This is to certify that the

thesis entitled

PLASMA LIPIDS MODIFIED BY PANTOTHENIC ACID DEFICIENCY IN GROWING RATS

presented by

Miyoung Jang

has been accepted towards fulfillment
of the requirements for

Master's degree in Human Nutrition

Major professor

Date Jan. 11th, 1994

PLASMA LIPIDS MODIFIED BY PANTOTHENIC ACID DEFICIENCY IN GROWING RATS

By

Miyoung Jang

A THESIS

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

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

1993

ABSTRACT

PLASMA LIPIDS MODIFIED BY PANTOTHENIC ACID DEFICIENCY IN GROWING RATS

By

Miyoung Jang

To elucidate how lipid metabolism is altered by pantothenic acid (PA) deficiency, weanling Sprague-Dawley rats were fed for 4 wks a PA deficient diet, ad lib; a PA supplemented diet, ad lib; or a PA supplemented diet, pair-fed to the PA deficient group. The pair-fed group exhibited a distinctive pattern of plasma [triglyceride, TG] and [free fatty acid, FFA] which can be explained by different feeding conditions. [TG] and [FFA] in 24 h fasting plasma were higher in the PA deficient group than in the PA supplemented, ad lib group at wk 2, but not at wk 4. At wk 4, plasma [TG] and [FFA] in the PA deficient group were comparable to those in the PA supplemented group and coincided with elevated plasma [PA] in response to the 24 h fast. At wk 4 the significantly decreased liver [CoASH] and [long chain acyl-CoA] seen in the PA deficient rats compared to those in the PA supplemented were not related to the altered plasma [TG] and [FFA] in 24 h fasting samples. PA deficiency alters plasma [TG] and [FFA] more severely at early stage of the vitamin deficiency by a mechanism other than through a reduction in tissue [total CoA].

To my husband

ACKNOWLEDGEMENTS

I would like to express my great appreciation to my advisor Dr. Won Song for her support and encouragement. I would like to thank Dr. Maurice Bennink and Dr. Dale Romsos for their willingness to serve on my committee and valuable input. I thank my colleagues Kathi Annous, Ritu Ummadi and Kati Chevaux for their friendship. I would like to thank my parents and family in Korea for their love. Finally I would like to thank my husband Seung-Jae for his love and understanding.

TABLE OF CONTENTS

LIST OF TABLES

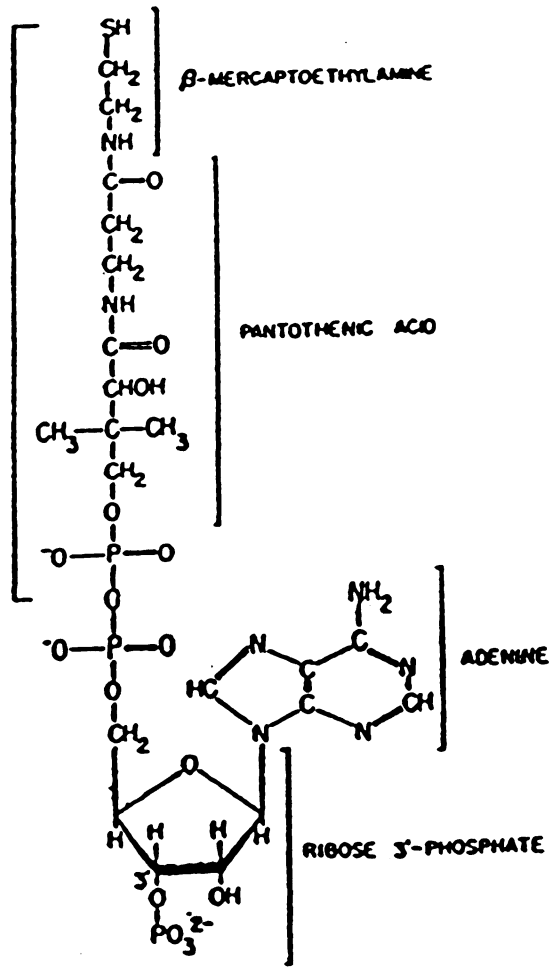
LIST OF FIGURES

INTRODUCTION

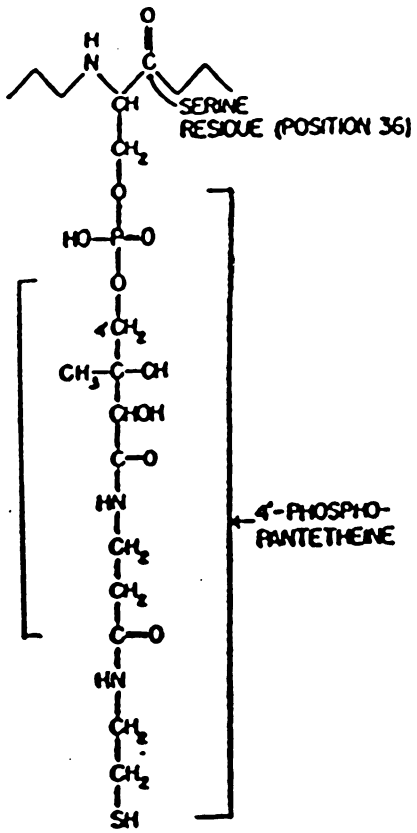
A close relationship is expected between pantothenic acid and lipid metabolism because the B vitamin is present in CoA and in acyl carrier protein (ACP) of fatty acid synthase as 4'-phosphopantetheine (Figure 1). The two pantothenic acid coenzymes transfer various lengths of acyl compounds as covalently bound thioesters in energy metabolism, and in biosynthesis of many vital compounds (Abiko, 1975; Robishaw and Neely, 1985; Wakil et al., 1983).

In general, pantothenic acid deficiency resulted in significantly decreased tissue CoA concentrations when weanling rats were studied (Moiseenok et al., 1987; Srinivasan and Belavady, 1976; Wittwer et al., 1990). It has also been reported that older rats are more difficult than younger rats to induce a pantothenic acid deficiency that is severe enough to decrease tissue CoA concentrations (Reibel et al, 1982; Robishaw and Neely, 1985). The pantothenic acid requirement of the rats has been reported to decrease with increasing age (Unna and Richards, 1942; Barboriak et al., 1956).

Only a few studies have reported on the pantothenic acid status related to the lipid metabolism in vivo. Carter and Hockaday (1962) reported that the capacity of liver



CoA



ACP

FIGURE 1 Structures of CoA and ACP

slices to oxidize exogenous energy substrate, octanoate, as measured by oxygen uptake and ketone body production, was lower in pantothenic acid deficient rats than in the normal control. Mild pantothenic acid deficient rats which did not show a growth retardation had significantly elevated serum triglyceride and free fatty acid concentrations (Wittwer et al., 1990). The mechanism for the altered lipid metabolism in pantothenic acid deficiency is uncertain yet. Schaefer et al (1942) reported that dogs fed a pantothenic acid deficient diet developed a fatty liver. Lower liver fat content has been observed in the pantothenic acid deficient rats (Carter and Hockaday, 1962; Guehring et al., 1952). Carcass fat was significantly less in pantothenic acid deficient rats (Carter and Hockaday, 1962; Srinivasan and Belavady 1975). These observations might suggest that pantothenic acid deficiency affects lipid metabolism in liver and thus causes changes in concentrations of circulatory lipids and body fat content.

The goal of this study was to elucidate how lipid metabolism is affected by pantothenic acid deficiency when the vitamin was deprived in weanling rats' diet. Hypothesis of this study is that lipid metabolism is impaired in pantothenic acid deficient rats due to limited availability of pantothenic acid and CoA in blood or tissues. The specific objectives were:

(1) to determine the triglyceride and free fatty acid concentrations in plasma,

(2) to determine the pantothenic acid concentrations in plasma and tissues,

(3) to determine the CoA concentrations in liver and muscle, organs that are important in lipid metabolism.

REVIEW OF LITERATURE

1. Pantothenic acid in lipid metabolism

CoA and ACP of fatty acid synthase are the major recognized coenzyme forms of pantothenic acid. As the universal carriers of acyl groups, the coenzymes are particularly important in both fatty acid oxidation and fatty acid synthesis (Abiko, 1975; Robishaw and Neely, 1985; Wakil et al., 1983). The lipid metabolism and content in pantothenic acid deficiency have been studied in rats and dogs by the researchers (Table 1).

In the study of Carter and Hockaday (1962), rats weighing 25-30 g were fed a pantothenic acid deficient diet for 80 days, and fasted for 17 h before sacrifice. Liver slices from the rats in the pantothenic acid deficient and control groups were measured for oxygen uptake and ketone production. Liver slices from the pantothenic deficient rats had significantly decreased oxygen uptake and ketone production, compared to those from the control group, when octanoate was added. No significant differences were seen between the groups when no substrate was added. The data suggest that the ability to utilize energy substrates is impaired in pantothenic acid deficiency.

Wittwer et al (1990) investigated the relationship

TABLE 1
Reported lipid metabolism modulated by pantothenic acid nutriture

Animals	Treatment	Major findings[1]	References
Rats, 55-60 g	PA- diet, 80 days	↓ oxygen uptake ↓ ketone production ↓ liver fat ↓ carcass fat	Carter and Hockaday 1962
Rats, Weanling	PA- diet, 2 wks	↑ serum TG ↑ serum FFA	Wittwer et al. 1990
Rats, Weanling	PA- diet, 5 wks	↓ liver fat	Guehring et al. 1952
Rats, Weanling	PA- diet, 6 wks	↓ carcass fat	Srinivasan & Belavady 1975
Dogs	PA- diet, 11 wks	↑ liver fat	Schaefer et al. 1942

[1] In comparison with the data of normal control group ($P < 0.05$).
Abbreviations: PA- (pantothenic acid deficient diet), TG (triglyceride), FFA (free fatty acid).

between pantothenic acid status of rats and circulating serum triglycerides and free fatty acids. Serum triglyceride and free fatty acid concentrations were significantly elevated when 6-wk-old rats were fed a pantothenic acid deficient diet for 2 wks of feeding. No similar observations were made when the rats were fed the same diet for 5 wks. The data indicated that an adaptive metabolic response to pantothenic acid deficiency occurred with increasing age.

The increased serum triglyceride concentrations could be due either to increased production of VLDL by the liver or to decreased removal of VLDL from the blood. Increased serum free fatty acid concentrations in pantothenic acid deficiency might result from either increased fat mobilization from the adipose tissue or decreased fatty acid oxidation in liver and other tissues under fasting condition. Pantothenic acid deficiency has been reported to decrease CoA concentrations in many tissues including liver and adipose tissues (Moiseenok et al., 1987; Smith et al., 1987; Srinivasan and Belavady, 1976). Decreased CoA availability in the tissues might result in the altered serum triglyceride and free fatty acid concentrations in pantothenic acid deficiency. The mechanism by which lipid metabolism is altered in the study of Wittwer et al (1990) and Carter and Hockaday (1962) is yet uncertain.

Dogs fed a pantothenic acid deficient diet for 11 wks showed fatty livers (Schaefer et al., 1942). The

pantothenic acid deficient dogs had 45 % fat of dry liver wt, whereas the control dogs had 13-17 % fat of dry liver. In contrast, lower fat content in liver has been observed in the pantothenic acid deficient rats than in control with 2.3 and 10.1 % fat of dry liver wt, respectively (Carter and Hockaday, 1962); 2.4 and 11.2 % fat of dry liver wt, respectively (Guehring et al., 1952). Carter and Hockaday (1962) observed a lower carcass fat content in rats fed for 11 wks a pantothenic acid deficient diet than in control (3.4 vs. 9.8 g/100 g wet wt, respectively). Srinivasan and Belavady (1975; Table 1) also reported that male Wistar rats fed a pantothenic acid deficient for 6 wks had significantly less carcass fat than the control (9.98 vs. 17.70 g /100 g wet wt, respectively). These observations suggest that pantothenic acid deficiency affects lipid metabolism in liver, and changes circulatory lipids and body fat content.

CoA metabolism was investigated in vitro in relation to energy source in the heart with elevated total CoA (Lopaschuk et al., 1986; Lopaschuk and Neely 1987). Total CoA concentrations were elevated from 537 ± 14 to 818 ± 44 nmol/g dry wt by perfusing hearts with Krebs-Henseleit buffer containing 0.1 mM cystein, 0.2 mM dithiothreitol, 15 uM pantothenic acid, and no energy substrate in the study of Lopaschuk et al (1986). The hearts with elevated concentrations of CoA had an increase in conversion of exogenous [U-^{14}C]-palmitate to triglyceride than the hearts with normal concentrations of CoA (20.6 ± 1.1 and 13.5 ± 1.9

umol/g dry wt, respectively, means±SE), and had a 50 % reduced apparent rate of oxidation of exogenous [U-^{14}C]-palmitate as measured by $^{14}CO_2$ production (0.47 ± 0.16 and 0.97 ± 0.17 umol/g min, respectively, means±SE). These data demonstrate that the elevated myocardial CoA concentrations increase palmitate conversion to triglyceride, and decrease palmitate oxidation. It is speculated that the reported increase in CoA concentrations in liver and heart under fast affect fatty acid metabolism.

When the hearts with elevated concentration of CoA (818 ± 44 nmol/g dry wt) were perfused for 3 min with buffer containing 11 mM glucose and 5 mM pyruvate for 3 min, CoA concentrations were not changed (789 ± 42 nmol/ g dry wt, means±SE; Lopaschuk and Neely, 1987). However, when the hearts were perfused with a buffer containing 11 mM glucose and 1.2 mM palmitate, CoA concentrations were significantly decreased within 3 min to 683 ± 34 nmol/g dry wt. The rapid loss of total cellular CoA in hearts perfused with palmitate, appeared to be localized in the cytosolic compartment (no substrate vs. 11 mM glucose and 5 mM pyruvate vs. 11 mM glucose and 1.2 mM palmitate ; mitochondrial CoA, 1.97 ± 0.1 vs. 1.90 ± 0.15 vs. 1.98 ± 0.1 nmol/mg mitochondrial protein, 53 mg mitochondrial protein/g wet wt; cytosolic CoA, 133 ± 14.7 vs. 107 ± 13.9 vs. 82 ± 4.2 nmol/g dry wt, means±SE). Perfusing the hearts containing elevated total CoA with 11 mM glucose and 1.2 mM palmitate resulted in significantly lower concentrations of

short-chain acyl-CoA and free CoA (159 ± 22 and 251 ± 14 nmol/g dry wt, respectively, means±SE) than perfusing the hearts with 11 mM glucose and 5 mM pyruvate (351 ± 33 and 343 ± 16 nmol/g dry wt, respectively, means±SE). The long-chain acyl-CoA concentrations were significantly increased in the hearts which were perfused with 1.2 mM palmitate (305 ± 6 nmol/g dry wt), compared to the hearts which were perfused with pyruvate (118 ± 21 nmol/g dry wt). The cytosolic long-chain acyl-CoA was elevated with a significant increase in [^{14}C]-palmitate incorporation into triglyceride (20.6 ± 1.1 in elevated CoA vs. 13.5 ± 1.9 umol/g dry wt in control, means+SE).

Lopaschuk and Neely (1987) concluded that the loss of total myocardial CoA, in the presence of palmitate, was associated with production of cytosolic acyl-CoA. This association suggests that abnormally high concentrations of long-chain acyl-CoA subsequent to fatty acid perfusion may stimulate CoA degradation. Whether increased concentrations of CoA in fasting in vivo alters fatty acid utilization or altered fatty acid metabolism in fasting results in increased concentrations of CoA can not be answered with the findings made in above in vitro studies.

Extensive studies have been done with pantethine, a disulfide form of pantetheine, in vivo or in vitro, to elucidate the mechanism by which altered lipid metabolism can be explained in pantothenic acid deficiency. Morisaki et al (1983) showed that [^{14}-C]-palmitate oxidation is

decreased in brain microvessels of spontaneously hypertensive rats (SHR). When SHR were fed a diet containing 0.2 % pantethine for 4 wks, fatty acid oxidation activity, measured by $^{14}CO_2$ production, was restored to the control concentrations in brain microvessels (control, SHR and SHR+pantethine, mean±SD; 2.34 ± 0.05, 1.25 ± 0.38 and 2.11 ± 0.54 $x10^6$ dpm/g wet wt, respectively). Pantethine and its derivatives (i.e., pantetheine and 4'-phosphopantetheine) activated fatty acid oxidation by increasing the activity of acyl-CoA synthetase (1.5 - 3.4 times), carnitine acyltransferase (1.2 - 2 times) and intramitochondrial ß-oxidation (1.2 - 1.8 times).

In the study of Noma et al (1984), male Wistar rats weighing 130 - 150 g were given either water (control) or pantethine (250 mg/ 0.4 ml/ rat) daily by a stomach tube for 3 wks. After an overnight fast, blood was collected for determination of the plasma lipids and post-heparin lipoprotein lipase activity. The pantethine-treated group showed significantly lower plasma concentrations of triglyceride, total cholesterol and phospholipids (142.8 ± 9.9, 45.9 ± 2, and 106.7 ± 3.1 mg/dl, respectively) compared to the control (186.0 ± 6.9, 58.7 ± 1.9, and 132.4 ± 3.4 mg/dl, respectively). Post-heparin plasma lipoprotein lipase activity which was separated from hepatic lipoprotein lipase by heparin-Sepharose affinity chromatography, was higher in pantethine-treated group than in the control (5.69 ± 0.21 vs. 4.14 ± 0.13 umol oleic acid/h/ml). The lower

plasma triglyceride concentrations in pantethine-treated group can be explained by higher post-heparin plasma lipoprotein lipase activity than in control.

The effect of pantethine, a pantothenate derivative, on lipogenesis and fatty acid oxidation, was studied with hepatocytes from 10-wk old White Leghorn chicks (Hsu et al., 1992). Lipogenesis was determined by incorporation of [^{14}C]-acetate into various lipid fractions (i.e., free and ester of cholesterol, triglyceride, diglyceride, monoglyceride, and phospholipid). The researchers reported that the lipogenesis was decreased as pantethine concentrations in the hepatocyte media increased in the range of 0, 0.1, 0.5, 1.0, 2.0, 5.0 mg pantethine/ml medium. The enzymes responsible for the fatty acid synthesis (i.e., acetyl-CoA synthetase and fatty acid synthase) were also significantly lower in the hepatocytes incubated in pantethine supplemented medium (5.0 mg/ml) than those without. CO_2 production from [^{14}C]-palmitate was also decreased in the hepatocytes incubated in 0.1 mg pantethine/ ml medium than those without pantethine supplementation.

Pantethine is marketed in Italy (Pantetina) and Japan (Pantosin) as a hypolipidemic drug (Wittwer et al., 1985). The administration of a hypolipidemic drug, clofibrate, increased the amount of hepatic total CoA from 2.27 nmol/mg protein to 4.83 nmol/mg protein. The increase in hepatic total CoA was mainly from increased concentrations of free CoA. Long-chain acyl-CoA was only 10 % of the total CoA

(0.48 nmol/mg protein) in the clofibrate-treated liver (Skrede and Halvorsen, 1979). Total CoA in rat liver was also increased by other hypolipidemic drugs such as bezafibrate, ciprofibrate, fenofibrate and tiadenol, but not by pantethine (Halvorsen, 1983). The hypolipidemic drugs increased the activity of carnitine palmitoyltransferase, which is responsible for the transport of activated fatty acids into the mitochondria, and pantothenate kinase in the liver (Halvorsen, 1983).

Oxidation of fatty acids is thus thought to be stimulated along with CoA synthesis by the hypolipidemic drugs. Although clinical relationships between pantothenic acid, CoA and lipid metabolism have continuously appeared in the literature (Carter and Hockaday, 1962; Lopaschuk et al., 1986; Lopaschuk and Neely, 1987; Morisaki et al., 1982; Wittwer et al., 1990), none of the studies examined the relation between pantothenic acid status (including pantothenic acid in plasma and tissues, and CoA in tissues) and the altered lipid metabolism in pantothenic acid deficiency.

2. Synthesis and degradation of CoA

The pathway for CoA synthesis was first proposed by Brown (1959) and later confirmed by Abiko (1967). CoA consists of 3'-phosphoadenosine coupled through the 5'-position of the ribose to pantothenic acid by a pyrophosphate linkage; the carboxyl end of pantothenic acid

is linked through a peptidic linkage to ß-mercaptoethylamine (cysteamine). Three substrates (pantothenic acid, ATP and cysteine) are needed to synthesize CoA (Abiko, 1975; Robishaw and Neely, 1985).

In the synthesis of CoA (Figure 2 and Table 2), pantothenic acid is first phosphorylated to 4'-phosphopantothenic acid by the action of pantothenate kinase. The apparent K_m is 0.011 mM for D-pantothenic acid and 1 mM for ATP in rat liver (Abiko, 1975). The second step is the condensation between 4'-phosphopantothenic acid and L-cysteine in the presence of CTP or ATP and MG^{2+} to form 4'-phosphopantothenoyl-cysteine. This step is catalyzed by phosphopantothenoyl-cysteine synthetase (K_m for 4'-phosphopantothenate is 0.071 mM in rat liver). 4'-phosphopantothenoyl-cysteine is then decarboxylated to 4'-phosphopantetheine by the action of phosphopantothenoyl-cysteine decarboxylase (K_m for 4'-phosphopantothenoyl-cysteine is 0.14 mM for rat liver enzyme). These first three enzymes in the pathway appeared to be located exclusively in the cytosol.

In the final two steps in the pathway, 4'-phosphopantetheine is adenylated to form dephospho-CoA, and dephospho-CoA is phosphorylated at the 3' position of ribose to form CoA. The enzymes catalyzing these reactions are dephospho-CoA pyrophosphorylase (Km for 4'-phosphopantetheine is 0.14 mM in rat liver) and dephospho-CoA kinase (K_m of mitochondrial dephospho-CoA kinase for

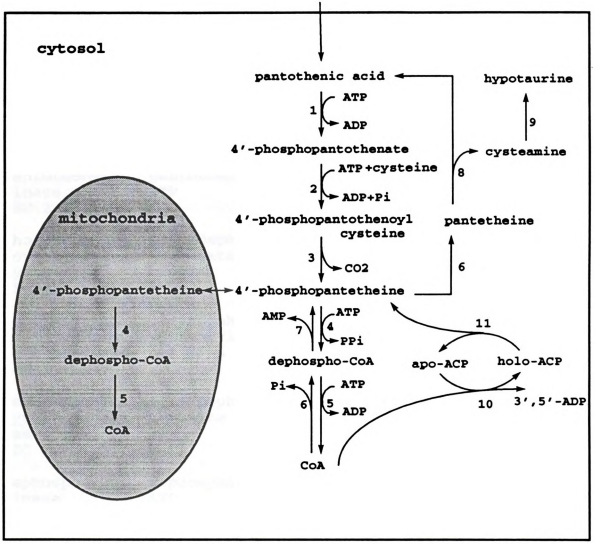


FIGURE 2. Synthetic and degradative pathways of CoA and ACP (From Song and Wyse, 1993)

1 Pantothenate kinase (EC 2.7.1.33)
2 Phophopntothenoylcysteine synthetase (EC 6.3.2.5)
3 Phophopantothenoylcysteine decarboxylase (EC 4.1.1.36)
4 Dephospho-CoA pyrophosphorylase (EC 2.7.7.3)
5 Dephospho-CoA kinase (EC 2.7.1.24)
6 Acid phosphatase (EC 3.1.3.2)
7 Dephospho-CoA pyrophosphatase (EC 3.6.1.15)
8 Pantetheinase
9 Oxygenase
10 Holo-ACP synthetase (EC 2.7.8.7)
11 ACP hydrolase (EC 3.1.4.14)

TABLE 2
Enzymes in CoA synthetic pathway and their K_m values for substrates

Enzymes	Substrates	K_m	Tissue	References
		mM		
Pantothenate kinase (EC 2.7.1.33)	pantothenate	0.011	rat liver	Abiko, 1975
	ATP	1		
Phosphopantheinoyl-cysteine synthetase (EC 6.3.2.5)	4'-phosphopantothenate	0.071	rat liver	Abiko,1975
Phosphopantheinoyl-cysteine decarboxylase (EC 4.1.1.36)	4'-phosphopantothenoyl-cysteine	0.14	rat liver	Abiko, 1975
Dephospho-CoA pyrophosphorylase (EC 2.7.7.3)	4'-phosphopantetheine	0.14	rat liver	Abiko, 1975
	ATP	1	rat liver	
Dephospho-CoA kinase (EC 2.7.1.24)	dephospho-CoA	0.01	rat liver	Skrede and Halvorsen 1979
	ATP	0.05	rat liver	
	dephospho-CoA	0.12	rat liver	Abiko, 1975
	ATP	0.36	rat liver	

dephospho-CoA is about 0.01 mM). Skrede and Halvorsen (1979) reported that these final two enzymes are present in the inner mitochondrial membrane as well as in the cytosol. Thus 4'-phosphopantetheine is the initial precursor for mitochondrial CoA synthesis.

It has been generally understood that CoA synthesis is controlled at the pantothenate kinase catalyzed step. In an in vitro experiment with partially purified pantothenate kinase from rat kidney, both CoA and dephospho-CoA had the most potent inhibitory effect on the enzyme followed by 4'-phosphopantetheine and its disulfide, 4'-phosphopantethine (Karasawa et al., 1972). Palmitoyl-CoA and 4'-phosphopantothenate had a low inhibitory effect at the concentration of 10^{-3} M. On the other hand, none of the nucleotides possessing adenylate moiety in their structure, such as AMP, ADP, 3' 5'-ADP and NAD inhibited the enzyme activity at all the concentration applied (10^{-3}, 10^{-4} and 10^{-5} M). Halvorsen and Skrede (1982) also investigated the inhibitory effect of CoA, dephospho-CoA and various acyl-CoA thioesters on partially purified pantothenate kinase from particle-free supernatant from the liver of normal or clofibrate-fed rats. All CoA derivatives tested at 0.02 mM inhibited 17-92 % of the pantothenate kinase activity of normal rats with varying degree: 43 % by CoA, 85-92 % by short-chain acyl-CoA derivatives. The activity of pantothenate kinase was more than doubled after clofibrate treatment (Skrede and Halvorsen, 1979). It has been found

that pantethine is much more effective than pantothenic acid in promoting the synthesis of CoA in rat liver (Branca et al., 1984). Pantethine (0.1 mM in the perfusion medium) increased the total CoA concentrations approximately 20-30 % in perfused rat liver. Under the same condition, pantothenic acid (0.1 mM in the perfusion medium which does not contain ATP and cysteine for CoA synthesis) had no effect on CoA concentrations in liver. They didn't measure the effect of any other intermediates on CoA synthesis.

The pathway and regulation of CoA degradation are less well understood than the synthetic pathway. Enzymatic degradation of CoA was first studied by Novelli and his coworkers (Novelli et al., 1953). The sequence of the intracellular degradation reactions of CoA seems to be nearly a reverse of synthetic pathway (Fig.2., Abiko, 1975): in rat liver, CoA is dephosphorylated at the 3' position of ribose to form dephospho-CoA by lysosomal acid phosphatase. Then, dephospho-CoA is subjected to pyrophosphate bond cleavage to yield 4'-phosphopantetheine and 5'-AMP by the action of a dephospho-CoA pyrophosphatase in the plasma membrane of microsomal and nuclear fractions. 4'-phosphopantetheine is dephosphorylated to form pantetheine by the action of a lysosomal acid phosphatase.

In the final step, pantetheine is degraded to pantothenic acid and cysteamine by the action of pantetheinase. Of the two products of the reactions, only cysteamine is further metabolized to hypotaurine by the

action of a specific oxygenase. It is not known at this point whether the pantothenic acid and other intermediates produced intracellularly as the result of CoA degradation reenter the synthetic pathway or are excreted (Robishaw and Neely, 1985).

3. Pantothenic acid and CoA concentrations

Pantothenic acid and CoA concentrations in many tissues (e.g., liver, heart, kidney, etc.) of rats and mice are changed under different dietary, metabolic and hormonal conditions.

(1) Dietary effect

Pantothenic acid deficiency results in low pantothenic acid and/or CoA concentrations in tissues (Table 3; Moiseenok et al., 1987; Smith et al., 1987; Srinivasan and Belavady, 1976). Albino rats, 80-90 g, which were fed a pantothenic acid deficient diet had decreased hepatic total CoA concentrations only after 11 days and throughout 44 days of feeding (Moiseenok et al., 1987). Weanling Wistar albino rats fed a pantothenic acid deficient diet for 6 wks had decreased hepatic total CoA concentrations (Srinivasan and Belavady, 1976). Smith et al (1987) reported that mice fed a pantothenic acid deficient diet for 65 days had 71 % to 91 % decreased free pantothenic acid concentrations, compared to the normal control mice, in all tissues examined (liver, kidney, brain, spleen, epididymal fat pads, heart, diaphragm and leg skeletal muscle). The total CoA concentrations were

TABLE 3
Reported data on CoA concentrations in tissues of pantothenic acid deficient and control groups

Animals	Treatment	Measurements	References
Rats, 80-90 g	11 days PA- PA+	hepatic total CoA 287±6.9 nmol/g 320±11.6 nmol/g	Moiseenok et al. 1987
	44 days PA- PA+	hepatic total CoA 223±12.6 nmol/g 306±15.5 nmol/g	
Rats, Weanling	6 wks PA- PA+	hepatic CoA 53.2±7.9 units/g 120.6±10.8 units/g	Srinivasan & Belavady 1976
Rats, 250-300 g	4 and 8 wks PA-	total CoA in tissues no change	Reibel et al. 1982
Mice	65 days PA-	Tissue total CoA 18-54 % decrease in liver, kidney, spleen, diaphragm and skeletal muscle compared to control	Smith et al. 1987

Abbreviations: PA- (pantothenic acid deficient diet), PA+ (pantothenic acid supplemented diet).

also decreased in all tissues except brain and epididymal fat pads (liver 18 % decrease, kidney 23 %, spleen 21 %, heart 38 %, skeletal muscle 66 %). The total tissue CoA concentrations of the pantothenic acid deficient mice did not decrease further after 105 days of feeding.

However, not all investigators have found that diet-induced pantothenic acid deficiency decreases CoA concentrations in tissues (Reibel et al., 1982; Karasawa et al., 1971). Sprague-Dawley rats weighing 250 - 300 g and fed a pantothenic acid deficient diet for 4 wks maintained CoA concentrations in many organs (liver, heart, kidney, gastrocnemius, testes, diaphragm and adrenal), even though pantothenic acid concentrations were significantly decreased (Reibel et al., 1982). It is possible that some tissues that were not studied had low concentrations of CoA, since CoA concentrations in the whole carcass were not determined by Reibel et al. (1982). Pantothenic acid deficiency that is severe enough to decrease tissue CoA concentrations is difficult to induce in adult rats and humans (Robishaw and Neely, 1985). This may be due to a relatively slow rate of CoA degradation, reutilization of pantothenic acid degraded from CoA and holo-ACP and conservation of body stores of pantothenic acid in many different forms.

As shown in Table 4, Wittwer et al (1990) investigated that the effect of dietary fat on the blood lipid abnormalities (i.e., increased serum triglyceride and free fatty acid concentrations) in pantothenic acid deficient

TABLE 4
Pantothenic acid and CoA concentrations modulated by diet, fast and diabetes

Treatment	Major findings[1]	References
High fat diet		
40 %	↑ hepatic total CoA compared to 20 % fat diet group	Wittwer et al. 1990
18 %	↓ hepatic total CoA compared to 6 % fat diet group	Williams et al. 1968
Fasting: 48 h	↑ PA and CoA in tissues	Smith et al. 1987 Reibel et al. 1982
Alloxan-induced diabetes	↑ hepatic total CoA	Smith et al. 1978 Reibel et al. 1982

[1] In comparison with the data of control ($P < 0.05$).

male rats. Three-wk-old rats were fed for 2 wks a 20, 30, or 40 energy % fat diet with or without pantothenic acid supplementation (10 mg pantothenic acid/kg diet, n=5/group). Hepatic total CoA concentrations were significantly depressed in pantothenic acid deficient groups. The rats fed a 40 % fat diet had higher total CoA concentrations in liver compared to the rats fed a 20 or 30 % fat in both pantothenic acid deficient and control groups. This observation may be explained by the fact that in a high fat diet fatty acid ß-oxidation in liver is accelerated by high concentrations of long-chain acyl CoA, so increased concentrations of CoA are needed in order to respond to the metabolic change in the body.

However, in the study of Williams et al (1968), liver total CoA concentrations in both control and deficient groups fed the 18 % fat diet by wt (175 ± 22 vs. 140 ± 8 nmol/g wet wt, respectively, mean+SE) were significantly lower than those in the rats fed the 6 % fat diet by wt (353 ± 39 vs. 235 ± 26 nmol/g wet wt, respectively, mean+SE). An explanation for the discrepancy in the findings between above two studies is not obvious. However, many conditions such as strain and age of rats, dietary component and analytical methods were different between the studies of William et al (1968) and Wittwer et al (1990).

The effects of high fat diets (20-30 % by wt) containing various oils on the hepatic long-chain acyl CoA concentrations were studied in rats by Nilsson et al.

(1984). Diets supplemented with a partially hydrogenated marine oil (5, 10 15, 20, or 30 % fat by wt) resulted in higher concentrations of long-chain acyl-CoA in the liver than the ones containing soybean oil. This difference was especially prominent with 25 % or 30 % oil in the diet. The same researchers found that peroxisomal ß-oxidation of fatty acid in the liver positively correlated with the amount of long-chain acyl-CoA in the liver. This finding supports the fact that increased concentrations of long-chain acyl-CoA in high fat diet facilitate the fatty acid oxidation (Voet and Voet, 1990). They didn't measure fatty acid ß-oxidation in the liver mitochondria.

(2) Metabolic and hormonal effects

Fasting and diabetes in rat increase CoA concentrations of liver and heart, regardless of the amount of pantothenic acid in the diet (Table 4; Beinlich et al., 1989; Reibel et al., 1981, 1982; Smith et al., 1987).

Smith et al (1987) reported that fast for 48 h significantly increased the total CoA concentrations in the heart and liver of both pantothenic acid deficient and control groups (heart, 19 and 32 %; liver, 45 and 39 %, respectively). Under the same experimental condition, total CoA concentrations of both pantothenic acid deficient and control groups decreased in epididymal fat pads (38 and 28 %, respectively), diaphragm (49 and 25 %, respectively) and skeletal muscle (22 and no change, respectively).

Compared to normal control rats, alloxan--induced diabetic rats have a significantly higher concentrations of liver total CoA (388 ± 54 and 723 ± 131 nmol/g wet wt, respectively, mean±SD); whereas a significantly lower concentration of heart total CoA (138 ± 36 and 118 ± 36 nmol/g wet wt, respectively, mean±SD) (Smith et al. (1978). Reibel et al (1981) also showed that a reduced uptake and incorporation of pantothenic acid into CoA in the diabetic heart.

Beinlich et al (1989) investigated if hearts from diabetic rats retain the ability to stimulate CoA synthesis when exposed to the condition known to stimulate CoA synthesis (perfusing hearts with Krebs-Henseleit buffer containing 0.1 mM cystein, 0.2 mM dithiothreitol, 15 uM pantothenic acid, and no energy substrate) which was developed by Lopaschuk et al (1986). Hearts from control rats increased their CoA concentrations by 287 nmol/g dry (the difference in tissue CoA at 10 and 70 min). Hearts removed from rats 18 h after diabetes was induced, had elevated tissue concentrations of CoA compared to the control (650 and 550 nmol/g dry wt, respectively). The heart from 18 h diabetic rats continued to increase CoA concentrations during perfusion for 1 h. The increase, however, was 68 % of that observed in the control hearts (196 and 287 nmol/g dry wt, respectively). Hearts removed after 48 and 84 h diabetes were not able to elevate total CoA concentration which was obtained after 18 h diabetes.

Hearts from fats that were diabetic for 9 days contained CpA concentrations similar to that of the control; CoA accumulation during the in vitro perfusion was 26 % of the control. The data indicated that the hearts from rats that had been diabetic for more than 9 days could not sustain the stimulated rate of CoA synthesis. This reduced CoA synthesis in diabetic hearts could have resulted from reduced pantothenic acid uptake and incorporation of pantothenic acid to CoA found in this study and the study of Reibel et al (1981).

The response of hepatic total CoA to insulin was obtained by measuring liver total CoA in alloxan-diabetic rats 2 h after the rats received 0.2 units Lente insulin by intraperitoneal injection (Smith et al. 1978). The insulin injection caused a rapid decrease of mitochondrial total CoA concentrations from 7.7 to 4.2 nmol/mg protein in liver. In exercised hearts from control rats receiving 11 mM glucose as the only exogenous substrate, addition of insulin to the perfusate resulted in 90 % inhibition of CoA synthesis (from 20.20 ± 1.8 to 2.10 ± 0.5 nmol/g dry wt/h, mean±SE) with no change in pantothenic acid uptake. Interestingly, glucagon had no effect on either uptake or incorporation of pantothenic acid into CoA in heart from the normal control rats (Reibel et al., 1981). The body sources of pantothenic acid for the elevated CoA concentrations in liver and heart in fasting and diabetes, and possible mechanisms for increased CoA concentrations need to be further studied.

4. Fatty acid metabolism

Fatty acids are oxidized to acetyl-CoA in the mitochondria and synthesized from acetyl-CoA in the cytosol. Although the chemical stages involved are comparable, the two pathways that take place in two separate compartments of the cell. The separation of fatty acid oxidation from synthesis allows each process to be individually controlled and integrated with tissue requirements (Guzman and Geelen, 1993; Wakil, 1983).

(1) Fatty acid oxidation

Fatty acids must be activated in the cytosol before they can be oxidized in the mitochondria. The activated long-chain acyl-CoA is transported into the mitochondria by the long-chain carnitine palmitoyl-transferase system. Fatty acyl-CoAs are then metabolized by ß-oxidation and the resulting acetyl-CoAs are further oxidized to CO_2 and H_2O (Guzman and Geelen, 1993; Mayes, 1990). CoA functions as an activator of long-chain fatty acids in cytosol and an acyl carrier in mitochondria in fatty acid oxidation.

Fatty acid oxidation is regulated largely by the concentration of free fatty acids in the blood, which is, in turn, controlled by the hydrolysis rate of triglyceride in adipose tissue by a hormone-sensitive lipase. Epinephrine and norepinephrine, as does glucagon, act to increase adipose tissue cAMP concentrations. The cAMP allosterically activates cAMP-dependent protein kinase which, in turn,

increases the phosphorylation of the hormone-sensitive lipase. The activated hormone-sensitive lipase by phosphorylation stimulates lipolysis in adipose tissue, raises blood concentrations of free fatty acid, and ultimately activates the ß-oxidation pathway in other tissues such as liver and muscle.

Insulin has the opposite effect of glucagon and epinephrine. Insulin decreases the cAMP concentration which leads to dephosphorylation and thus inactivation of the hormone-sensitive lipase. Insulin thereby reduces the amount of free fatty acid in blood that is available for oxidation. Malonyl-CoA in cytosol inhibits carnitine acyltransferase I, and thereby reduces the transfer of acyl-CoA into the mitochondria for ß-oxidation (Moir and Zammit, 1993).

(2) Fatty acid synthesis

Acetyl-CoA, formed from pyruvate by the action of pyruvate dehydrogenase in mitochondria, is the major building block for long-chain fatty acid synthesis in cytosol. The cell needs to transport out acetyl-CoA through the mitochondrial membrane by allowing acetyl-CoA to form citrate in the citric acid cycle and transporting the citrate out of the mitochondria by tricarboxylate transporter system. Citrate transported out to cytosol produce acetyl-CoA for fatty acid synthesis by the action of an ATP-citrate lyase. Acetyl-CoA is then carboxylated to

malonyl-CoA in the presence of ATP by acetyl-CoA carboxylase. Then, the malonyl group is elongated by sequential addition of two carbon units derived from malonyl-CoA. Palmitate is produced as an end product by the action of thioesterase in the fatty acid synthase system (Mayes, 1990; Volpe and Vagelos, 1973; Wakil, 1983).

Fatty acid synthase There are two types of fatty acid synthase systems in the soluble portion of the cell. In bacteria, plants and lower forms, the individual enzymes of the system are separate, and acyl intermediates are found in combination with the ACP which contains 4'-phosphopantetheine. However, the synthase system in yeast, mammals and birds, is a multifunctional enzyme complex which is resistant to dissociation. Dissociated component proteins lose the catalytic activity. ACP containing 4'-phosphopantetheine has not yet been isolated in a pure form (Roncari et al., 1972).

The fatty acid synthase in yeast, mammals, and birds is a dimer with identical two monomers. Monomer consists of one stable polypeptide chain containing all six enzymes of fatty acid synthase (ketoacyl synthase, transacylase, enoyl reductase, hydratase, ketoacyl reductase and thioesterase) and an ACP with a 4'-phosphopantetheine (Mayes, 1990; Wakil et al., 1983). In close proximity to the -SH end of 4'-phosphopantetheine is another thiol of a cysteine residue attached to the pair that interacts in a head-to-tail manner.

The intermediates in fatty acid synthesis are bound in thioester linkage to the -SH end of a 4'-phosphopantetheine which is attached as a phosphodiester to a serine residue of the ACP (Majerus et al. 1965). The 4'-phosphopantetheine arm enables the growing fatty acid chain to come into close contact with the active site of each enzyme in the fatty acid synthase complex. The efficiency of the overall synthetic process of fatty acid synthase is high because intermediates are directly transferred from one active site to the next without being diluted in the cytosol or interfered by competing processes (Mayes, 1990; Volpe and Vagelos, 1973).

Only the fatty acid synthase with the ACP containing 4'-phosphopantetheine (holo-ACP) is active. The holo-ACP is synthesized by holo-ACP synthetase which transfers 4'-phosphopantetheine from reduced free CoA to apo-ACP (Figure 2). This enzyme isolated from E.Coli had apparent K_m's of 0.4 uM and 150 uM for apo-ACP and free CoA, respectively and requires Mg^{++} or Mn^{++} for activity (Elovson and Vagelos, 1968). It is noteworthy that holo-ACP has about 400 times higher K_m value for free CoA than apo-ACP. Partially purified rat liver holo-ACP synthetase utilizes rat liver apo-ACP and either CoA or E.coli ACP as substrates (K_m for CoA is approximately 0.6 uM, Burton et al., 1979).

ACP hydrolase isolated from E.coli breaks down E.Coli holo-ACP to a 4'-phosphopantetheine and an apo-ACP (Fig.2). The ACP hydrolase is very specific for intact E.Coli holo-

ACP and is inactive with mammalian holo-ACP (Vagelos and Larrabee, 1967). Rat liver ACP hydrolase also converts a holo-ACP to an apo-ACP and a 4'-phosphopantetheine, the prosthetic group. The rat liver ACP hydrolase is also specific for the rat liver holo-ACP. It does not hydrolyze 4'-phosphopantetheine from pigeon liver holo-ACP or from CoA (Burton et al., 1979).

The turnover rate of 4'-phosphopantetheine prosthetic group between CoA and fatty acid synthase in rat brain, liver and adipose tissue was determined after the injection of [^{3}H]-pantothenate at various time intervals until 48 h (Volpe and Vagelos, 1973). The turnover rate was extremely rapid in all three tissues. In brain, the prosthetic group was incorporated into fatty acid synthase in 10-11 h which was much faster than the turnover of the fatty acid synthase complex (i.e., a half-life of 6.4 days). The prosthetic group of the fatty acid synthase in liver and adipose was replaced faster than in brain. Incorporation of the labeled pantothenic acid into CoA always preceded that into ACP, which supported the notion that the prosthetic group of ACP comes from CoA.

The rate of prosthetic group exchange between CoA and ACP in brain and liver was also determined after feeding a fat-free diet and fasting for 48 h (Volpe and Vagelos, 1973) The efficiency of 4'-phosphopantetheine exchange between CoA and fatty acid synthase was determined by the ratio of fatty acid synthase specific radioactivity to CoA specific

radioactivity. A higher ratio of 3.28 was determined in liver of rats after fed a fat-free diet than after fasting with a ratio of 0.57. The efficiency in brain did not differ between fed and fasting states. The rapid turnover of 4'-phosphopantetheine was also seen in radiolabelled experiments with auxotroph of E.Coli (Powell et al., 1969).

Regulation of fatty acid synthesis The enzymes involved in fatty acid synthesis appear to be controlled in two ways: (a) short-term or acute control by allosteric and covalent modification of enzymes which occurs within min or less; (b) long-term or adaptive control by changes in rates of synthesis and degradation of enzymes which occur within hours or days (Mayes, 1990; Volpe and Vagelos, 1973: Wakil et al., 1983).

(a) Short-term control

Acetyl-CoA carboxylase catalyzes the first rate-controlling step of fatty acid synthesis. The polymeric form of the enzyme is catalytically active but the protomer is not. The rate of fatty acid synthesis is therefore controlled by the equilibrium between the two forms, polymeric and protomer. Citrate activates acetyl-CoA carboxylase by shifting the equilibrium towards polymer formation. Palmitate, the end product of fatty acid synthesis, inactivates acetyl-CoA carboxylase by promoting disaggregation of polymer to protomer. The cAMP allosterically activates cAMP-dependent protein kinase which, in turn, inactivates acetyl-CoA carboxylase by

shifting the equilibrium in favor of the inactive protomer. Acetyl-CoA carboxylase is also subject to hormonal regulation. Glucagon, epinephrine and norepinephrine increase cAMP concentrations and thus inactivate acetyl-CoA synthetase.

Acyl-CoA also inhibits the mitochondrial tricarboxylate transporter, thus preventing transport of citrate from the mitochondria into the cytosol. The synthesis of long chain fatty acyl-CoA by fatty acid synthase from rat epididymal adipose tissue requires malonyl-CoA along with acetyl-CoA and NADPH (Martin et al., 1964; Guynn et al., 1972). Omission of malonyl-CoA completely inhibited fatty acid synthesis determined by spectrophotometric method. Methylmalonyl-CoA can inhibit fatty acid synthesis as an active-site competitor with malonyl-CoA.

Fatty acid synthase in mammals is not known to be regulated by allosteric effectors or by covalent modification (Wakil, 1989). However, the mycobacterial fatty acid synthase is highly sensitive to palmitoyl-CoA with K_i= 10 uM (Bloch, 1977).

(b) Long-term control

Both adaptive enzymes, acetyl-CoA carboxylase and fatty acid synthase, increase their activities in fed state; and decrease in fasting, diabetes and after feeding a high fat diet (Allmann et al., 1965; Tweto and Larrabee 1972; Yu and Burton 1974). Fasting for 48 h decreases the half-life of fatty acid synthase from about 70 h to 20 h. When rats were

fasted for 48 h and refed a fat-free diet, activity of fatty acid synthase in liver cytosol increased in 6 h (Tweto and Larrabee, 1972). The authors determined the fatty acid synthase activity by the rate of incorporation of [^{14}C]-acetate, [^{14}C]-acetyl-CoA, or [^{14}C]-malonyl-CoA into long-chain fatty acids.

5. Summary

Pantothenic acid is the precursor for the synthesis of CoA and is present in CoA in the form of 4'-phosphopantetheine. CoA functions as an acyl-carrier in fatty acid oxidation and in many other reactions affecting lipid metabolism (i.e., citric acid cycle, cholesterol synthesis, etc.). A close relationship between pantothenic acid and lipid metabolism is thus expected.

Only a few studies have reported altered lipid metabolism in the pantothenic acid deficiency in vivo: A lower ability to utilize energy substrate by the liver, and elevated circulatory triglyceride and free fatty acid concentrations. In general, tissue CoA concentrations were significantly decreased in pantothenic acid deficient weanling rats.

It was hypothesized that the altered lipid metabolism reported in pantothenic acid deficiency was due to decreased limited availability of [tissue CoA] in pantothenic acid deficiency. Clinical observations on relationship between pantothenic acid, CoA and lipid metabolism have continuously

appeared in the literature (Carter and Hockaday, 1962; Lopaschuk et al., 1986; Lopaschuk and Neely, 1987; Morisaki et al., 1982; Wittwer et al., 1990). None of the studies, however, examined the pantothenic acid status (including pantothenic acid in plasma and tissues, and CoA in tissues) and its relationship with the altered lipid metabolism in pantothenic acid deficiency. Thus it is necessary to study how altered lipid metabolism in pantothenic acid deficient rats is related to pantothenic acid status and CoA concentrations.

MATERIALS AND METHODS

1. Materials

Chemicals: Enzymatic triglyceride diagnostic kits (procedure No. 336 and 337), triglyceride standards (250 and 500 mg/100 mL), glycerol standards (250 and 500 mg/100 mL), 1.54 N tetrabutylammonium hydroxide (TBAH), thymolphthalein, D-pantothenic acid (hemicalcium salt), rabbit serum albumin, CoASH (sodium salt), ATP (disodium salt), and Triton X-100 were purchased from Sigma Chemical Company (St. Louise, MO). [^{14}C]-pantothenic acid (40-60 mCi/mmol) and [^{14}C]-palmitic acid (57 mCi/mmol) were from New England Nuclear (Boston, MA). Dithiothreitol (DTT) was from Boehringer Manheim Biochemicals (Indianapolis, IN). EDTA was from JT Baker Chemical Company (Phillipsburg, NJ). Soluene was from Packard Instruments Company (Downer's Grove, IL). Scintillation fluid, Safety Solve, was from Research Products International (Indianapolis, IN). All rat diet ingredients were purchased from ICN Biochemicals (Irvine, CA). All other reagents used were of the highest analytical purity available from commercial sources.

Enzymes: Alkaline phosphatase (EC. 3.1.3.1), acyl-CoA synthetase (EC. 6.2.1.3) were purchased from Sigma Chemical Company (St. Louis, MO). Pantetheinase was obtained from

Drs. Wittwer and Wyse's laboratory at Utah State University.

2. Preliminary study

(1) Plasma triglyceride concentrations during fasting

Triglycerides in human plasma or serum are normally determined in samples drawn from subjects fasted for 12-16 hours, since triglyceride concentrations increase as soon as 2 hours postprandially and reach a maximum at 4 to 6 hours (Naito and David, 1984). Blood samples drawn from nonfasting subjects are not suitable for triglyceride analysis, because elevated triglyceride concentrations from normal assimilation of food cannot be distinguished from abnormal lipid metabolism. A preliminary experiment was conducted to see how plasma triglyceride concentrations are changed during fasting to decide how long rats need to be fasted for the subsequent experiment.

Seven male Sprague-Dawley rats weighing 150 - 200 g were fed laboratory chow diet for 3 days and fasted for 3, 7, 12, 24, and 48 h, starting from 8:30 in the morning. Water was given ad lib during fasting. Right after food withdrawal at 8:30 in the morning (0 h) and after each fasting time (3, 7, 12, 24, and 48 h), blood, 0.3 - 0.5 ml/rat, was collected from tail artery of the rats after ether anesthesia. Plasma triglyceride concentration was determined to see the effect of fasting length on plasma triglyceride concentrations.

(2) Determination of food intake pattern

Ad libitum food intake of Sprague-Dawley rats (n=7) weighing 100 - 150 g was recorded throughout the day to see if the length of food withdrawal is the same as that of fasting. Food consumption was measured every 4 hours throughout the day starting from 8:30 in the morning.

3. Main study

(1) Protocol

Weanling male Sprague-Dawley rats (43.8 ± 3.0 g) were divided into three equal wt groups (n=8/group), housed for 4 wks individually in stainless steel hanging cages, maintained on a 12 h light-dark cycle (light cycle, 7 am - 7 pm), and given water ad libitum. The experimental protocol for the rats was reviewed and approved by the University Laboratory Animal Research at Michigan State University and was in compliance with the Guide for the Care and Use of Laboratory Animals (NRC 1985). One group was given a pantothenic acid deficient, semipurified diet, ad lib and the other two groups were given the same diet supplemented with pantothenic acid, ad lib or pair-fed with pantothenic acid deficient, ad lib group. Since rats fed a pantothenic acid deficient diet have been reported to eat less food than rats fed a pantothenic acid supplemented diet, a pantothenic acid supplemented, pair-fed group was included in the present study.

All diet ingredients were mixed with a rotary mixer

(Table 5). The diet contained 50 % of energy from sucrose, 20 % from vitamin-free casein and 30 % from corn oil. Pantothenic acid, 10.6 mg, was added to each kg supplemented diet and less than 0.3 mg pantothenic acid was determined per kg deficient diet.

Food intake of pantothenic acid deficient, ad lib group was measured daily to calculate the amount of daily food to be given for pantothenic acid supplemented, pair-fed group. Food intake of pantothenic acid supplemented, ad lib group and the body weight of all three groups were recorded weekly.

The rats were fasted at wk 2 and wk 4 for 3 h (starting from 8:30 am) and 24 h, anesthetized with inhalation of diethyl ether. Blood sample, about 0.7 - 1 ml/ rat, was collected from the tail artery into a 1 ml syringe rinsed with sodium heparin. At wk 4, after 24 h fasting, the rats were anesthetized and killed by cardiac puncture. Blood was collected into vacutainer tubes containing sodium heparin. The blood samples collected were kept on ice, and centrifuged at 2000 x g for 15 min in a table-top centrifuge. Plasma was removed immediately and stored at -20 °C for later determination of triglyceride, free fatty acid and pantothenic acid. Liver, leg skeletal muscle, epididymal fat pad, kidneys and adrenals were also removed, rinsed in ice cold phosphate buffered saline (PBS, PH 7.4), and stored at -20 °C.

TABLE 5
Compositon of pantothenic acid deficient diet[1]

Ingredient	% by weight	% by Kcal
Sucrose	54.3	50
Vitamin-free casein	21.4	20
DL-methionine	0.3	
Corn oil	14.3	30
Fiber - Celufil	5.0	
AIN-76 mineral mix	3.5	
ICN vitamin mix[2]	1.0	
Choline bitartrate	0.2	

[1] Diet contained 432.7 kcal/100 g. Pantothenic acid deficient (PA-) diet contained 0.3 mg PA/ kg and pantothenic acid supplemented diet was the PA- plus 10.6 mg PA/ kg.
[2] ICN vitamin mixture except calcium pantothenate.

(2) Pantothenic acid in diet

Pantothenic acid deficient and supplemented diets were analyzed for the amount of total pantothenic acid by means of radioimmunoassay (RIA; Wyse et al., 1979). Two grams of pantothenic acid deficient and supplemented diets were weighed into a test tube in duplicate. The diets were mixed with 0.4 ml 1.0 M Tris-buffer, pH 7.4, containing 30 units alkaline phosphatase and 40 units pantetheinase, and incubated overnight at 37 °C in a water-shaker bath. The contents were then filtrated by use of Whatman #1 filter paper into a 100 ml volumetric flask, and the final volume adjusted to 100 ml with distilled water. This extract was used for the measurement of pantothenic acid via RIA.

(3) Triglyceride and free fatty acid in plasma

Plasma triglyceride concentrations were determined by a spectrophotometric enzymatic method using a modified method of Bucolo and David (1973; triglyceride kits, procedure No. 336). The assay in which triglyceride is reacted with lipoprotein lipase, glycerol kinase and glycerol-1-phosphate dehydrogenase coupled with reduction of 2-[p-iodophenyl]-3-p-nitrophenyl-5-phenyltetrazolium (INT) to formazan which can be detected with absorbance at 500 nm. The triglyceride reagent containing all of the above enzymes and cofactors (ATP, NAD and $MgCl_2$) was reconstituted with distilled water. One ml of the reconstituted triglyceride reagent was mixed with 10 ul of either triglyceride standards (0, 50, 100,

200, 250 mg/100 mL) or plasma samples. After incubation for 20 min at room temperature, the mixture was determined for absorbance at 500 nm in a Spectronic 21 spectrophotometer (Bausch and Lomb). The increase in absorbance at 500 nm is directly proportional to the triglyceride concentration of the standards from which a standard curve was derived. Sample triglyceride values are read from the standard curve.

Since the modified method of Bucolo and David (1973) measures endogenous glycerol in the plasma to be measured as triglyceride, 24 h fast samples were again assayed for true triglyceride and glycerol separately by the modified method of McGowan et al (1983; triglyceride kits, procedure No. 337). The procedure is also based on the enzymatic reactions of lipoprotein lipase, glycerol kinase, glycerol phosphate oxidase and peroxidase with the formation of quinoneimine dye. Triglyceride reagent A containing all enzymes except lipoprotein lipase, and triglyceride reagent B containing lipoprotein lipase only were reconstituted with distilled water. Triglyceride reagent A, 0.8 ml, was mixed with 10 ul of either glycerol standard (0, 50, 100, 200, 250 mg/100 ml) or plasma samples. After incubation for 15 min at room temperature, the samples were read for absorbance at 540 nm in Spectronic 21 spectrophotometer to get endogenous glycerol value. The reaction mixture was added with 0.2 ml of triglyceride reagent B containing lipoprotein lipase, incubated for 15 more min at room temperature, and then read for absorbance at 540 nm to get total triglyceride value

including true triglyceride and glycerol. The true triglyceride value was obtained by subtracting the glycerol value from the total triglyceride value.

Free fatty acid in plasma was determined by the method of Ko and Royer (1967). Hundred ul of either plasma or palmitic acid standards in methanol (0, 200, 400, 600, 800, 1000 ueq/L) were vortexed vigorously for at least 1 min with 3.0 ml of extraction mixture (isopropyl alcohol:heptane:1.0 N H_2SO_4 = 40:10:1, by volume), 0.6 ml of heptane and 2.9 ml of distilled water. One ml of upper heptane layer was transferred to another tube and 100 ul of 0.01 % thymolphthalein in acetone was added as an indicator. Titration was carried out with 5 x 10^{-3} N tetrabutylammonium hydroxide (TBAH) using a Hamilton syringe under nitrogen to remove CO_2 in the reaction mixture. TBAH, 1.54 N, in methanol was diluted with isopropyl alcohol to 5 x 10^{-3} N. The indicator, thymolphthalein, changes from colorless to blue at pH 10.5. Free fatty acid concentration in plasma was obtained from a standard curve which was drawn with the amount of TBAH titrant used for palmitate standards. Linear range of standard curve was obtained from 0 to 1200 ueq/L concentrations of palmitate standards.

(4) Pantothenic acid in plasma and tissues

Tissue preparation

For measurement of free pantothenic acid, 0.2 g tissue (liver, leg skeletal muscle, and epididymal fat pad) was

homogenized in 4 to 5 volume (w/v) of distilled water (0.8 ml for muscle and fat; 1 ml for liver) with a Polytron homogenizer (Brinkmann Co., Westbury, New York) at the speed setting of 5 for 30 s. One hundred ul of homogenate was deproteinized by equimolar concentration of saturated $Ba(OH)_2$ and 10 % $ZnSo_4$ added in that order, vortexed, and then centrifuged at 5000 x g for 10 min in a Sorvall ultraspeed centrifuge (RC-5B, Du Pont Instrument). The resulting supernatant was separated and stored at -20 °C until analysis for free pantothenic acid by RIA.

To measure total pantothenic acid, 100 ul of the tissue homogenate was mixed with 50 ul 1 M Tris-buffer, pH 7.4, containing 4 units alkaline phosphatase and 6 units pantetheinase. The tissue and enzyme mixture was incubated at 37 °C for over 5 hours in a water-shaker bath, and then deproteinized by the same procedure as for the free pantothenic acid.

RIA protocol

In the RIA procedure for pantothenic acid, 50 ul of either pantothenic acid standards, 0 - 1.0 nmol, or samples were combined with 250 ul mixture of [^{14}C]-pantothenic acid (40-60 mCi/mmol, 8000 dpm/sample), antisera (1:25 dilution) and 1.5 % rabbit serum albumin in PBS. After incubation for 15 min on a rotary shaker, 300 ul saturated $(NH_4)_2SO_4$ solution was added to the reaction mixture, vortexed briefly, and centrifuged at 12000 x g for 15 min in a Sorvall ultraspeed centrifuge. The supernatant containing

pantothenic acid unbound to the antibody was aspirated, and the precipitate was resuspended with 300 ul of 50 % $(NH_4)_2SO_4$ solution. After centrifugation again at 12000 x g for 15 min, the supernatant was again aspirated. The resulting pellet was vortexed with 300 ul tissue solubilizer, Soluene (Packard Co.), and incubated at 60 °C for 30 min. After cooling to room temperature, 3 ml of scintillation fluid was added to each vial and counted for radioactivity in a liquid scintillation counter (Tri Carb 4000 series, Packard Co.).

The amount of pantothenic acid in samples was determined from a standard curve based on the percent binding of radiolabelled pantothenic acid to antibody at different concentrations of unlabelled pantothenic acid standards. The higher percent binding of the radiolabelled pantothenic acid to antibody occurs with lower concentration of pantothenic acid in a standard solution, since both radiolabelled pantothenic acid and unlabelled pantothenic acid compete for the binding sites of the antibody.

(5) CoA in tissues

Tissue preparation

For the measurement of free CoA, the tissue sample (liver and muscle) was homogenized with less than 10 volume (w/v) of chilled 6 % perchloric acid (w/v) for muscle, and 8 % for liver which contained final concentration of 2 mM DTT, and homogenized with a Polytron homogenizer at the speed of

5 for 30 s. Samples were then centrifuged at 6000 x g for 15 min. The supernatant was adjusted to pH 6.5 - 7.5 with 3 N KOH on ice and centrifuged again at 6000 x g for 15 min to remove potassium perchlorate. The resulting supernatant was removed and stored at -20 °C for free CoA performed within three days (Knight and Drew, 1988).

For the measurement of total long chain acyl-CoA's, 0.5 g tissue samples (liver and muscle) were combined with 1 ml of chilled 6 % (w/v) perchloric acid containing a final concentration of 6 mM DTT, and homogenized with a Polytron homogenizer at the speed of 5 for 30 s. The homogenized liver and muscle samples were then centrifuged at 6000 x g for 15 min and 10 min, respectively. The supernatant was removed and discarded. The pellet was resuspended with 1 ml 30 mM DTT, adjusted to pH 11.5 - 12.5 with 3 N KOH, and then heated at 55 °C for 10 min for liver, and 15 min for muscle. After alkaline hydrolysis of long chain acyl-CoA's, the samples were cooled, neutralized to pH 6.5 - 7.5 with 0.5 M triethanolamine in 6 % perchloric acid, placed on ice for 30 min, and then centrifuged again at 6000 x g for 15 min for liver, and 10 min for muscle (Williamson and Corkey, 1969). The resulting supernatant was separated and stored at -20 °C for long chain acyl-CoA's measured within three days. All sample preparations were carried out on ice and under refrigerate centrifugation.

<u>CoA assay</u>

Free CoA was assayed by the radioisotopic method using

[^{14}C]-palmitic acid and bacterial acyl-CoA synthetase (Knight and Drew, 1988). The total long chain acyl-CoA's were also assayed by the same procedure after the tissue samples were subjected to an alkaline hydrolysis by the method of Williamson and Corkey (1969) as described above.

Substrate preparation: 400 ul of [^{14}C]-palmitic acid in ethanol (40 uCi, 57 mCi/mmol) was evaporated to dryness under nitrogen, and resuspended in 2 ml 100 mM Tris-HCl, pH 8, containing 0.5 % Triton X-100. Unlabelled palmitic acid in methanol was added to the [^{14}C]-palmitic acid to give a final concentration of 1 mM palmitic acid with a specific activity of 20 mCi/mmol.

Lyophilized Acyl-CoA synthetase was resuspended in 50 mM potassium phosphate buffer, pH 7.4, containing 2 mM DTT, 1 mM EDTA, and 0.005 % Triton X-100 to give an activity of 0.034 units/20 ul.

CoASH standards: 12.5 uM CoA stock solution was prepared in 1 mM Tris-HCl, pH 8.4, containing 0.1 M DTT. Working CoA standards (0-250 pmol/20 ul) were prepared at the time of assay with 1 mM Tris-HCl buffer, pH 8.4.

Assay procedure: Twenty ul of either CoA standards or samples were combined with 180 ul of reaction mixture to achieve the final reaction mixture concentration in 200 ul to be 150 mM Tris-HCl, pH 8.4, 6.2 mM $MgCl_2$, 0.05 % Triton X-100, 2 mM EDTA, 2.5 mM ATP, 1 mM DTT, 100 uM [^{14}C]-palmitic acid. After the mixture was equilibrated at 37 °C for 1 min, the reaction was initiated by the addition of 20

ul (0.034 units) of the acyl-CoA synthetase solution. After incubation at 37 °C for 10 min, the reaction was terminated by mixing with 3.25 ml of methanol/chloroform/heptane (1.41/1.25/1, by volume). To ease the separation of the solvent layer, 1.05 ml 0.1 M sodium acetate, pH 4, was added. Each tube was tightly sealed with parafilm and then vortexed vigorously. After mixing on a rotary shaker for 20 min, the tubes were centrifuged at 500 x g at room temperature for 10 min. One ml of the upper aqueous phase (mixture of methanol and sodium acetate) containing [^{14}C]-palmitoyl-CoA was transferred into 20 ml scintillation vial, mixed with 10 ml of scintillation fluid, and determined for radioactivity. CoA concentration in the sample was obtained from a standard curve of radioactivity (dpm) vs. working CoA standards (1-250 pmol/20 ul).

4. Statistical analysis

Data are expressed as means±SD. One-way ANOVA was used to detect the effect of different diets on the variables determined. If the difference was significant at $P < 0.05$, Tukey's multiple comparison test was used to determine differences among the groups. Student's t-test was used for the comparison between two data at the significance level of $P < 0.05$. The statistical tests were conducted with statistical software, Statistical Graphics System (V3.0, Statistical Graphics Co., Rockville, Maryland).

RESULTS

1. Preliminary study

(1) Plasma triglyceride concentrations during fasting

The average plasma triglyceride concentration was reduced from 125.2 ± 19.6 mg/100 mL to 82.5 ± 13.3 mg/100 mL in 3 h fast (Figure 3). The reduction in plasma triglyceride concentrations may be explained by the clearance of chylomicron in rats. Surprisingly, plasma triglyceride concentrations increased gradually during 7, 12 and 24 h fast and the increase at 24 h fast was significant compared to that at 3 h fast. Glycerol which is not readily utilized in adipose tissue diffuses into the plasma, and is utilized by liver and kidney under fasting condition. It was questioned if the elevated triglyceride concentrations at 24 h fast was due to glycerol. This elevated plasma triglyceride concentration at 24 h fast was not due to glycerol as confirmed later, but due likely to VLDL production by the liver.

Under fasting free fatty acids are released from the adipose tissue by lipolysis, some of them are transported to the liver and either oxidized or reesterified to triglyceride. When the supply of free fatty acids exceeds the need or the capacity of hepatic cells to oxidize them,

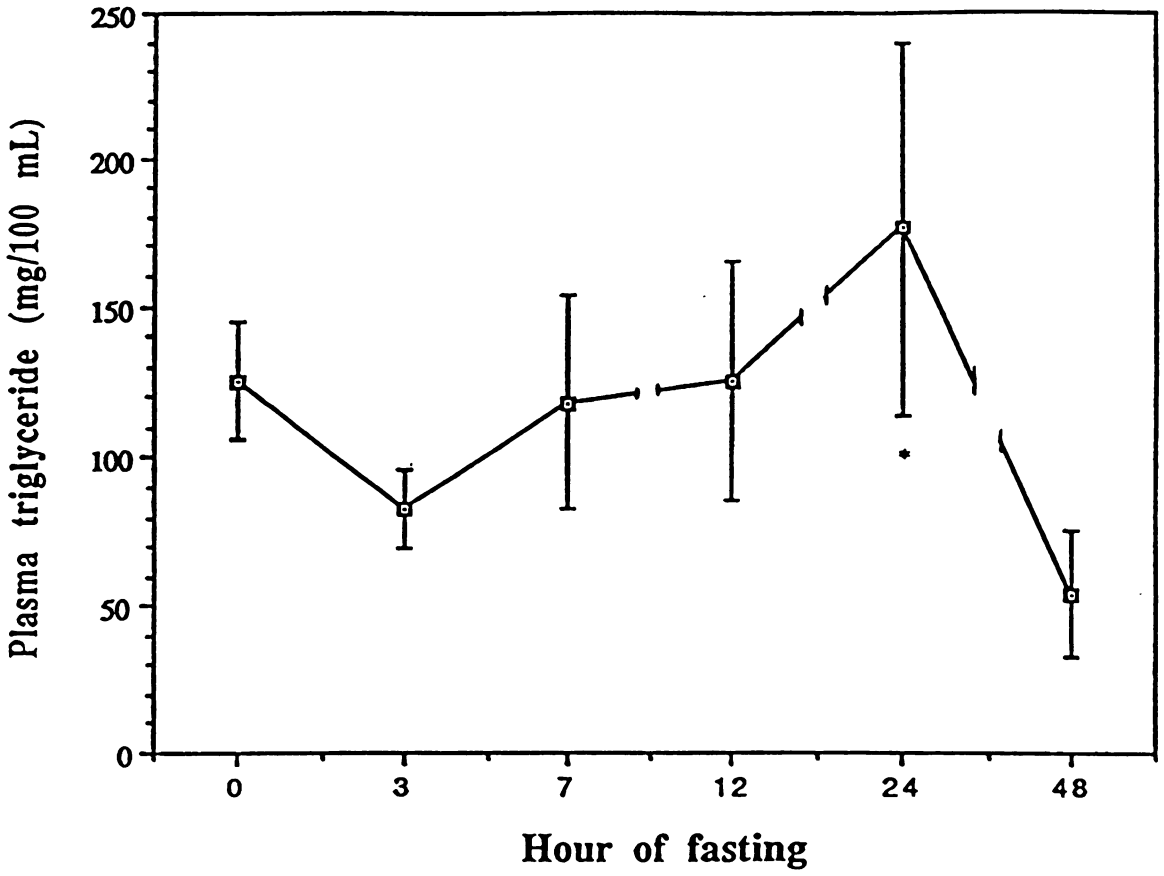


FIGURE 3 The effect of fasting on plasma triglyceride concentrations. Sprague-Dawley rats (n=7) were food-deprived for 0, 3, 7, 12, 24, and 48 h and plasma triglyceride concentrations were determined. Error bar represents SD.
* Significantly higher compared to 3 h fast and 48 h fast plasma by Tukey's multiple comparison test ($P<0.001$).

the formation of triglyceride in the liver relieves the potentially toxic effects of free fatty acids.

Elia et al (1987) reported that fat mobilization from adipose tissue increased 3-fold in 4 day-fasting compared to overnight fasting as determined by a two-stage nonisotopic glycerol infusion. Surprisingly, the resterification of free fatty acid to triglyceride increased 6-fold in 4 day-fasting compared to overnight fasting. Wolfe et al (1985) found that the appearance of free fatty acid in VLDL was the same as that of free fatty acid in plasma and the oxidation of VLDL was same as that of plasma free fatty acid in 24 h fasting rats. The finding suggests the importance of free fatty acids in VLDL in energy metabolism even in fasting state. After 48 h fast, the average plasma triglyceride concentration was markedly reduced to 53.3 ± 21.2 mg/100 mL. This suggests that the reduction of triglyceride deposit in the adipose tissue which is the source of free fatty acids for VLDL synthesis in liver or increased utilization of free fatty acids by the tissues under prolonged fasting. To see the effect of pantothenic acid on lipid metabolism, the present study determined plasma lipids at 3 h and 24 h fast, when clearance and production of lipoproteins are apparent.

(2) Pattern of daily food intake

Rats ate about 70 % of daily intake from evening to morning (8:30 pm to 8:30 am) with the peak consumption between 12:30 and 4:30 during dark cycle (Figure 4). Since

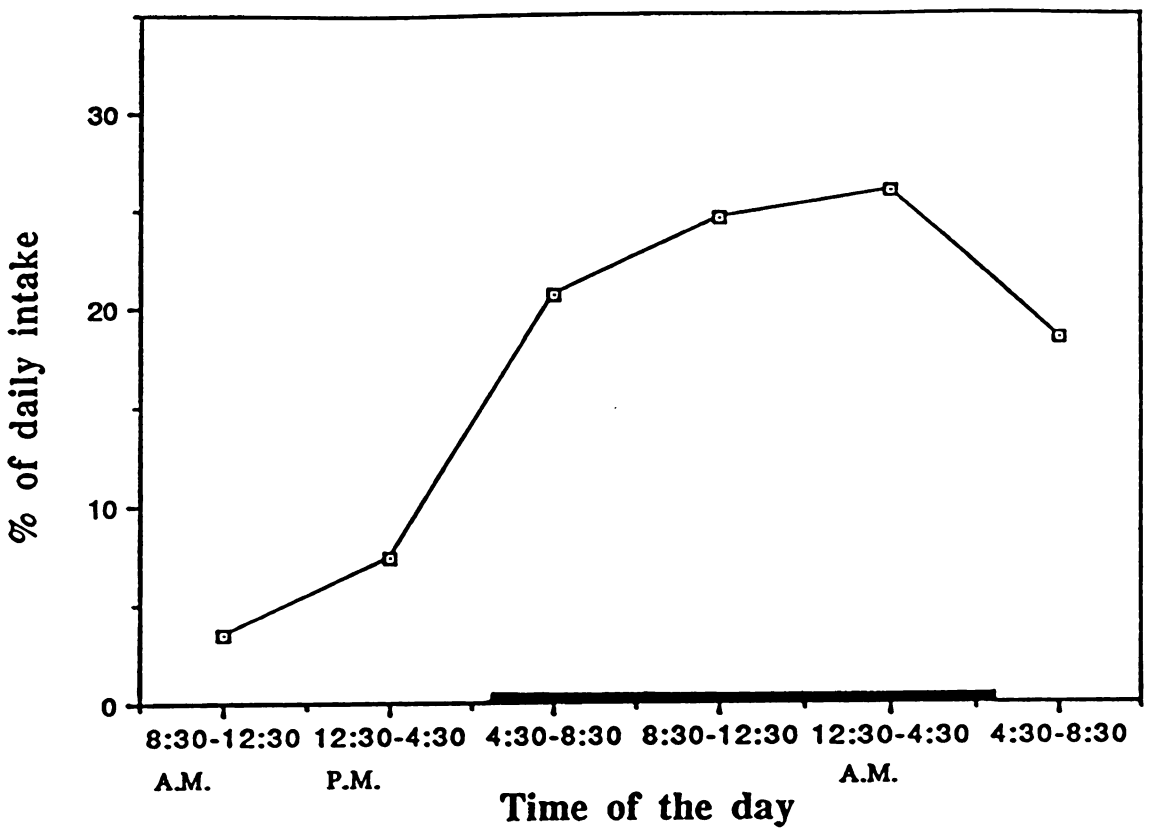


FIGURE 4 Food consumption pattern of rats fed ad libitum throughout the day. Food intake of Sprague-Dawley rats (n=7) was determined every 4 hours throughout the day. The rats consumed 70 % of daily food intake from 8:30 pm to 8:30 am with the peak consumption between 12:30 and 4:30 am during dark cycle (7 pm - 7 am; black line).

the rats continuously ate during dark cycle and until 8:30 in the morning, food withdrawal for 3 h, starting from 8:30 in the morning, was confirmed as the 3 h fast in this experiment.

2. Main study

(1) Food intake, body weight, and signs of pantothenic acid deficiency

Weekly food intake of pantothenic acid deficient, ad lib group was less compared to that of pantothenic acid supplemented, ad lib group starting wk 2 throughout the study ($P < 0.001$; Figure 5). The body weight of the rats in pantothenic acid deficient, ad lib group was significantly lower than that of pantothenic acid supplemented, ad lib group beginning wk 1 ($P < 0.001$), and less than that of pantothenic acid supplemented, pair-fed group beginning wk 2 ($P < 0.001$; Figure 6). The average weight of pantothenic acid supplemented, pair-fed group was also significantly lower than that of pantothenic acid supplemented, ad lib group from wk 1 to the end of the study, except at wk 2. The total weight gain of the rats in pantothenic acid deficient, ad lib group for 4 wks were 70 % of that of pantothenic acid supplemented, pair-fed group and only 52 % of that of pantothenic acid supplemented, ad lib group (97.8 ± 14.2 g, 140.6 ± 15.3 g, 189.3 ± 12.5 g, respectively).

Hair loss was observed in rats fed a pantothenic acid deficient diet beginning wk 3 of the study. In the present

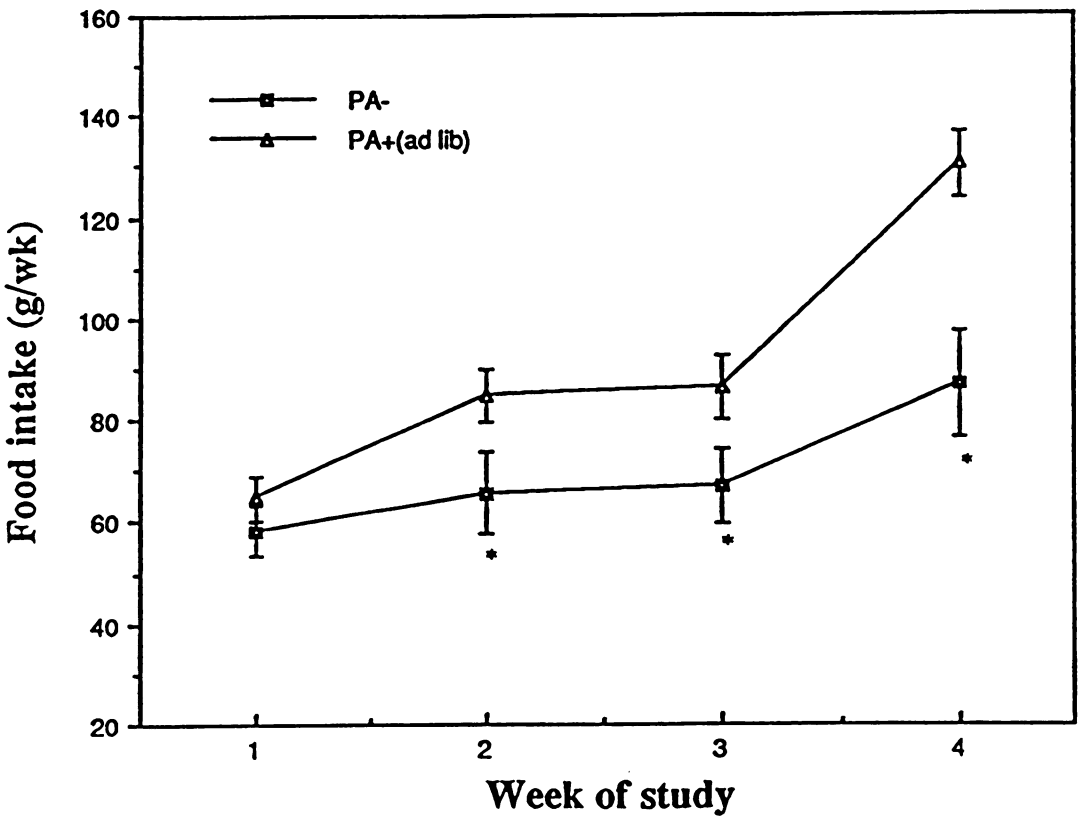


FIGURE 5 Weekly food intake of rats fed, ad libitum, a pantothenic acid deficient (PA-) or supplemented diet (PA+). Weanling Sprague-Dawley rats (n=8/group) were fed either a PA- or PA+ for 4 wks. The rats in the pair-fed group were given the PA+ diet daily in the same amount consumed by the PA- group the day before. Error bar represents SD (n=8/group).
* Significantly less compared to the datum of PA+, ad lib group by Tukey's multiple comparison test ($P<0.001$).

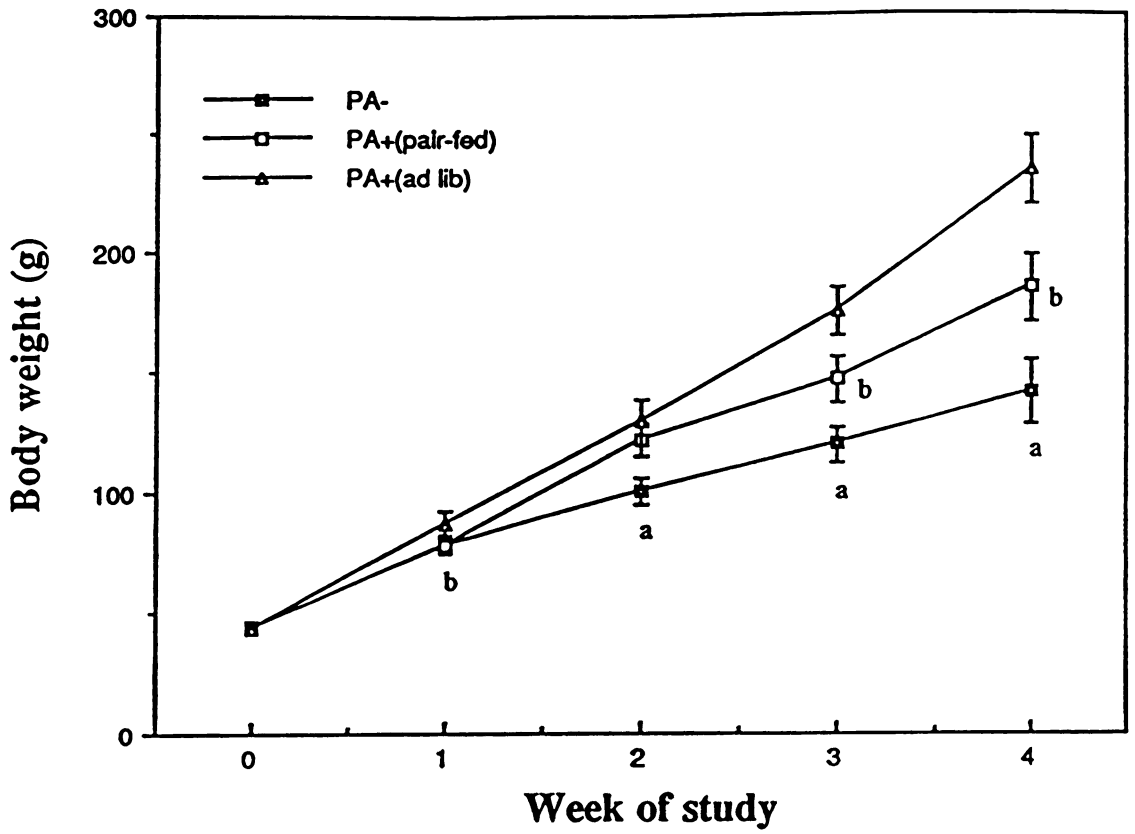


FIGURE 6 Body weight of rats fed either a pantothenic acid deficient diet (PA-), or supplemented diet (PA+), ad libitum or pair-fed to deficient group. Error bar represents SD (n=8/group).
[a] Significantly lower compared to PA+ groups by Tukey's multiple comparison test ($P<0.001$).
[b] Significantly lower compared to PA+, ad lib by Tukey's multiple comparison test ($P<0.001$).

study, the weanling rats fed a pantothenic acid deficient diet for 4 wks survived 100 % to the end of the study without any other signs of pantothenic acid deficiency reported in Wilhem (1988).

(2) Organ weight

Liver and kidney weighed significantly less in the pantothenic acid deficient group compared to those of the controls at the end of the 4 wk study ($P < 0.001$; Table 6). However, the pantothenic acid deficient group had a significantly higher % body weight of liver compared to the controls. Liver and kidney weights of pantothenic acid supplemented, pair-fed group were also significantly lower than those of pantothenic acid supplemented, ad lib group ($P < 0.001$). Even though no significant differences were seen in adrenal weights among the groups, individual variations (SD) were high in pantothenic acid deficient group. The adrenals were quite enlarged and darker in the pantothenic acid deficient group compared to the controls.

(3) Plasma triglyceride and free fatty acid concentrations

At wk 2, there were no significant differences among the groups in 3 h fast plasma triglyceride and free fatty acid concentrations (Table 7). The 24 h fast plasma triglyceride and free fatty acid concentrations were significantly higher in pantothenic acid deficient group than in pantothenic acid supplemented groups in 24 h fast

TABLE 6
Organ weight of rats in pantothenic acid deficient and supplemented groups[1]

Tissues		Groups		
		PA-, ad lib	PA+, ad lib	PA+, pair-fed
Liver	g	5.05 ± 0.44[a]	7.10 ± 0.32	6.08 ± 0.71[b]
	% BW	3.57 ± 0.31[a]	3.04 ± 0.14	3.30 ± 0.39
Kidney	g	1.23 ± 0.12[a]	1.81 ± 0.18	1.51 ± 0.11[b]
	% BW	0.87 ± 0.08	0.78 ± 0.08	0.82 ± 0.06
Adrenal	mg	0.043 ± 0.025	0.041 ± 0.013	0.045 ± 0.011
	% BW	0.030 ± 0.018	0.018 ± 0.006	0.024 ± 0.006

[1] Weanling Sprague-Dawley rats (n=8/group) were fed either a pantothenic acid deficient (PA-, ad lib) or supplemented diet (PA+, ad lib and PA+, pair-fed) for 4 wks. Rats were killed by cardiac puncture after 24 h fast at wk 4 of the study. Organs were removed, rinsed in PBS, weighed and stored at -20 °C. Organ weights were also expressed as % of body weight (% BW). Means±SD (n=6-8/group).
[a] Significantly different compared to PA+ groups by Tukey's multiple comparison test ($P<0.05$).
[b] Significantly lower compared to PA+, ad lib group by Tukey's multiple comparison test ($P<0.001$).

TABLE 7
Plasma triglyceride and free fatty acid concentrations of pantothenic acid deficient and supplemented groups at wk 2[1]

	Groups		
	PA-, ad lib	PA+, ad lib	PA+, pair-fed
Triglyceride (mg/100 mL)			
3 h fast	77.4 ± 28.6	60.6 ± 17.3	59.8 ± 7.8
24 h fast	213.3 ± 33.5[ab]	138.1 ± 28.0[b]	51.2 ± 7.5[c]
Free fatty acid (ueq/L)			
3 h fast	963.8 ± 113.0	846.0 ± 101.2	648.5 ± 188.2
24 h fast	1201.2 ± 88.8[a]	745.8 ± 83.3	931.0 ± 182.7

[1] Weanling Sprague-Dawley rats (n=8/group) were fed either a pantothenic acid deficient (PA-, ad lib) or supplemented diet (PA+, ad lib and PA+, pair-fed) for 4 wks. Blood samples were collected at wk 2 from tail artery of the rats in PA-, ad lib and PA+ groups after 3 h and 24 h fast. Plasma triglyceride concentrations were determined by an enzymatic spectrophotometric method (Modified method of Bucolo and David, 1973; Sigma TG kits, No. 336). Plasma free fatty acid was determined by the method of Ko and Royer (1967). Means±SD (n=4-8/group).
[a] Significantly higher compared to PA+ groups by Tukey's multiple comparison test ($P<0.001$).
[b] Significantly increased compared to 3 h fast by Tukey's multiple comparison test ($P<0.001$).
[c] Significantly lower compared to PA+, ad lib group by Tukey's multiple comparison test ($P<0.001$).

plasma ($P < 0.001$). In both pantothenic acid deficient and pantothenic acid supplemented, ad lib groups, 24 h fast plasma triglyceride concentrations were elevated significantly compared to 3 h fast plasma ($P < 0.001$), as was seen in the preliminary study.

The 24 h fast plasma were analyzed again without and then with lipoprotein lipase to determine glycerol and total triglyceride which includes glycerol and true triglyceride. In both pantothenic acid deficient and pantothenic acid supplemented, ad lib groups, glycerol accounted for only 7-19 % of total triglyceride. The plasma triglyceride concentrations determined by the first assay were not significantly different from true triglyceride values obtained by the second assay (Table 8). In pantothenic acid supplemented, pair-fed group, however, the amount of glycerol was 40 % of total triglyceride. The reason why triglyceride concentrations were increased after 24 h fast needs to be further investigated.

The pantothenic acid supplemented, pair-fed group exhibited very distinctive pattern of plasma triglyceride and free fatty acid concentrations from the two ad lib groups. Differences in plasma triglyceride concentrations between pantothenic acid supplemented, pair-fed group and pantothenic acid supplemented, ad lib group were significant at all time points measured except at wk 2, 3 h fast ($P < 0.001$; Table 7 and 9). Even though no significant differences in plasma free fatty acid concentrations were

TABLE 8
Comparison of triglyceride concentrations in 24 h fasting plasma at wk 2 determined by two different assays[1]

Assays	Groups		
	PA-, ad lib	PA+, ad lib	PA+, pair-fed
	mg/100 mL		
Assay 1			
TG	213.3 ± 33.5	138.1 ± 28.0	51.2 ± 7.5
Assay 2[2]			
Total TG	217.9 ± 33.7	156.1 ± 34.6	44.9 ± 4.8
Glycerol	16.4 ± 4.3	29.9 ± 20.7	18.0 ± 7.5
True TG	201.5 ± 36.5	126.2 ± 27.1	26.9 ± 7.9[a]

[1] Weanling Sprague-Dawley rats (n=8/group) were fed either a pantothenic acid deficient (PA-, ad lib) or supplemented diet (PA+, ad lib and PA+, pair-fed) for 4 wks. Blood samples collected at wk 2 after 24 h fast were analyzed for triglyceride (TG) concentrations by two assays. By assay 1, plasma TG concentrations were determined by enzymatic spectrophotometric method (Modified method of Bucolo and David, 1973; Sigma TG kits, No. 336).
[2] Endogenous glycerol was determined separately from true triglyceride by running the assay without and with lipoprotein lipase (McGowan et al, 1983; Sigma TG kits, No. 337). Means±SD (n=5-8/group)
[a] Significantly different from TG obtained by Assay 1 by Student's t-test ($P<0.001$).

TABLE 9
Plasma triglyceride and free fatty acid concentrations of pantothenic acid deficient and supplemented groups at wk 4[1]

	Groups		
	PA-, ad lib	PA+, ad lib	PA+, pair-fed
Triglyceride (mg/100 mL)			
3 h fast	115.4 ± 31.5[a]	80.1 ± 15.7	136.2 ± 36.1[a]
24 h fast	117.5 ± 46.2[b]	110.3 ± 21.6[b]	43.0 ± 11.2
Free fatty acid (ueq/L)			
3 h fast	1015.2 ± 215.9[ab]	577.4 ± 70.0	454.5 ± 138.9
24 h fast	985.0 ± 169.9	834.3 ± 196.5	885.7 ± 49.7

[1] Weanling Sprague-Dawley rats (n=8/group) were fed either a pantothenic acid deficient (PA-, ad lib) or supplemented diet (PA+, ad lib and PA+, pair-fed) for 4 wks. Blood samples were collected at wk 2 from tail artery of the rats in PA-, ad lib and PA+ groups after 3 h and 24 h fast. Plasma triglyceride concentrations were determined by an enzymatic spectrophotometric method (Modified method of Bucolo and David, 1973; Sigma TG kits, No. 336). Plasma free fatty acid was determined by the method of Ko and Royer (1967). Means±SD (n=4-8/group).
[a] Significantly higher compared to PA+, ad lib group by Tukey's multiple comparison test ($P<0.05$).
[b] Significantly higher compared to PA+, pair-fed group by Tukey's multiple comparison test ($P<0.05$).

seen between two pantothenic acid supplemented groups, the variations (SD) were higher in pantothenic acid supplemented, pair-fed group than in pantothenic acid supplemented, ad lib group (Table 7 and 9). We observed that the rats in pantothenic acid supplemented, pair-fed group finished food within 5 hours after food was given daily between 11:00 and 12:00 noon. This observation indicates that the rats in pantothenic acid supplemented, pair-fed group experienced repeated fasting every day.

At wk 4, pantothenic acid deficient group had significantly higher 3 h fast plasma triglyceride and free fatty acid concentrations than the pantothenic acid supplemented, ad lib group ($P < 0.02$; Table 9). No significant differences were seen between pantothenic acid deficient and supplemented, ad lib groups in 24 h fast plasma triglyceride and free fatty acid concentrations. The normalized plasma lipid concentrations in 24 h fasted pantothenic acid deficient rats at wk 4 coincided with increased plasma pantothenic acid concentrations in response to 24 h fast.

(4) Pantothenic acid in plasma and tissues

The pantothenic acid deficient group had significantly lower plasma pantothenic acid concentrations than the controls at all time points measured ($P < 0.001$; Table 10). Pantothenic acid concentration of pantothenic acid supplemented, pair-fed group was higher in 3 h fast plasma

TABLE 10
Pantothenic acid concentrations in plasma of pantothenic acid deficient and supplemented groups[1]

	Groups		
	PA-, ad lib	PA+, ad lib	PA+, pair-fed
	umol/L		
Wk 2			
3 h fast	0.11 ± 0.04[a]	0.41 ± 0.07	0.67 ± 0.17[b]
24 h fast	0.15 ± 0.03[a]	0.54 ± 0.21	0.55 ± 0.14
Wk 4			
3 h fast	0.05 ± 0.02[a]	0.19 ± 0.07	0.25 ± 0.09
24 h fast	0.22 ± 0.03[ac]	1.45 ± 0.31[c]	0.89 ± 0.34[bc]

[1] Weanling Sprague-Dawley rats (n=8/group) were fed either a pantothenic acid deficient (PA-, ad lib) or supplemented diet (PA+, ad lib and PA+, pair-fed) for 4 wks. Blood samples were collected from the rats at wk 2 and wk 4 after 3 h and 24 h fast. Plasma pantothenic acid was determined by RIA (Wyse et al., 1979). Means±SD (n=5-8/group).
[a] Significantly lower compared to PA+ groups at the same time points by Tukey's multiple comparison test ($P<0.001$).
[b] Significantly different from PA+, ad lib group by Tukey's multiple comparison test ($P<0.001$).
[c] Significantly increased after 24 h fast compared to 3 h fast plasma by Tukey's multiple comparison test ($P<0.001$).

at wk 2 and lower in 24 h fast plasma at wk 4 compared to that of pantothenic acid supplemented, ad lib group. This observation suggests that plasma pantothenic acid is affected by feeding and fasting cycle as reported by others.

Blood and tissue pantothenic acid concentrations were reported to increase by fasting (Reibel et al., 1982; Smith et al., 1987). The 24 h fast plasma pantothenic acid concentrations significantly increased compared to the 3 h fast sample at wk 4, but not at wk 2 in all groups ($P < 0.001$). The extent of the increase in plasma pantothenic acid concentration after 24 h fast was the highest in the pantothenic acid supplemented, ad lib group (pantothenic acid supplemented, ad lib, 7.6 times and pair-fed, 3.6 times; deficient, 4.4 times). The observation that plasma pantothenic acid concentrations did not change after 24 h fast at wk 2, while markedly increased at wk 4 indicates that the pantothenic acid pool (source of increased pantothenic acid in plasma under fasting) is being developed as rats grow older, regardless of pantothenic acid status, and the larger pantothenic acid pool exists in pantothenic acid supplemented groups than pantothenic acid deficient group.

Pantothenic acid in liver, leg skeletal muscle and epididymal fat pad was assayed after 24 h fast at wk 4 (Table 11). The free pantothenic acid concentrations in the tissues were significantly lower in pantothenic acid deficient group than in controls ($P < 0.001$). Liver total

TABLE 11
Pantothenic acid concentrations in tissues of pantothenic acid deficient and supplemented groups[1]

	Groups		
	PA-, ad lib	PA+, ad lib	PA+, pair-fed
	nmol/g ww		
Liver			
free	207.7 ± 39.1[a]	351.3 ± 47.8	300.7 ± 28.6
total	330.7 ± 32.5[b]	433.6 ± 41.9	373.7 ± 38.0[b]
Skeletal muscle			
free	10.2 ± 0.6[a]	29.9 ± 5.3	23.7 ± 3.1
Epididymal fat pad			
free	10.5 ± 0.9[a]	22.7 ± 2.6	20.6 ± 2.3

[1] Weanling Sprague-Dawley rats (n=8/group) were fed either a pantothenic acid deficient (PA-, ad lib) or supplemented diet (PA+, ad lib and PA+, pair-fed) for 4 wks. The rats were killed by cardiac puncture after 24 h fast at wk 4 of the study. Organs were removed, rinsed in PBS, weighed and stored at -20 °C until analysis of tissue pantothenic acid. Means±SD (n=5-8/group). Tissue PA was determined by RIA (Wyse et al., 1979)
[a] Significantly lower compared to PA+ groups by Tukey's multiple comparison test ($P<0.001$).
[b] Significantly lower compared to PA+, ad lib group by Tukey's multiple comparison test ($P<0.001$).

pantothenic acid concentrations (the sum of free plus bound forms of pantothenic acid) were also significantly less in pantothenic acid deficient and supplemented, pair-fed groups compared to pantothenic acid supplemented, ad lib group ($P < 0.001$). Liver free pantothenic acid concentrations of all three groups were higher in the present study than those in other studies of our laboratory. The rats in the present study, however, differ from others because they were fasted for 24 h whereas others were not fasted or fasted only overnight.

(5) CoA concentrations in tissues

Both liver free CoA and long chain acyl-CoA concentrations of pantothenic acid deficient group were 50 % of those of pantothenic acid supplemented, ad lib group ($P < 0.001$; Table 12). Pantothenic acid deficiency resulted in decreased concentrations of liver CoA concentrations as reported (Moiseenok et al., 1987; Smith et al., 1987; Srinivasan and Belavady, 1976).

Lower liver free CoA concentration was also seen in pantothenic acid supplemented, pair-fed group than in pantothenic acid supplemented, ad lib group. No significant differences were observed in muscle free CoA and long chain acyl-CoA concentrations among the treatment groups.

TABLE 12
Free CoA and long chain acyl-CoA concentrations in tissues of pantothenic acid deficient and supplemented groups[1]

	Groups		
	PA-, ad lib	PA+, ad lib	PA+, pair-fed
	nmol/g ww		
Liver			
long chain	10.6 ± 4.2[a]	22.1 ± 4.6	20.2 ± 2.9
free	33.3 ± 10.9[b]	68.0 ± 12.0	36.0 ± 9.9[b]
Skeletal muscle			
long chain	5.9 ± 2.1	6.3 ± 2.9	8.4 ± 3.7
free	61.2 ± 6.1	61.1 ± 8.3	56.0 ± 5.4

[1] Weanling Sprague-Dawley rats (n=8/group) were fed either a pantothenic acid deficient (PA-, ad lib) or supplemented diet (PA+, ad lib and PA+, pair-fed) for 4 wks. The rats were killed by cardiac puncture after 24 h fast at wk 4 of the study. Organs were removed, rinsed in PBS and stored at -20 °C until analysis of tissue CoA. Means±SD (n=6-8/group). Tissue CoA was determined by the method of Knights & Drew (1988).
[a] Significantly lower compared to PA+ groups by Tukey's multiple comparison test ($P<0.001$).
[b] Significantly lower compared to PA+, ad lib group by Tukey's multiple comparison test ($P<0.001$).

3. Summary of results

(1) Triglyceride and free fatty acid concentrations were significantly elevated in 24 h fast plasma at wk 2 and in 3 h fast plasma at wk 4 in the pantothenic acid deficient group compared to the pantothenic acid supplemented, ad lib group ($P < 0.05$).

(2) Plasma and tissue pantothenic acid concentrations were significantly decreased in the pantothenic acid deficient group ($P < 0.001$).

(3) Plasma pantothenic acid concentrations were significantly increased after 24 h fast at wk 4 in all three groups, but not at wk 2 ($P < 0.001$).

(4) The pantothenic acid deficient rats had significantly decreased free CoA and long chain acyl-CoA concentrations in the liver ($P < 0.001$), but not in the muscle, showing tissue specific response to pantothenic acid deficiency.

(5) The normalized triglyceride and free fatty acid concentrations in 24 h fast plasma of pantothenic acid deficient group coincided with the increased concentration of plasma pantothenic acid, and could not be explained by the decreased liver CoA concentrations.

DISCUSSION

In the present study, a significant reduction in weight gain was seen in the pantothenic acid deficient group only after 1 wk of feeding and followed by a significant decrease in food intake beginning wk 2 of the study. The slower growth rate of the pantothenic acid deficient group is thus interpreted as due to pantothenic acid deficiency itself, not as a consequence of a decreased food intake. Growth retardation followed by a decrease in food intake have been also reported in pantothenic acid deficient rats and dogs as the first signs of pantothenic acid deficiency (Barboriak et al, 1956; Schaefer et al, 1942; Srinivasan and Belavady, 1975). Young, male rats have also been reported to be very susceptible to pantothenic acid deficiency based on food intake, body weight, liver pantothenic acid concentrations and carcass fat content (Srinivasan and Belavady, 1975).

The observation that only liver, % body weight, was significantly higher in pantothenic acid deficient group than in controls, suggests that liver may be affected most in short term of pantothenic acid deficiency and thus changes in lipid metabolism occur. Development of a fatty liver in the pantothenic acid deficient dogs (45 % fat of dry liver compared to 13-17 % in the control; Schaefer et

al.,1942) and a lower liver fat content in pantothenic acid deficient rats (Guehring et al., 1952; Carter and Hockaday, 1962) suggest that the liver was affected in pantothenic acid deficiency.

In the present study, the pantothenic acid supplemented, pair-fed group exhibited a distinctive pattern of plasma triglyceride and free fatty acid from the ad lib groups which can be explained by the adaptive metabolic response to repeated fasting in pair-fed group due to the idiosyncratic feeding schedule. Our postulation is substantiated by the reports of others. As fasting extends, adaptive metabolic events occur to maintain glucose homeostasis and to conserve body protein (Saudek, 1976). Lipid metabolism in rats is affected by food intake pattern such as fasting, refeeding and duration of fasting, and dietary ingredients (Allmann et al., 1965; Luo et al., 1992; Moir and Zammit, 1993; Tweto and Larrabee, 1972). The rats fed a high fat diet (70 % energy from fat) showed the adaptive response to the high fat diet by increasing the activity of enzymes involved in fatty acid oxidation such as ß-hydroxyacyl-CoA dehydrogenase and thiolase, and by decreasing the activity of phosphofructo-kinase which is the regulatory enzyme in glycolysis (Nemeth et al., 1992). With these points, plasma lipids of pantothenic acid supplemented, pair-fed group could not be compared to those of other two, ad lib groups. The finding of this study indicates that pair-feeding may not be an appropriate model

to study plasma lipids in comparison with ad lib groups unless the feeding schedule is controlled.

Plasma triglyceride and free fatty acid concentrations were elevated in pantothenic acid deficient rats fasted for 24 h (not for 3 h) at wk 2. Wittwer's study (1990) also reported that serum triglyceride and free fatty acids were elevated in mild pantothenic acid deficiency. The mechanism by which plasma triglyceride and free fatty acid concentrations are elevated in the pantothenic acid deficient rats is yet unclear.

Significantly increased triglyceride concentrations seen in 24 h fast plasma of pantothenic acid deficient group at wk 2 could be caused by either increased reesterification of free fatty acids to triglyceride in liver or defect in VLDL removal from blood. Elevated concentrations of plasma free fatty acid at wk 2 in pantothenic acid deficient group may be used for the synthesis of triglyceride in the liver and thus more VLDL is secreted into the blood. Even though tissue CoA concentrations were not determined at wk 2, it has been reported that liver CoA concentrations were significantly decreased in pantothenic acid deficient rats only after 11 days of feeding (Moiseenok et al., 1987). If CoA concentrations were decreased in pantothenic acid deficient group at wk 2, limited availability of CoA in liver might result in reesterification of free fatty acids to triglyceride rather than oxidation of free fatty acids. This possibility is based on the fact that no net

utilization of CoA occurs for triglyceride synthesis in the cytosol, while 8 CoAs are needed for palmitate oxidation.

It is also possible that a defect in VLDL removal by tissues resulted in the increased triglyceride concentrations in 24 h fast plasma of pantothenic acid deficient group at wk 2. The rats treated with one of the pantothenic acid derivatives, pantethine (250 mg/0.4 ml), for 3 wks have been reported to lower plasma triglyceride concentrations along with 1.5-fold increase in post-heparin lipoprotein lipase and adipose tissue lipoprotein lipase activities (Noma et al., 1984). Considering pantethine increases pantothenic acid concentrations in the blood, it is possible that lipoprotein lipase activity is increased by pantothenic acid in pantethine-treated rats. Even though there are no supporting data for explaining elevated plasma triglyceride concentrations in pantothenic acid deficient group, it is possible that lipoprotein lipase activity is decreased in pantothenic acid deficiency, resulting in high plasma triglyceride concentrations. Increased triglyceride concentration in 3 h fast plasma of pantothenic acid deficient group at wk 4 may be due to the defect in removal of chylomicron through the low lipoprotein activity in adipose tissue, since chylomicron seemed to be cleared from blood within 3 h of fast.

Under fasting condition, plasma free fatty acid concentrations are determined by the rate of lipolysis in adipose tissue and the rate of free fatty acid uptake from

blood and utilization by other tissues such as muscle and liver. Elevated free fatty acid concentrations in 24 h fast plasma of pantothenic acid deficient group at wk 2 could be due to both increased lipolysis and decreased utilization of free fatty acid.

Srinivasan and Belavady (1976) reported that gluconeogenesis was impaired in pantothenic acid deficient group as measured by the activity of hexose diphosphatase and incorporation of labelled alanine to blood glucose. It is expected that if gluconeogenesis was impaired in pantothenic acid deficient group, lipolysis in adipose tissue is more stimulated, resulting in increased free fatty acid concentrations, and finally result in lower body fat as reported (Carter and Hockaday, 1962; Srinivasan and Belavady, 1975).

Accumulation of triglyceride in plasma seen in pantothenic acid deficient group might imply an impairment in utilization of free fatty acid, resulting in increased free fatty acid concentration in pantothenic acid deficient group. Carter and Hockaday (1962) also reported that utilization of extra energy substrate, octanoate, was decreased in 17 h fasted liver of pantothenic acid deficient group as measured by oxygen uptake and ketone production. Decreased ability of fatty acid oxidation can result in increased plasma free fatty acid concentrations.

At wk 4, normalized lipid concentrations in 24 h fast plasma of the pantothenic acid deficient group might be

related to the ability to increase plasma pantothenic acid concentrations in response to 24 h fast. Plasma triglyceride concentrations were inversely related with plasma pantothenic acid concentrations regardless of the groups (3 h fast, r = - 0.4420; 24 h fast, r = - 0.3668). It has been reported that serum triglyceride and free fatty acid concentrations varied inversely with serum pantothenate (Peterson et al., 1987; Wittwer et al., 1990). The significantly decreased liver free and long chain acyl-CoA concentrations seen in pantothenic acid deficient group at wk 4 compared to those in pantothenic acid supplemented, ad lib group were not related to the altered plasma triglyceride and free fatty acid concentrations in 24 h fast plasma. It is obvious from this study that pantothenic acid deficiency alters lipid metabolism by a mechanism other than through reduction in CoA as suggested by Reibel et al (1982).

If plasma lipids at wk 4 in pantothenic acid deficient group were normalized with increased plasma pantothenic acid concentrations in response to 24 h fast, the source of increased pantothenic acid (pantothenic acid pool) needs to be identified. Even though tissue pantothenic acid concentrations were not measured at fed state along with fasted state in the present study, tissue pantothenic acid concentrations are known to increase or decrease after fasting depending on the tissues (Reibel et al., 1982; Smith et al., 1987). Smith et al (1987) reported that pantothenic

acid concentrations only in epididymal fat pad were decreased after 48 h fast while pantothenic acid concentrations in other tissues such as liver, heart, diaphragm and leg skeletal muscle were increased in mice. Adipose tissues in whole body may have a pool of pantothenic acid which can be directed to blood and other tissues to be a source of increased pantothenic acid and be used for the synthesis of CoA to adapt to fasting.

The amount or activity of fatty acid synthase in rat liver and adipose tissue decreases after fasting and increases after refeeding (Allmann et al., 1965; Volpe and Vagelos, 1973; Yu and Burton, 1974). Fatty acid synthase which is abundant in liver and adipose tissue contains ACP which has 4'-phosphopantethine as prosthetic group. It is possible that 4'-phosphopantethine released from ACP of fatty acid synthase during fasting state is further broken down to pantothenic acid in adipose tissue and released into blood. The speculation that fatty acid synthase is a source of increased pantothenic acid under fasting needs to be clarified.

CONCLUSIONS

1. The fasting plasma lipid concentrations are dependent on the feeding cycle and metabolic adaptation as evidenced by the distinctive pattern of fasting plasma lipids in pantothenic acid supplemented, pair-fed group.
2. Lipid metabolism is affected severely in early stage of pantothenic acid deficiency in growing rats and may be dependent on the pool of pantothenic acid in the longer period of pantothenic acid deficiency.
3. Pantothenic acid deficiency alters lipid metabolism by a mechanism other than through reduction in tissue CoA concentrations.

LIMITATIONS

A few limitations that were encountered in the present study need to be carefully considered in the future studies with similar research questions.

1. The present study included a pantothenic acid supplemented group that was pair-fed to the pantothenic acid deficient group. This control group was included in an attempt to exclude the confounding effect of decreased food intake in pantothenic acid deficiency on lipid metabolism as well as biochemical parameters for pantothenic acid status. The pantothenic acid supplemented, pair-fed group rats, however, exhibited distinctive pattern of circulatory lipids. The specific causes and mechanisms of the different plasma lipid concentrations of the pair-fed group have not been investigated in the present study.
2. Fresh plasma or serum samples are recommended for the analysis of triglyceride and free fatty acids (Naito and David, 1984). In the present study, lipids were assayed in fresh as well as frozen and thawed plasma samples. However, all frozen samples were thawed only once for the assays, avoiding repeated freeze-thaw

steps. In the present study, the frozen samples yielded plasma lipid concentrations that were not statistically different from the fresh samples by the assays we used.

3. Free fatty acids in plasma was determined in the present study by micromethod of extraction and titration. Standard curves and a reference plasma sample yielded consistent data among assays that were run on different days. Difficulties, however, existed in delivering small volumes of the titrant with accuracy (usually less than 60 ul/sample), and determining the end point by the indicator, thymolphthalein.

RECOMMENDATIONS

1. In the experiment for the study of lipid metabolism in pantothenic acid deficiency, pair-feeding group needs to be carefully planned to avoid the unexplainable circulatory lipid concentrations responding to fast that were observed in the present study. Possibly, rats in all treatment groups may be trained to a certain feeding condition that can be used throughout the study.
2. A significantly elevated triglyceride concentration in 24 h fasting plasma samples was observed in the present study. Although the experiment was repeated in our preliminary study, we offers no explanation for the mechanism. We suggest that the experiment should be repeated with a larger sample size with a shorter time intervals for 48 h. Determination of plasma lipoproteins of each sample will provide possible clues to explain the mechanism. Also the rate of triglyceride hydrolysis in adipose tissue may be assayed by nonradioactive glycerol infusion technique; the rate of triglyceride oxidation is determined by indirect calorimetric method using a paramagnetic oxygen analyzer and an infrared CO_2 analyzer (Elia et

al., 1987).

3. The present study offers no definitive answers on the role of pantothenic acid in lipid metabolism. We speculated that altered lipid metabolism in pantothenic acid deficient rats is dependent on the pantothenic acid pool in the body. Fatty acid synthase is suspected as the source of increased pantothenic acid in blood and tissues under fasting state. Should be investigated first is the relationship between pantothenic acid and fatty acid synthase in the tissues that are important in lipid metabolism such as liver and adipose tissues after fasting and refeeding the rats.

4. The elevated plasma triglyceride concentration in the pantothenic acid deficiency needs to be investigated in relation to lipoprotein lipase activity or to impaired gluconeogenic ability which has been reported (Srinivasan and Belavady, 1976).

LIST OF REFERENCES

LIST OF REFERENCES

Abiko, Y. (1967) Investigation on pantothenic acid and its related compounds. J. Biochem. 61: 290-299.

Abiko, Y. (1975) Metabolism of coenzyme A. In: Metabolic Pathways, Vol. 7 (Greenberg, D.M., ed.), pp. 1-25. Academic Press, New York, NY.

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

Barboriak, J. J., Krehl, W. A. & Cowgill, G. R. (1965) Pantothenic acid requirement of the growing and adult rat. J. Nutr. 61: 13-21.

Beinlich, C. J., Robishaw, J. D. & Neely, J.R. (1989) Metabolism of pantothenic acid in hearts of diabetic rats. J. Mol. Cell. Cardiol. 21: 641-649.

Bloch, K. (1977) Control mechanisms in the synthesis of saturated fatty acids. Ann. Rev. Biochem. 46: 263-298.

Branca, D., Scutari, G. & Siliprandi, N. (1984) Pantethine and pantothenate effect on the CoA content of rat liver. Internat. J. Vit. Nutr. Res. 54: 211-216.

Brown, G. M. (1958) The metabolism of pantothenic acid. J. Biol. Chem. 234: 370-378.

Bucolo, G. & David, H. (1973) Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem. 19: 476-482.

Burton, D. N., Sobhy, C., Jenik, R. A. & Porter, J. W. (1979) Interconversion of apo- and holofatty acid synthetases of rat and pigeon liver. In: Methods in Enzymology, Vol. 62 (Lowenstein, J.M. ed.), pp. 249-262. Academic Press, New York, NY.

Carter, C. W., & Hockaday, T. D. R. (1972) Liver lipids and ketone-body formation in rats deficient in pantothenate. Biochem. J. 84: 275-280.

Elia, M., Zed, C. Neeale, G. & Livesey, G. (1987) The energy cost of triglyceride-fatty acid recycling in nonobese subjects after an overnight fast and four days of starvation. Metabolism, 36: 251-255.

Elovson, J. & Vagelos, P. R. (1968) ACP synthetase. J. Biol. Chem. 243: 3603-3611.

Guehring, R. R., Hurley, L. S. & Morgan, A. F. (1952) Cholesterol metabolism in pantothenic acid deficiency. J. Biol. Chem. 197: 485-493

Guynn, R. W., Veloso, D. & Veech, R. L. (1972) The concentration of malonyl-coenzyme A and the control of fatty acid synthesis in vivo. J. Biol. Chem. 247: 7325-7331.

Guzman, M & Geelen, M. J. H. (1993) Regulation of fatty acid oxidation in mammalian liver. Biochim. Biophy. Acta. 1167: 227-241.

Halvorsen, O. (1983) Effects of hypolipidemic drugs on hepatic CoA. Biochem. Pharmacol. 32: 1126-1128.

Halvorsen, O. & Skrede, S. (1982) Regulation of the biosynthesis of CoA at the level of pantothenate kinase. Europ. J. Biochem. 124: 211-215.

Hsu, JC., Tanaka, K., Inayama, I. & Ohtani, S. (1992) Effects of pantethine on lipogenesis and CO_2 production in the isolated hepatocytes of the chick (Gallus Domesticus). Comp. Biochem. Physiol. 102: 569-572.

Karasawa, T., Yoshida, K., Furukawa, K. & Hosoki, K. (1972) Feedback inhibition of pantothenate kinase by coenzyme A and possible role of the enzyme for the regulation of cellular coenzyme A level. J. Biochem. 71: 1065-1067.

Knights, K. M. & Drew, R. (1988) A radioisotopic assay of picomolar concentrations of coenzyme A in liver tissue. Anal. Biohem. 168: 94-99.

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

Lopaschuk, G. D., Hansen, C. A. & Neely, J. R. (1986) Fatty acid metabolism in hearts containing elevated levels of CoA. Am. J. Physiol. 250: H351-H359.

Lopaschuk, G. D. & Neely, J. R. (1987) Stimulation of myocardial coenzyme A degradation by fatty acids. Am. J. Physiol. 253: H41-H46

Luo, J., Rizkalla, S. W., Alamowitch, C., Boillot, J., Bruzzo, F., Chevalier, A. & Slama, G. (1992) Neither dietary fructose, dextrose nor starch modifies in vitro glycerol release by adipocytes from streptozotocin-diabetic rats. J. Nutr. 122: 2361-2366.

Majerus, P. W., Alberts, A. W. & Vagelos, P. W. (1965) Acyl carrier protein - The primary structure of the substrate-binding site. J. Biol. Chem. 240: 4723-4726.

Martin, D. B., Horing, M. G. & Vagelos, P. R. (1964) Fatty acid synthesis in adipose tissue. J. Biol. Chem. 236: 663-668.

Mayes, P. A. (1990) Chap. 23-29. In: Harper's Biochemistry, 22nd ed. (Langan, C., ed.), pp. 199-266. Appleton & Lange, East Norwalk, CT.

McGowan, M. W., Artiss, J. D., Strandbergh, D. R. & Zak, B. (1983) A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem. 29: 538-542.

Moir, A. M. B. & Zammit, V. A. (1993) Rapid switch of hepatic fatty acid metabolism from oxidation to esterification during diurnal feeding of meal-fed rats correlates with changes in the properties of acetyl-CoA carboxylase, but not of carnitine palmitoyltransferase I. Biochem. J. 292: 241-246.

Moiseenok, A. G., Sheibak, V. M. & Gurinovich, V. A. (1987) Hepatic CoA, S-acyl-CoA, biosynthetic precursors of the coenzyme and pantothenate-protein complexes in dietary pantothenic acid deficiency. Int. J. Vit. Nutr. Res. 57: 71-77.

Morisaki, N., Matsuoka, N., Shirai, K., Sasaki, N., Saito, Y. & Kumakai, A. (1982) Effects of pantethine on fatty acid oxidation in microvessels of rat brain. Tohoko J. Exp. Med. 141: 41-45.

Naito, H. K. & David, J. A. (1984) Lipid analysis in blood and tissue. In: Lipid Research Methodology (Story, J. A., ed.), pp. 4-11. Alan R Liss Inc., New York, NY.

National Research Council (1985) Guide for the Care and Use of Laboratory Animals. Publication no. 85-23 (rev.), National Institutes of Health, Washington, DC.

Nemeth, P. M., Rosser, B. W. C., Choksi, R. M., Norris, B. J. & Baker, K. M. (1992) Metabolic response to a high-fat diet in neonatal and adult rat muscle. Am. J. Physiol. 262: C282-C286.

Nilsson, A., Thomassen, M. S. & Christiansen, E. (1984) Long-chain acyl-CoA levels in liver from rats fed high fat diets: Is it of significance for an increased peroxisomal ß-oxidation? Lipids 19: 187-194.

Noma, A., Kita, M. & Okamiya, T. (1984) Effect of pantethine on post-heparin plasma lipolytic activities and adipose tissue lipoprotein lipase in rats. Horm. Metabol. Res. 16: 233-236.

Novelli, G. D., Schmetz, F. J. & Kaplan, N. O. (1954) Enzymatic degradation and resynthesis of coenzyme A. J. Biol. Chem. 206: 533-545.

Peterson, M. A., Wittwer, C. T. Jorgensen, E. Windham, C. T. & Wyse, B. W. (1987) The effect of pantothenate status on serum triglycerides and free fatty acids in rats fed a high fat diet. Fed. Proc. 46:1489.

Powell, G. L. Elovson, J. & Vagelos, P. R. (1969) Acyl carrier protein - Synthesis and turnover of the prosthetic group of acyl carrier protein in vivo. J. Biol. Chem. 244: 5616-5624.

Reibel, D. K., Wyse, B. W., Berkich, D. A. & Neely, J. R. (1981) Regulation of coenzyme A synthesis in heart muscle: effects of diabetes and fasting. Am. J. Physiol. 240: H606-H611.

Reibel, D. K., Wyse, B. W., Berkich, D. A., Palko, W. M. & Neely, J. R. (1981) Effects of diabetes and fasting on pantothenic acid metabolism in rats. Am. J. Physiol. 240: E597-601.

Reibel, D. K., Wyse, B. W., Berkich, D. A. & Neely, J. R. (1982) Coenzyme A metabolism in pantothenic acid-deficient rats. J. Nutr. 112: 1144-1150.

Robishaw, J. D. & Neely. J. R. (1985) Coenzyme A metabolism. Am. J. Physiol. 248: E1-E9.

Roncari, D. A. K., Bradshaw, R. A. & Vagelos, P. R. (1972) ACP - Amino acid sequence of liver fatty acid synthetase peptides containing 4'-phosphopantetheine. J. Biol. Chem. 247: 6234-6242.

Saudek, C. D. (1976) The metabolic events of starvation. Am. J. Med. 60: 117-126.

Schaefer, A. E., Mckibbin, J. M. & Elvehjem, C. A. (1942) Pantothenic acid deficiency studies in dogs. J. Biol. Chem. 143: 321-330.

Skrede, S. & Halvorsen, O. (1979) Mitochondrial biosynthesis of coenzyme A. Biochem. Biophy. Res. Comm. 91: 1536-1542.

Skrede, S. & Halvorsen, O. (1979) Increased biosynthesis of CoA in the liver of rats treated with clofibrate. J. Biochem. 98: 223-229.

Smith, C. M., Cano, M. & Potyraj, J. (1978) The relationship between metabolic state and total CoA content of rat liver and heart. J. Nutr. 108: 854-862.

Smith, C. M., Narrow, C. M., Kendrick, Z. V. & Steffen, C. (1987) The effects of pantothenate deficiency in mice on their metabolic response to fast and exercise. Metabolism, 36: 115-121.

Song, W. & Wyse, B. (1993) Physiology of pantothenic acid. In: Encyclopedia of Food Science, Food Technology and Nutrition, Academic press, London, United Kingdom.

Srinivasan, V. & Belavady, B. (1975) Sex differences in susceptibility to experimental induction of pantothenic acid deficiency in rats. Indian J. Biochem. Biophys. 12: 185-187.

Srinivasan, V. & Belavady, B. (1976) Alterations in gluconeogenesis in experimental pantothenic acid deficiency. Indian J. Biochem. Biophy. 13: 387-389.

Tweto, J. & Larrabee, A. R. (1972) The effect of fasting on synthesis and 4'-phosphopantetheine exchange in rat liver fatty acid synthetase. J. Biol. Chem. 247: 4900-4904.

Unna, K. & Richards, G. (1942) Relationship between pantothenic acid requirement and age in the rat. J. Nutr. 23: 545-553.

Vagelos, P. W. & Larrabee, A. R. (1967) ACP - Acyl carrier protein hydrolase. J. Biol. Chem. 242: 1776-1781.

Voet, D & Voet, J. G. (1990) Lipid metabolism. In: Biochemistry, pp. 618-677. John Wiley & Sons.

Volpe, J. J. & Vagelos, P. R. (1973) Saturated fatty acid biosynthesis and its regulation. Ann. Rev. Biochem. 42: 21-60.

Volpe, J. J. & Vagelos, P. R. (1973) Fatty acid synthetase of mammalian brain, liver and adipose tissue regulation by prosthetic group turnover. Biochim. Biophys. Acta. 326: 293-304.

Wakil, S. J. (1989) Fatty acid synthase, a proficient multifunctional enzyme. Biochem. 28: 4523-4530.

Wakil, S. J. Stoops, J. K. & Joshi, V. C. (1983) Fatty acid synthesis and its regulation. Ann. Rev. Biochem. 52: 537-579.

Wilhem, F. (1988) Pantothenic acid. In: Vitamins (Gruyter, W., ed), pp. 807-835. Berlin, Germany.

Williams, M. A., Chu, L.-C., McIntosh, D. J. & Hincenbergs, I. (1968) Effects of dietary fat level on pantothenate depletion and liver fatty acid composition in the rat. J. Nutr. 94: 377-382.

Williamson, J. R. & Corkey, B. E. (1969) Assays of intermediates of the citric acid cycle and related compounds by fluorometric enzyme methods. In: Methods in Enzymology, Vol. 13 (Lowenstein, J. M., ed.), pp. 434-445. Academic Press, New York, NY.

Wittwer, C. T., Beck, S., Peterson, M., Davidson, R., Wilson, D. E. & Hansen, R. G. (1990) Mild pantothenate deficiency in rats elevates serum triglyceride and free fatty acid levels. J. Nutr. 120: 719-725.

Wittwer, C. T., Gahl, W. A., Butler, J. D., Zatz, M. & Thoene, J. G. (1985) Metabolism of pantethine in cystinosis. J. Clin. Invest. 76: 1665-1672.

Wolfe, R. R. & Durkot, M. J. (1985) Role of very low density lipoproteins in the energy metabolism of the rat. J. Lipid. Res. 26: 210-216.

Wyse, B. W., Wittwer, C. T. & Hansen, R. G. (1979) Radioimmunoassay for pantothenic acid in blood and other tissues. Clin. Chem. 25: 108-111.

Yu, H. L. & Burton, D. N. (1974) Adaptive synthesis of rat liver fatty acid synthetase: Evidence for in vitro formation of active enzyme from inactive protein precursors and 4'-phosphopantetheine. Biochem. Biophy. Res. Comm. 61: 483-488.