of PA from the intestine, or possibly increased copraphagy. Decreased PA content in

other unmeasured tissues is another possibility which is presently being explored in this laboratory. As well, while the diet was deficient in PA, it was not completely devoid of the vitamin, offering still another source of PA to maintain total PA concentrations per gram tissue at constant levels. Tissue concentrations of CoA which are normally maintained within a narrow range have been shown to change under some pathological conditions (e.g. diabetes) (Robishaw and Neely, 1985). These changes could have a significant effect on fatty acid metabolism or other pathways utilizing CoA. Apart from measuring total PA it would be useful to have information on the ratio of free CoA to acyl CoA as well as on the distribution of CoA between the cytosol and mitochondria as these factors can alter certain metabolic functions.

It appears from this study that PA has a hypotriglyceridemic effect for elevated plasma lipids induced by feeding a high fat diet. This effect has been proposed to be as a result of a vitamin related effect of PA versus a pharmacologic one (Peterson et al., 1987). Proposed mechanisms for the hypotriglyceridemic effect include fatty acid oxidation stimulation (Kameda and Abiko, 1980), activation of the citric acid cycle (Prisco et al., 1984) and lipoprotein lipase activation (Noma et al., 1984).

Hypertriglyceridemia can be caused by increased secretion of VLDL by the liver or decreased degradation by lipoprotein lipase or both. As PA is incorporated into CoA which is extensively involved in lipid metabolism, certain reactions may be suppressed by an inadequate supply of CoA as a result of insufficient PA in the diet. This could result in elevated triglycerides in rats not receiving PA in their diet.

SUMMARY

Urinary PA excretion dropped significantly ($p<.01$) by day 2 of experiment 1 in rats on diets deficient in PA. No differences were found between high-fat and high-CHO PA deficient treatment groups.

Significant differences ($p<.01$) were found beginning at week 0.5 of experiment 2 in whole blood (total and free) and in plasma (free) between PA supplemented and PA deficient treatment groups. No significant differences were seen between high-fat and high-CHO PA deficient treatment groups.

Free PA concentrations in the liver, kidney, and heart were significantly lower ($p<.01$) in the PA deficient groups beginning at week 1.5 of experiment 2. No significant differences were found among treatment groups with respect to total PA in any organ analyzed.

Plasma triglycerides were significantly higher ($p<.01$) in the high-fat PA deficient group than in the other three treatment groups at weeks 2.5 and 4 in experiment 2.

The high-fat PA deficient group had significantly lower PA in tissue and blood and higher triglyceride concentrations ($p<.01$) than the high-fat PA supplemented pair fed or ad libitum fed groups. The underlying mechanisms are unknown at this time.

Neither food intake nor weight gain differed significantly between the high-fat and high-CHO PA deficient groups. Food intake of the high-fat PA supplemented ad libitum fed group was significantly greater ($p<.01$) than all other treatment grous beginning at week 3 of experiment 2. Weight of the ad libitum and pair fed PA supplemented groups was significantly greater ($p<.01$) than the PA deficient groups beginning at week 2 of experiment 2.

CONCLUSIONS

The results of these studies do not demonstrate significant differences in total and free PA in blood and tissue, or urine PA levels between high fat PA deficient and high carbohydrate PA deficient groups, though the differences were significant between PA supplemented and PA deficient groups beginning at week 0.5 of the second experiment. From these studies, it was concluded that the PA requirement in rats as determined by blood, tissue, and urinary PA levels is not altered significantly by the quantity of fat and carbohydrate in the diet.

RECOMMENDATIONS

The relationship between high-fat and high-carbohydrate diets and PA status needs to be further examined by using diets containing marginal PA, that is, inadequate amounts to support growth and maintenance. In this way, the presence and/or absence of PA would not be such a strong factor and other more subtle effects could be observed. As well, it might prove beneficial to examine other tissues in rats fed experimental diets as in this study. Since kidney, heart, and liver are all quite important tissues metabolically, it is likely that these are the same tissues which would be affected last in a deficiency state in the rat. Muscle and fat tissue are less active and thus might provide interesting information about what happens when rats are fed high-fat or high-carbohydrate PA deficient diets vs rats fed these same diets supplemented with PA. These tissues could potentially be acting as stores of PA from which liver, kidney, and heart draw to maintain their CoA levels as was observed. More generally, in order to more accurately determine the correlation between dietary intake of PA and blood levels of the vitamin, measuring blood PA content of rats daily for the first three or four days they are fed experimental diet is recommended. This information will be useful in helping to determine a Recommended Dietary Allowance for pantothenic acid.

APPENDICES

APPENDIX A

HIGH CARBOHYDRATE PANTOTHENIC ACID DEFICIENT
DIET COMPOSITION[1,2,3]

INGREDIENT	WEIGHT	KCAL
Vitamin Free Casein	20	21%
DL-Methionine	0.3	
Cornstarch	15	67.5%
Sucrose	50	
Fiber - Celufil	5	
Corn oil	5	12%
AIN-76 Mineral mix	3.5	
AIN-76 Vitamin mix without calcium pantothenate	1.0	
Choline bitartrate	0.2	

1
PA supplemented diet mixed as above with the addition of 12 ug PA/g diet (312 ug PA/ 100 kcal) D-calcium pantothenate

2
this diet contains 385 kcal ME/100 g

3
all ingredients purchased from United States Biochemical Corporation

HIGH FAT PANTOTHENIC ACID DEFICIENT

DIET COMPOSITION [1,2,3]

INGREDIENT	WEIGHT	KCAL
Vitamin Free Casein	20	21%
DL-methionine	0.3	
Cornstarch [1]	11.25	12%
Sucrose [1]		
Fiber - Celufil	5	
Corn oil	5 [1]	
Crisco *	23.89 [1]	67.5%
AIN-76 Mineral mix	3.5	
AIN-76 Vitamin mix without calcium pantothenate	1.0	
Choline bitartrate	0.2	

[1] pantothenic acid supplemented diet mixed as above with the addition of 17 ug PA/ g diet (312 ug PA/ 100 kcal) D-calcium pantothenate

[2] this diet contains 385 kcal ME/ 70 g

[3] all ingredients purchased from United States Biochemical Corporation

* Crisco is made of partially hydrogenated soybean, palm, and sunflower oils

APPENDIX B

Food Intake - Experiment 1

Day [1]		Treatment		
		High Fat PA-	High CHO PA- *	High CHO PA+
1	g	5± 1	5± 1	4± 1
2	kcal	33± 9	27± 4	27± 1
3	"	28± 9	34± 3	25± 3
4	"	31± 3	39± 2	33± 2
5	"	32± 4	39± 2	38± 4
6	"	35± 2	42± 2	40± 5
7	"	37± 3	39± 1	37± 2
8	g	8± 1	8± 1	8± 1
9	"	11± 1	11± 2	12± 2
10	"	13± 1	14± 1	14± 1
11	"	15± 1	16± 1	17± 1
12	"	15± 1	15± 1	15± 1
13	"	13± 2	14± 2	15± 1
14	"	16± 1	16± 2	17± 1
15	"	18± 3	18± 2	18± 1
16	"	19± 1	19± 1	19± 1
17	kcal	79± 6	62± 5	61± 6
18	"	76± 5	73± 6	63± 1
19	"	65± 8	74± 6	69± 4
20	"	65± 4	67± 5	66± 3
21	"	68± 6	68± 5	63± 4
22	"	71± 3	70± 6	68± 4
23	"	67± 6	73± 6	69± 5

* food spilt into urine not accounted for, therefore reported values are higher than actual intake.

[1] day 1- 7 and 17- 23 rats fed experimental diets
day 8- 16 rats fed stock diet

Weight - Experiment 1

Week	Treatment		
	High Fat PA-	High CHO PA-	High CHO PA+
0	37± 2	38± 2	38± 3
1	68± 7	71± 5	70± 5
2	111± 9	120± 10	118± 7
2'*	116± 10	117± 12	117± 6
3	157± 12	156± 12	152± 7

* rats randomly divided into three new equal groups at this time

URINE PA CONTENT ug/24 h	TREATMENT HIGH FAT PA DEFICIENT						HIGH CHO PA DEFICIENT						HIGH CARBOHYDARTE PA SUPPLEMENTED					
RAT ID	1	2	3	4	5 MEAN/STD DEV		1	2	3	4	5 MEAN/STD DEV		1	2	3	4	5 MEAN/STD DEV	
DAY 1	99.58	97.14	109.54	150.19	115.26	99.84	87.61	55.82	85.04	112.48	59.42	81.10	73.44	104.24	43.25	99.27	89.78	96.86
	75.46	115.78	65.63	85.71	108.89	17.90	78.41	83.89	75.35	103.36	69.57	16.78	87.57	132.11	61.15	144.85	73.29	32.26
DAY 2	14.89	16.33	6.72	24.87	14.59	12.67	9.88	7.79	25.35	39.43	12.46	12.95	110.79	101.81	62.08	103.13	35.80	89.46
	6.16	12.55	16.35		13.21	2.12	4.94	14.74	26.68	49.62	7.35	8.97	69.56	116.82	118.67	68.47	50.80	9.57
DAY 3	1.79	2.95	4.92	4.71	2.48	3.62	0.59	9.49	4.94	1.73	9.74	7.17	11.82	11.39	9.07	9.07	12.65	10.74
	4.33	2.46	7.60	2.56	2.39	1.54	1.62	11.62	11.78	10.41		3.79	11.68	10.93	10.32	8.36	12.74	1.51
DAY 4	5.51	8.64		4.11	4.35	6.86	3.17	2.18	9.73	8.50	5.02	5.79	10.85	15.17	14.92	15.58	7.15	11.59
	8.01	9.99		6.53	6.20	0.68	2.60	4.11	7.73	7.13	5.73	2.61	16.35	5.87	10.00	10.31	11.92	2.45
DAY 5	3.19	5.70	5.13	3.64	6.89	4.66	1.20	1.67	1.10	2.40	0.97	1.43	21.44	9.89	19.33	7.58	15.67	14.51
	4.00	3.84	3.25	4.92	6.11	0.88	0.70	2.20	0.70	1.72	2.27	0.49		10.83	19.95	8.19	16.80	5.88
DAY 6	1.77	4.18	2.19	1.34	3.64	2.94	0.44	3.65	1.69	2.67	0.61	1.20	39.83	16.44	18.45	18.73	15.09	15.15
	3.79	4.21	3.03	4.80	2.37	0.65	0.76	2.74	1.61	1.99	1.21	0.58	42.44	13.10	11.25	15.15	16.10	1.61
DAY 7	4.04	1.72	1.42	2.80	3.67	2.41	0.27	0.65	1.41	0.75	0.55	0.79	10.47	9.67	12.99	8.15		9.43
	2.11		1.76	1.92	2.47	0.93	0.51	0.78	1.05	0.76	0.48	0.20	10.10	7.42	11.94	6.91		1.30
DAY 8	10.80	9.62	5.01	8.13	12.87	9.11	5.68	9.03	4.98	15.22	5.97	8.42	10.37	26.29	20.29	12.35	25.99	19.13
	10.00	14.18	7.23	6.95	9.54	2.66	6.02	8.72	3.89	8.16	7.38	3.28	9.84	22.84	20.08	9.26	23.99	5.92
DAY 9	24.43	23.91	12.83	9.98	15.39	14.80	11.28	24.43	12.92	23.40	26.02	19.22	33.07	104.56	65.83	55.34	165.75	83.41
	32.44	21.00	11.66	10.26	13.34	5.39	12.27	27.04	10.36	22.32	18.87	7.28	34.10	82.70	68.12	53.45	167.29	50.57

(continued)

DAY 10	~~18.76~~	23.21	~~12.84~~	13.77	13.65	17.72	17.57	33.66	9.39	23.61	28.01	20.65	111.70	145.70	162.47	89.83	166.21	143.58
	36.21	23.47	12.14	14.86	15.27	6.45	14.32	33.94	9.08	22.97	15.60	7.66	121.58	166.66	178.41	90.30	136.84	32.79
DAY 11	71.95	51.38	46.87	48.45	46.32	55.02	96.94	44.24	64.45	88.63	55.51	70.80	227.45	200.83	259.38	195.06	212.14	219.80
	81.88	47.65	46.47	45.75	42.67	16.89	106.83	60.30	62.80	90.77	53.32	18.03	224.28	205.02	232.63	209.15	227.28	19.34
DAY 12	80.74	155.81	92.96	91.87	138.04	106.53	137.03	152.61	80.74	88.69	65.72	111.13	174.05	187.82	204.08	191.76	194.69	194.28
	90.71	150.79	94.57	94.57	137.59	29.50	145.85	149.96	104.25	95.63	71.84	36.09	175.92	174.38	200.18	183.15	214.21	10.56
DAY 13	230.32	200.90	129.59	163.49	171.88	193.19	217.60	195.16	216.45	198.11	183.40	203.68	262.52	195.16	243.06	278.99	235.99	241.78
	223.89	229.06	123.29	155.30	203.02	29.12	175.30	230.56	197.79	219.55	163.69	18.97	214.59	231.31	267.89	248.53	171.34	23.21
DAY 14	197.79	236.14	203.44	160.48		195.17	254.38	178.53	225.51	220.03	242.85	223.04	273.06	235.22	292.08	283.92	261.70	261.63
	196.72	239.44	216.39	156.88		30.79	245.69	172.56	201.46	244.49	222.93	17.74	266.54	234.50	279.44	285.43	233.18	14.61
DAY 15	216.31	257.69	231.45	205.40	185.22	231.76	248.79	226.89	206.56	230.53	240.84	229.59	288.70	250.62	241.80	246.00	248.63	229.11
	235.15	251.61	266.18	222.03	211.86	17.52	254.38	234.59	190.06	230.56	225.42	17.83	220.39	253.56	229.11	239.97	187.68	14.30
DAY 16	207.16	270.75	216.86	223.34	286.99	268.02	187.18	212.65	217.57	264.33	221.89	247.15	233.85	291.29	254.57	229.27	265.24	247.15
	231.19	309.15	225.60	234.45	245.37	45.88	206.69	258.60	244.51	201.80	232.82	7.10	255.00	232.29	231.19	236.93	232.82	7.10
#DAY 17	159.14	159.45	160.37	153.18	85.71	158.53	164.14	180.24	197.85	144.65	210.35	184.84	286.56	281.14	292.70	280.06	295.45	307.87
	154.85	184.30	158.05	154.85	83.78	5.62	177.38	187.70	228.46	185.60	219.10	27.41	343.74	311.19	318.70	325.35	326.47	7.41
DAY 18	29.77	41.30	34.15	38.12	29.62	34.96	22.41	23.02	15.38	23.17	36.57	26.43	225.34	322.78	244.95	324.70	323.74	315.15
	51.74	29.21	34.09	34.09	27.54	4.37	34.77	22.67	15.89	33.26	35.16	7.60	343.29	362.93	343.29	343.29	345.42	17.78
DAY 19	38.85	32.81	41.41	22.18	29.06	31.83	18.93	26.99	22.30	37.08	23.85	29.87	319.62	378.80	323.57	271.83	303.55	337.57
	42.77	45.13	22.08	20.29	26.51	8.10	35.00		28.96		34.17	4.86	388.55	316.87	315.65	375.60	369.29	14.37
DAY 20	11.46	7.90	10.92	7.17	8.47	11.37	13.08	7.71	5.71	8.34	20.22	11.45	299.66	289.45	349.57	331.22	342.28	338.95
	11.56	12.87	13.20	13.16		2.44	15.17	10.71	10.85	17.84	11.74	2.01	367.76	379.07	328.67	343.62	379.07	12.59
DAY 21	8.65	23.31	11.88	8.03	5.81	12.78	5.73	19.77	4.04	5.88	13.78	10.69	376.89	407.07	397.22	291.69	376.89	338.32
	7.96	20.47	21.89	12.00	13.85	4.47	14.61	16.42	10.46	7.70	7.73	2.57	279.60	297.73	317.15	356.77	314.26	14.14
DAY 22		11.33	9.48	6.73	6.46	8.99	15.45	21.93	3.13	2.74	4.08	12.85	322.88	312.65	324.23	372.06	359.85	340.82
		12.31			4.08	1.57	21.70		7.06	8.21	16.43	7.66	373.74	353.90	382.83	314.61	277.81	6.12
DAY 23		6.83	9.22	6.28	18.03	15.47	8.54	6.32	4.97	5.89	3.49	11.79	425.87	338.23	350.84	403.33	424.49	392.92
		22.16	21.69	21.72	5.49	2.04	23.26	22.13	11.38	22.57	9.38	4.20	332.66	417.64	430.65	335.99	352.00	10.15

1 = EXPERIMENTAL DIET
PLACED ON STOCK DIET

HEART: TOTAL PA CONTENT

nmol PA/g tissue

BASELINE

WEEK 0

RAT I.D.		
A	342.60	221.69
B	420.38	243.85
C	387.36	249.81
D	355.01	265.35
E	439.31	229.37
F	375.94	266.82
G	385.40	247.17
MEAN	312.31	
STD DEV	23.65	

TREATMENT GROUP

RAT I.D.	HIGH FAT PA-		HIGH FAT PA+ PR FED		HIGH CHO PA-		HIGH FAT PA+ AD LIB	
WEEK 0.5								
1	218.68	295.26	296.39		199.57	219.01	233.95	260.34
2	228.30	199.14	208.84	302.74	229.81	265.25	238.07	239.74
3	230.39	275.25	275.15	295.01	290.40	261.89	234.18	255.35
4	306.30	280.12	216.62	246.45	240.29	295.78	211.10	251.08
5	222.79	212.22	277.64	300.49	232.50	276.82	227.75	253.46
MEAN	246.48		271.61		245.13		237.00	
STD DEV	32.49		27.23		30.10		10.66	
WEEK 1.5								
11	158.96	190.35	214.65	260.17	200.04	250.35	186.97	237.33
12	264.08	279.45	208.60	209.87	158.67	152.59	145.92	196.67
13	150.56	230.45	200.41	163.14	172.68	222.49	250.04	266.24
14	222.84	199.65		227.18	185.56	164.26	216.30	240.79
15	170.36	206.07	234.53	219.04	202.95	215.17	218.28	213.99
MEAN	207.30		216.42		192.48		217.25	
STD DEV	38.33		21.81		27.56		31.38	
WEEK 2.5								
6	191.19	187.62	188.80	228.81	209.72	214.96	199.21	181.90
7	176.93	196.78	197.49	218.40	177.27	197.51	205.89	213.37
8	173.88	173.13	202.91	203.49	164.34	194.78	191.57	184.34
9	173.72	205.48	178.82	192.12	157.17	187.09	161.72	180.58
10	229.96	244.26	189.08	189.58	213.40	227.03	196.07	205.59
MEAN	195.30		197.99		194.33		192.86	
STD DEV	24.30		9.58		20.94		14.44	
WEEK 4.0								
16	222.43	168.73	220.65	210.45	184.01	190.22	206.63	179.01
17	177.97	211.57	192.39	197.55	186.22	190.33	202.56	183.71
18	217.16	202.99	213.86	189.84	217.37	197.57	209.62	167.65
19	219.35	277.47	217.73	204.26	233.25	181.56	218.26	189.74
20	226.73	198.26	197.39	221.98	144.13	150.76	190.44	186.07
MEAN	211.57		206.60		187.54		198.37	
STD DEV	22.48		8.62		24.59		6.36	

HEART:FREE FA CONTENT

BASELINE — nmol FA/g tissue

WEEK 0

RAT I.D.		
A	66.35	55.62
B		49.62
C	44.7	57.21
D	18.49	21.41
E	23.22	36.35
F	33.88	39.72
G	24.87	33.63
	MEAN	41.76
	STD DEV	15.46

TREATMENT GROUP

	HIGH FAT FA-			HIGH FAT FA+ PR FED		HIGH CHO FA-		HIGH FAT FA+ AD LIB	
RAT I.D.			WEEK 0.5						
1	53.28	64.08		42.78	40.19	39.47	47.65	57.73	72.75
2	54.44	53.43		39.93	72.19	99.65	41.31		39.30
3	68.44	82.49		60.90	71.35	68.37	37.62	53.27	55.97
4	35.00	40.64		48.87	45.75	37.01	26.00	62.88	56.73
5				70.49	72.37	23.18	23.11	60.57	62.60
	MEAN	56.60			56.48		35.42		60.15
	STD DEV	15.29			12.51		8.24		3.84
			WEEK 1.5						
11	10.54	6.84		81.70	71.44	9.26	7.59	78.50	62.81
12	3.62	3.03		70.36	81.66	30.49	27.73		86.53
13	3.09	2.11		75.01	99.37	20.03	16.25	98.49	85.27
14	5.06	2.88		69.41	68.63	3.98	3.79	108.39	94.06
15	2.62	1.53		66.89	74.82	11.36	6.7	94.61	75.15
	MEAN	4.13			71.52		13.73		87.13
	STD DEV	2.65			4.19		10.04		11.19
			WEEK 2.5						
6	6.35	7.88		99.21	97.69	6.27	13.15	106.03	122.63
7	3.85	12.65		35.71	99.66	9.88	18.47		171.12
8	2.25	4.30		115.53	93.60	11.39	12.00	159.09	129.02
9	4.45	4.85		86.91	100.06	2.95	7.42	71.62	92.29
10	4.15	4.67		73.33	72.32	3.50	7.78	109.94	110.17
	MEAN	4.66			89.50		9.29		124.30
	STD DEV	1.47			13.50		3.88		34.20
			WEEK 4.0						
16	2.82	2.73		88.81	106.13	7.20	7.09	119.33	112.96
17	9.30	5.79		85.48	86.90	5.63	2.12	105.57	101.80
18	19.47	12.17		49.80	65.23	9.68	6.34	113.22	106.46
19	3.94	5.05		63.23	75.99	9.18	7.44	92.35	99.73
20	5.05	10.55		86.14	86.26	2.00	1.18	128.12	122.44
	MEAN	7.75			77.88		5.75		110.20
	STD DEV	5.01			18.80		3.00		11.24

KIDNEY: TOTAL PA CONTENT

BASELINE — nmol PA/g tissue

WEEK 0

RAT I.D.		
A	250.67	172.90
B	240.42	164.25
C	245.32	178.17
D	265.71	187.04
E	268.42	202.18
F	241.67	185.61
G	236.14	173.01
MEAN	233.40	
STD DEV	12.60	

TREATMENT GROUP

RAT I.D.	HIGH FAT PA-		HIGH FAT PA+ PR FED		HIGH CHO PA-		HIGH FAT PA+ AD LIB	
	WEEK 0.5							
1	179.08	161.71	225.62	207.38	217.48	182.42	200.33	192.70
2	189.28	172.28	262.40	229.34	217.97	191.08	219.44	176.99
3	221.81	175.88	272.65	212.15	200.29	160.78	206.09	168.99
4	193.19	166.02	233.39	187.22	224.13	167.38	247.94	191.19
5	237.87	197.25	272.01	251.95	213.56	155.20	149.52	122.95
MEAN	189.45		225.43		190.65		189.40	
STD DEV	18.79		21.49		10.28		29.30	
	WEEK 1.5							
11	189.36	215.38	200.67	203.85	200.25	189.37	215.44	206.05
12	219.67	252.05	210.61	212.78	198.67	183.91	210.23	180.10
13	185.44	188.36	212.46	241.73	172.15	169.29	218.32	202.38
14	201.34	204.31	187.38	217.79	185.15	188.35	216.34	213.76
15	187.29	205.05	206.62	227.76	181.84	188.70	217.88	209.68
MEAN	204.82		214.26		184.79		209.08	
STD DEV	18.38		10.92		9.29		7.97	
	WEEK 2.5							
6	156.69	155.38	170.08	150.78	154.21	148.85	158.10	155.34
7	176.82	175.48	148.10	163.07	145.85	163.08	153.88	165.12
8	161.79	159.51	149.47	148.17	148.09	147.62	148.07	142.70
9	152.02	150.81	152.92	144.98	160.28	163.61	154.45	150.99
10	173.27	142.05	157.91	158.17	167.61	139.69	136.15	148.18
MEAN	160.40		153.25		155.50		150.50	
STD DEV	9.41		5.95		8.28		7.58	
	WEEK 4.0							
16	144.70	157.79	176.88	177.71	182.94	179.47	181.27	148.92
17	135.31	148.67	171.42	158.56	128.13	162.94	185.21	169.57
18	164.41	146.62	158.04	176.41	176.22	155.14	141.14	126.41
19	150.85	204.39	204.14	206.29	155.69	173.46	150.22	164.10
20	166.76	177.67	164.22	161.43	165.09	168.87	162.18	131.72
MEAN	163.56		174.71		168.19		157.17	
STD DEV	11.30		17.28		16.89		15.29	

KIDNEY:FREE PA CONTENT

nmol PA/g tissue

	BASELINE	
	WEEK 0	
RAT I.D.		
A	120.46	119.38
B		100.44
C	72.95	85.61
D	78.28	96.14
E	89.17	93.86
F	84.62	81.73
G	66.63	77.35
MEAN	90.65	
STD DEV	15.89	

	TREATMENT GROUP							
	HIGH FAT PA-		HIGH FAT PA+ PR FED		HIGH CHO PA-		HIGH FAT PA+ AD LIB	
RAT I.D.	WEEK 0.5							
1	96.23	114.69	75.89	107.96	83.06	88.59	100.03	149.15
2	60.96	72.11	103.52	100.61	41.19	59.74	96.64	109.26
3	90.05	115.85	116.20	140.38	66.94	69.13	83.84	113.75
4	81.41	111.21	95.85	121.38	74.41	90.49	103.86	110.22
5	63.32	68.41	117.46	130.44	64.24	86.97	103.00	102.33
MEAN	88.22		112.90		72.48		100.01	
STD DEV	20.38		16.15		14.07		11.87	
	WEEK 1.5							
11	12.63	23.81	100.67	122.78	83.75	110.62	104.34	135.34
12	9.30	16.29	130.91	142.09	80.22	107.77	126.65	128.88
13	58.69	64.36	126.61	136.45	22.22	38.18	133.46	130.89
14	28.32	40.13	124.44	146.84	30.75	42.20	124.17	122.89
15	6.82	17.03	127.85	138.27	63.64	77.30	141.47	133.66
MEAN	27.77		135.91		63.07		131.28	
STD DEV	20.90		11.97		28.55		8.63	
	WEEK 2.5							
6	28.99	27.74	113.33	86.43	46.47		122.03	94.11
7	46.62	31.03	106.70	102.22	30.23	30.62	120.57	97.19
8	27.53	27.35	104.43	97.66	43.80	39.69	100.39	98.73
9	34.88	48.44	108.77	96.14	34.63	44.19	106.01	96.33
10	62.69	45.57	106.22	110.22	29.66	23.64	116.85	119.09
MEAN	40.11		108.22		39.14		107.22	
STD DEV	12.52		3.30		9.67		7.34	
	WEEK 4.0							
16	22.84	19.60	92.44	98.73	45.15	36.94	90.95	114.67
17	35.20	32.19	89.69	89.88	38.63	47.93	96.84	110.24
18	27.48	19.73	85.69	84.04	31.20	25.88	101.69	91.72
19	19.71	21.44	98.18	101.77	35.35	51.71	115.22	98.67
20	34.52	63.68	88.22	94.06	21.68	25.69	99.70	106.20
MEAN	29.64		90.98		38.24		102.09	
STD DEV	12.89		5.64		11.73		3.08	

LIVER: TOTAL PA CONTENT

BASELINE — nmol PA/g tissue

WEEK 0

RAT I.D.		
A	366.75	435.86
B	269.60	288.23
C	289.62	308.80
D	347.80	372.67
E	298.33	408.06
F	229.95	257.39
G	250.82	285.14
MEAN	316.36	
STD DEV	54.59	

TREATMENT GROUP

	HIGH FAT PA-		HIGH FAT PA+ PR FED		HIGH CHO PA-		HIGH FAT PA+ AD LI	
RAT I.D.			WEEK 0.5					
1	313.60	331.15	305.42	297.51	246.27	325.62	353.85	365.24
2	263.99	297.70	309.60	330.01	316.16	341.21	331.30	346.71
3	268.55	288.34	331.65	254.19	277.36	332.15	332.19	324.14
4	314.97	357.42	303.39	307.93	279.88	241.85	297.24	292.72
5	277.68	272.48	343.51	311.42			301.55	298.45
MEAN	290.69		309.46		295.09		324.34	
STD DEV	28.34		13.99		28.75		27.03	
			WEEK 1.5					
11	287.15	302.46	266.23	318.87	218.85	295.26	311.75	321.71
12	297.26	332.87	268.20	297.61	211.48	278.76	296.49	325.49
13	282.92	310.62	324.53	368.79	241.94	273.47	306.56	336.29
14	293.18	291.21	232.07	287.69	316.51	313.22	258.26	292.63
15	325.57	313.28	263.75	295.19	228.63	227.15	262.22	312.92
MEAN	303.63		292.29		260.53		304.48	
STD DEV	12.61		32.63		32.69		22.62	
			WEEK 2.5					
6	247.39	231.62	248.76	222.81	289.30	280.97	364.19	316.36
7	235.48	242.65	287.89	303.93	257.24	247.75	291.59	288.65
8	262.65	245.82	262.70	253.85	285.29	274.19	262.54	251.36
9	314.18	291.55	351.22	316.76	234.25	217.39	311.42	259.44
10	272.82	258.37	369.98	332.99	298.43	239.18	343.57	323.75
MEAN	260.19		295.20		262.43		300.81	
STD DEV	26.33		48.76		23.91		35.24	
			WEEK 4.0					
16	311.85	352.97	278.42	351.36	269.82	341.19	289.33	376.88
17	276.19	335.78	299.38	450.11	232.54	244.99	376.69	418.42
18	241.72	304.43	319.91	431.56	209.19	270.92	302.93	350.24
19	211.69	322.13	380.39	454.42	283.40	339.34	309.75	322.19
20	274.03	317.04	330.29	444.35	274.08	284.21	325.67	341.62
MEAN	296.88		374.02		274.97		341.37	
STD DEV	27.68		37.28		34.66		32.20	

LIVER:FREE FA CONTENT

BASELINE nmol FA/g tissue

WEEK 0

RAT I.D.		
A	144.99	160.95
B	129.41	127.1
C	182.27	193.44
D	105.89	85.64
E	81.07	99.23
F	51.32	57.98
G	75.48	71.81
MEAN	111.48	
STD DEV	47.13	

	TREATMENT GROUP							
	HIGH FAT FA-		HIGH FAT FA+ PR FED		HIGH CHO FA-		HIGH FAT FA+ AD LIB	
RAT I.D.	WEEK 0.5							
1	180.15	204.52	83.36	119.38	128.41	163.06	250.60	281.98
2	189.13	183.52	93.29	169.46	132.77	154.41	133.29	162.44
3	159.00	162.40	100.00	123.17	101.98	104.57	130.93	167.58
4	128.82	132.86	118.89	108.38	119.85	121.79	234.68	257.67
5	169.55	164.79	144.73	151.92	100.51	116.96		127.98
MEAN	167.48			114.76		124.43		187.51
STD DEV	24.30			19.42		19.54		63.69
	WEEK 1.5							
11	12.50	12.70	74.03	86.64	1.77	4.79	58.01	81.35
12	3.30	13.02	30.98	39.92	8.14	11.46	36.41	39.15
13	14.25	20.74	53.81	74.49			55.42	48.14
14	12.00	19.64	29.07	47.84	28.13	15.14	39.27	64.91
15	12.86	18.53	57.41	66.19	16.39	14.58	52.82	80.72
MEAN	13.96			56.04		12.25		61.64
STD DEV	3.70			18.86		7.84		7.16
	WEEK 2.5							
6	24.40	24.21	48.98	37.50	10.42	15.32	63.88	75.81
7	20.93	14.99	54.74	62.37	8.53	15.08	70.83	78.63
8	10.86	14.12	87.11	101.82	15.32	22.47	117.45	81.35
9	41.30	32.23	95.39	88.85	28.08	24.30	105.41	126.68
10	12.50	10.40	33.54	30.05	8.42	13.29	92.97	128.22
MEAN	20.39			64.06		16.22		94.14
STD DEV	10.39			28.38		6.50		20.89
	WEEK 4.0							
16	5.23	10.93	24.44	53.58	17.61	45.56	23.27	22.12
17	9.24	9.77	21.61	58.63		35.11	123.96	142.49
18	7.58	14.25	89.89		24.22	20.67	92.72	102.28
19	19.74	22.04	26.38	57.17	28.41	36.61	22.01	28.44
20	14.24	22.76	102.50		6.47	6.21	56.56	48.74
MEAN	13.70			62.62		30.41		66.26
STD DEV	5.88			30.99		5.52		48.03

WHOLE BLOOD: TOTAL PA CONTENT

nmol PA/ ml BLOOD

BASELINE

WEEK 0

RAT I.D.	
A	8.34
B	8.35
C	
D	
E	
F	
G	
MEAN	8.45
STD DEV	

	TREATMENT GROUP							
	HIGH FAT PA-		HIGH FAT PA+ PR FED		HIGH CHO PA-		HIGH FAT PA+ AD LIB	
RAT I.D.			WEEK 0.5					
1	0.31		2.35	1.10	0.69	0.49	2.64	3.83
2	0.37	0.34	1.43	1.22	0.21	0.33	1.45	1.28
3		0.49	3.25	1.85	0.36	0.44	1.56	0.78
4	0.77	0.39	2.62	2.40	0.90	0.83	1.95	1.92
5	0.53	0.61	1.02	2.75	0.26	0.37	0.83	0.75
MEAN		0.44		2.00		0.49		1.66
STD DEV		0.13		0.58		0.24		0.95
			WEEK 1.5					
11	1.01	0.85	6.11	3.27	0.92	0.88	4.37	4.09
12	0.82	0.51	3.89	2.27	0.72	0.75	3.41	2.89
13	1.13	1.18	6.48	2.94	1.03	0.47	3.66	4.12
14	1.74	0.89	3.38	2.92			4.72	3.52
15	1.25	0.75	2.36	2.35	1.00	0.78	5.57	5.06
MEAN		0.91		3.64		0.82		4.13
STD DEV		0.19		1.91		0.09		0.79
			WEEK 2.5					
6	1.40	0.87	2.78	3.27	0.85	0.56	2.92	2.91
7	0.78	0.91	1.88	3.35	0.69	0.70	1.71	1.99
8	0.81	0.48	2.47	2.44	0.87	0.79	3.20	5.35
9	0.78	0.42	2.54	1.79	1.26	1.21	4.01	3.47
10	0.90	0.74	2.80	2.07	0.78	1.02	2.62	2.27
MEAN		0.81		2.35		0.87		3.07
STD DEV		0.21		0.38		0.22		1.01
			WEEK 4.0					
16	0.57	0.56	1.25	1.09	0.91	0.94	0.82	1.88
17	0.50	0.69	1.12	0.82	0.60	0.94	0.81	1.44
18	0.75	0.43	0.95	1.27	0.66	0.56	0.79	0.98
19	0.83	0.71	1.29	1.01	0.81	0.99	1.48	1.34
20	0.60	0.72	1.36	0.72	0.74	0.71	0.86	0.63
MEAN		0.64		1.09		0.79		1.10
STD DEV		0.08		0.08		0.13		0.29

MOBILE BLOOD: FREE PA CONTENT

BASELINE — nmol PA/ ml BLOOD

WEEK 0

RAT I.D.	
A	3.25
B	3.38
C	2.58
D	3.29
E	3.61
F	4.33
G	
MEAN	3.32
STD DEV	0.56

TREATMENT GROUP

RAT I.D	HIGH FAT PA-		HIGH FAT PA+ PR FED		HIGH CHO PA-		HIGH FAT PA+ AD LIB	
WEEK 0.5								
1	0.64		2.83	1.21	1.32	0.37	1.94	1.43
2	0.44	0.53	0.89	0.75	0.50	0.44	2.30	1.03
3	0.50	0.44	2.46	2.19	0.73	0.23	1.55	1.27
4	1.25	0.44	1.70	1.50	0.74	0.27	1.68	1.90
5	1.23	0.43	1.47	1.81	0.65	0.46	0.64	0.58
MEAN		0.50		1.32		0.48		1.48
STD DEV		0.08		0.38		0.07		0.34
WEEK 1.5								
11	0.20	0.08	3.01		0.45		1.81	
12	0.26	0.10	2.26		0.25		1.98	
13	0.31	0.10	2.28		0.46		2.19	
14	0.36	0.65	1.44		0.41		2.76	
15	0.28	0.08	1.76		0.38		3.10	
MEAN		0.18		2.15		0.39		2.37
STD DEV		0.03		0.60		0.08		0.54
WEEK 2.5								
6	0.38	0.48	2.42	1.43	0.31	0.47	1.77	
7	0.42	0.35	0.89	2.49	0.52	0.29		1.80
8	0.26	0.72	1.19	2.95	0.55	0.32	1.47	1.56
9	0.38	0.45	1.37	1.84	0.47		0.52	1.49
10	0.34	0.41	0.89	1.54	0.46	0.60	1.56	2.17
MEAN		0.42		1.82		0.45		1.75
STD DEV		0.04		0.22		0.06		0.17
WEEK 4.0								
16	0.59		1.34	1.19	0.64	0.86		1.78
17	0.53	0.74	1.08	1.14	0.88	0.96	1.08	2.03
18	0.48	0.50		1.58	0.46	0.64	0.77	1.58
19	0.43	0.74	1.20	1.01	0.37	0.59	1.06	1.30
20	0.40	0.84		1.20	0.42	0.54	1.12	0.79
MEAN		0.58		1.27		0.64		1.44
STD DEV		0.06		0.24		0.19		0.28

PLASMA: PA CONTENT

BASELINE — nmol PA/ ml PLASMA

WEEK 0

RAT I.D.	
A	1.86
B	1.94
C	1.82
D	1.85
E	1.84
F	1.66
G	2.13
MEAN	1.87
STD DEV	0.14

TREATMENT GROUP

	HIGH FAT PA-		HIGH FAT PA+ PR FED		HIGH CHO PA-		HIGH FAT PA+ AD LIB	
RAT I.D.	WEEK 0.5							
1	0.45	0.60	0.89	1.09	0.46	0.48	5.45	6.99
2	0.38		3.03		0.32	0.48	3.45	5.15
3	0.68	0.87	5.89	5.45	0.41	0.45	2.89	2.74
4	0.54	0.71	2.68		0.74	1.05	2.64	2.84
5	1.01	1.41	2.94		0.41	0.62	2.05	2.88
MEAN		0.70		2.44		0.45		2.99
STD DEV		0.32		0.91		0.05		0.44
	WEEK 1.5							
11	0.15	0.16	5.65	4.51	0.15	0.20	3.12	3.36
12	0.13	0.15	5.13	6.78	0.17	0.22	3.73	
13	0.12	0.14	3.01	4.63	0.21	0.16	3.61	3.85
14	0.14	0.17	5.80	5.15	0.17	0.22	4.32	4.64
15	0.13	0.16	4.37	4.99	0.18	0.14	7.98	
MEAN		0.15				0.19		3.90
STD DEV		0.02				0.04		0.54
	WEEK 2.5							
6	0.14	0.27	1.78	2.93	0.28	0.29	3.95	4.28
7	0.17	0.33	2.96	2.93	0.27	0.29	1.98	3.35
8	0.09	0.23	2.75	3.34	0.32	0.44	3.20	3.12
9	0.31	0.34	4.16	4.15	0.29	0.32	2.72	3.56
10	0.18	0.38	3.29	1.82	0.29	0.22	3.94	3.37
MEAN		0.24		2.66		0.30		2.88
STD DEV		0.06		0.29		0.05		0.91
	WEEK 4.0							
16	0.32	0.29	1.54	1.52	0.36	0.30	3.13	2.92
17	0.52	0.59	1.58	1.66	0.30	0.42	1.33	0.87
18	0.43		0.62	0.99	0.27	0.42	1.04	0.96
19	0.32	0.56	2.06	2.65	0.37	0.40	1.43	1.17
20	0.32	0.26	2.66	2.30	0.41	0.49	1.08	1.23
MEAN		0.40		1.62		0.37		1.14
STD DEV		0.11		0.53		0.05		0.13

PLASMA TRIGLYCERIDES

BASELINE — dl /ml PLASMA

WEEK 0

RAT I.D.
A
B
C
D
E
F
G

MEAN 76.22
STD DEV 4.04

RAT I.D.	HIGH FAT PA-		HIGH FAT PA+ PR FED		HIGH CHO PA-		HIGH FAT PA+ AD LIB	
			TREATMENT GROUP					
WEEK 0.5								
1	79.89		90.43	101.74	148.98	175.62	140.39	142.21
2	48.91	0.55	80.00		130.78	145.20	120.11	
3	56.25	84.78	145.22	126.09	128.11		145.72	145.45
4	80.71	91.30	139.13	138.26	194.84	175.09	168.56	143.51
5	75.00	66.85						
MEAN	71.25			115.11		159.33		132.63
STD DEV	14.07			22.38		25.51		12.68
WEEK 1.5								
11	137.01	138.96	132.95	149.56	101.58	78.80	92.39	75.42
12	144.16	123.38	118.45		94.94		36.52	98.23
13	133.77	108.44	127.70	153.06	86.29	119.62	97.19	100.88
14	124.03	118.83	111.95	122.45	104.43	109.18	11.86	10.67
15	111.04	107.14	125.95	140.82	100.63		53.39	86.35
MEAN	124.68			130.23		99.11		80.67
STD DEV	11.47			11.53		6.59		14.58
WEEK 2.5								
6	94.38	143.36	120.79	84.96	88.76	125.84	24.16	
7	123.60	102.12	35.96	67.43	62.36	59.47	25.84	
8	94.38	110.44	58.99	66.37	20.79	39.29	24.16	58.41
9	38.76	35.58	22.47	20.18	99.44	69.36	38.15	37.70
10	57.30	152.92	57.86	61.39	10.67		35.95	85.49
MEAN	119.81			59.66		70.69		29.11
STD DEV	23.18			29.20		33.06		6.65
WEEK 4.0								
16	75.18	53.70	10.84	15.25	23.63	12.39	139.62	67.13
17	93.08	189.30	35.11	40.68			20.76	50.09
18	120.29	81.07	11.46	20.34	83.65	52.54	32.22	33.65
19	97.37	120.31	34.20	59.32	33.56		37.23	23.23
20	70.88	72.29	20.65	39.83	17.18		44.39	20.14
MEAN	97.37			28.71		32.71		32.64
STD DEV	30.91			14.33		25.09		2.13

HEART: TISSUE WEIGHT AT SACRIFICE

BASELINE GRAMS

WEEK 0

RAT I.D.	
A	0.21
B	0.20
C	0.24
D	0.29
E	0.21
F	0.28
G	0.25
MEAN	0.21
STD DEV	0.03

	TREATMENT GROUP			
	HIGH FAT PA-	HIGH FAT PA+ PR FED	HIGH CHO PA-	HIGH FAT PA+ AD LIB
RAT I.D.	WEEK 0.5			
1	0.38	0.37	0.38	0.40
2	0.37	0.39	0.40	0.40
3	0.38	0.35	0.16	0.42
4	0.32	0.41	0.35	0.37
5	0.38	0.35	0.35	0.36
MEAN	0.37	0.37	0.33	0.39
STD DEV	0.02	0.03	0.09	0.03
	WEEK 1.5			
11	0.66	0.56	0.56	0.55
12	0.44	0.48	0.57	0.54
13	0.51	0.45	0.47	0.41
14	0.48	0.50	0.54	0.56
15	0.43	0.48	0.47	0.52
MEAN	0.50	0.49	0.52	0.51
STD DEV	0.09	0.04	0.05	0.06
	WEEK 2.5			
6	0.66	0.66	0.52	0.58
7	0.58	0.58	0.47	0.68
8	0.60	0.67	0.62	0.74
9	0.63	0.65	0.49	0.75
10	0.62	0.54	0.54	0.62
MEAN	0.60	0.62	0.53	0.67
STD DEV	0.06	0.06	0.06	0.08
	WEEK 4.0			
16	0.62	0.67	0.62	0.81
17	0.53	0.72	0.57	0.72
18	0.58	0.61	0.52	0.91
19	0.55	0.67	0.59	0.82
20	0.63	0.67	0.63	0.85
MEAN	0.58	0.67	0.59	0.82
STD DEV	0.01	0.04	0.04	0.07

KIDNEY: TISSUE WEIGHT AT SACRIFICE

BASELINE		GRAMS
	WEEK 0	
RAT I.D.		
A	0.48	
B	0.45	
C	0.43	
D	0.38	
E	0.48	
F	0.47	
G	0.48	
MEAN	0.45	
STD DEV	0.04	

	TREATMENT GROUP			
	HIGH FAT PA-	HIGH FAT PA+ PR FED	HIGH CHO PA-	HIGH FAT PA+ AD LIB
RAT I.D.	WEEK 0.5			
1	0.84	0.72	0.79	0.84
2	0.72	0.69	0.82	0.76
3	0.77	0.77	0.78	0.83
4	0.63	0.71	0.80	0.62
5	0.69	0.69	0.74	0.68
MEAN	0.73	0.72	0.79	0.74
STD DEV	0.08	0.03	0.03	0.10
	WEEK 1.5			
11	0.93	1.02	1.15	1.01
12	0.81	0.97	1.07	1.12
13	0.87	0.94	1.05	0.99
14	1.02	1.09	1.11	1.07
15	0.93	1.00	1.12	1.01
MEAN	0.91	1.00	1.10	1.04
STD DEV	0.08	0.06	0.04	0.05
	WEEK 2.5			
6	1.25	1.19	1.12	1.27
7	1.02	1.03	1.03	1.22
8	1.00	1.40	1.19	1.54
9	1.29	1.28	1.07	1.27
10	1.08	1.07	1.10	1.38
MEAN	1.13	1.20	1.10	1.33
STD DEV	0.13	0.15	0.06	0.13
	WEEK 4.0			
16	1.16	1.34	1.34	1.68
17	1.16	1.38	1.27	1.50
18	1.24	1.28	1.42	1.87
19	1.06	1.11	1.21	1.63
20	1.38	1.70	1.20	1.80
MEAN	1.20	1.36	1.29	1.70
STD DEV	0.12	0.22	0.09	0.15

LIVER: TISSUE WEIGHT AT SACRIFICE

GRAMS

BASELINE WEEK 0	
RAT I.D.	
A	1.36
B	1.26
C	1
D	1.125
E	1.36
F	1.38
G	1.39
MEAN	1.27
STD DEV	0.15

	TREATMENT GROUP			
	HIGH FAT PA-	HIGH FAT PA+ PR FED	HIGH CHO PA-	HIGH FAT PA+ AD LIB
RAT I.D.	WEEK 0.5			
1	2.78	2.72	2.95	3.01
2	2.71	2.57	3.21	2.79
3	2.95	2.88	2.92	3.01
4	2.58	2.78	2.78	2.30
5	2.69	2.81	2.68	2.51
MEAN	2.74	2.75	2.91	2.72
STD DEV	0.14	0.12	0.20	0.31
	WEEK 1.5			
11	4.21	4.36	4.47	4.22
12	3.70	4.26	4.69	4.59
13	3.94	4.42	4.92	3.93
14	4.10	4.59	4.28	5.09
15	3.73	4.11	4.17	3.91
MEAN	3.94	4.39	4.51	4.35
STD DEV	0.22	0.18	0.30	0.50
	WEEK 2.5			
6	4.89	4.96	4.27	5.10
7	4.73	4.61	4.49	5.33
8	4.70	4.94	5.09	5.85
9	4.86	4.90	4.78	5.17
10	4.44	4.31	5.06	5.06
MEAN	4.72	4.74	4.73	5.3
STD DEV	0.18	0.28	0.36	0.32
	WEEK 4.0			
16	4.74	5.09	5.30	6.24
17	4.73	5.00	4.94	6.01
18	4.67	4.78	5.92	7.15
19	3.97	4.50	5.07	7.86
20	5.33	5.95	5.85	7.79
MEAN	4.69	5.06	5.42	7.01
STD DEV	0.48	0.54	0.45	0.86

WEIGHT AT SACRIFICE

BASELINE — GRAMS

WEEK 0

RAT I.D.	
A	43.30
B	39.00
C	41.60
D	32.20
E	41.40
F	37.90
G	42.00
MEAN	39.63
STD DEV	3.76

TREATMENT GROUP

RAT I.D.	HIGH FAT PA-	HIGH FAT PA+ PR FED	HIGH CHO PA-	HIGH FAT PA+ AD LIB
	WEEK 0.5			
1	70.50	70.30	69.00	76.80
2	61.90	66.10	69.90	74.40
3	70.70	69.70	64.00	76.20
4	59.60	66.90	61.90	56.90
5	64.10	70.30	58.80	60.40
MEAN	65.36	68.66	64.72	68.94
STD DEV	5.04	2.01	4.71	9.52
	WEEK 1.5			
11	107.00	121.50	104.00	114.50
12	93.50	105.10	104.30	117.00
13	96.40	99.00	100.80	107.10
14	112.60	115.00	103.40	129.80
15	95.00	107.70	94.40	106.10
MEAN	100.90	109.66	101.38	114.90
STD DEV	8.42	8.76	4.14	9.55
	WEEK 2.5			
6	135.20	135.40	110.80	146.40
7	114.50	121.80	105.40	144.50
8	113.90	147.90	122.00	168.50
9	122.20	140.70	100.90	136.50
10	121.10	131.50	104.00	146.20
MEAN	121.38	135.46	108.62	148.42
STD DEV	8.59	9.80	8.29	11.93
	WEEK 4.0			
16	129.80	171.00	146.20	204.10
17	126.50	160.10	133.00	192.10
18	136.60	139.40	130.90	225.00
19	115.50	136.40	124.80	219.20
20	144.90	190.90	118.80	217.60
MEAN	130.66	159.56	130.74	211.60
STD DEV	11.02	22.67	10.27	13.32

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