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Effects of Dietary Cis and Trans Unsaturated Fatty Acids on Blood Ethanol Clearance in Rats

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Mei-Shin Mong
May 1999

DEDICATION

This dissertation is dedicated to my parents,

Dr. Chih-Shih Mong

and

Mrs. Shin-Yu Chen Mong,

for giving me inspiration and invaluable support during my education.

ACKNOWLEDGMENTS

My appreciation to Dr. Dileep S. Sachan, the chairperson of my Ph.D. committee for his direction, expertise, and support throughout the graduate program. Sincere gratitude is also forwarded to the members of my Ph.D. committee: Dr. Jack Ellison, Dr. Naima Moustaid Moussa, and Dr. Jay Whelan for their tremendous encouragement, interest, time, and constructive suggestions throughout the process.

Genuine appreciation goes to Dr. Jay Whelan for providing laboratory facilities and assistance for fatty acid analysis. Acknowledgment is given to the Department of Nutrition for providing both the opportunity to pursue a Doctoral degree and financial support via graduate research assistantship. A special note of thanks is also extended to many graduate students in the Department of Nutrition as well as many friends in the United States and my homeland for their help and friendship.

My gratefulness to the members of my husband's family for their love, prayer, and support. A special thank you to my younger brothers and sister for their confidence and encouragement. Most gracious thanks to my parents, Chih-Shih Mong and Shih-Yu Chen Mong, for their love, support, understanding, and inspiration during my studies abroad.

With love and deepest gratitude, I am thankful to my husband, Chun-Hung Chiu, for standing by me while I confronted challenges and achieved my goals. This moment is shared with our boy, Linus, whose arrival adds a whole new meaning to our lives.

ABSTRACT

The effects of saturated fatty acids (SFA), cis fatty acids (CFA), and trans fatty acid (TFA) on blood ethanol clearance were determined in 5-week-old male Sprague-Dawley rats. In the first phase, it was established that the blood ethanol concentrations (BEC) of the SFA and CFA groups were higher than those of the TFA group after 2 weeks of dietary treatment. While the concentrations of liver triglyceride were significantly lower in the TFA group, the levels of plasma triglyceride were not affected by the types of dietary fatty acids.

Possible mechanisms of dietary fat related changes on BEC were investigated in these rats. It was found that TFA enhanced blood ethanol clearance in comparison to SFA or CFA; however, the hepatic alcohol dehydrogenase activity was not affected after either 2 or 6 weeks of dietary treatment. The activity of carnitine palmitoyl transferase-I was also not affected by the types of dietary fatty acid. The excretion of ethanol in urine was higher in the TFA group than in the CFA group during the first 4 hours post- ethanol administration and might partially explain the dietary fatty acids related changes in BEC. In addition, the concentrations of acylcarnitine were changed parallel with BEC of the dietary groups. Thus, the reduction in BEC in rats fed the TFA may be at least in part mediated through parallel changes in carnitine concentrations.

Differences in ethanol uptake and metabolism were examined in small intestines of rats fed dietary SFA, CFA, or TFA. The uptake and/or metabolism of ethanol was measured in everted intestinal sacs. Ethanol transport expressed as the ratio of serosal to mucosal ethanol concentrations and the *in vitro* absorption rate of ethanol was not

significantly affected by dietary fatty acids. However, when the amount of ethanol metabolized to CO_2 during the transport process was taken into account, uptake and metabolism of ethanol by the proximal segments of the small intestine were significantly higher in the rats fed TFA and CFA than in those fed SFA. Because ethanol oxidation was not significantly different between the CFA and TFA group, some other mechanism must be responsible for the differences in BEC of CFA and TFA animals. Nonetheless, it is noted that ethanol metabolism in extrahepatic tissues can not be ignored for the effects of SFA and unsaturated fatty acids on ethanol pharmacokinetics.

The fatty acid composition data revealed that the fatty acid profiles of diets were reflected in the fatty acid content of various tissues. The phospholipids fraction of the liver and small intestine of rats fed the TFA diet for 6 weeks had a higher percentage of linoleic acid and lower percentages of palmitic, stearic, and arachidonic acids than those fed the SFA and CFA diets. Compared with rats fed the CFA diet, the fatty acid composition in the liver microsomes of those fed the TFA diet was characterized by a significant decrease in palmitic, stearic, and oleic acids and an increase in linoleic acid. These fatty acid changes were similar in plasma. The fatty acid composition data supports the suggestion that elaidic acid interfered with the conversion of linoleic acid to arachidonic acid which has been shown to protect liver from alcohol-induced injury.

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ABBREVIATIONS

ADH	alcohol dehydrogenase
AIAC	acid insoluble acylcarnitine
ALD	alcoholic liver disease
ALDH	aldehyde dehydrogenase
ASAC	acid soluble acylcarnitine
BEC	blood ethanol concentrations
CE	cholesterol esters
CETP	cholesterylester transfer protein
CFA	cis fatty acids
CHD	coronary heart disease
CoA	coenzyme A
CPT-I	carnitine palmitoyl transferase-I
CPT-II	carnitine palmitoyl transferase-II
CYP	cytochrome P-450
F	females
FAMEs	fatty acid methyl esters
GC	gas chromatography
GLC	gas-liquid chromatography
HDL	high-density-lipoprotein
IR	infrared chromatography
LCAT	lecithin:cholesterol acyltransferase
LCT	long-chain triglyceride

LDL	low-density-lipoprotein
Lp(a)	lipoprotein (a)
M	males
MCT	medium-chain triglyceride
MEOS	microsomal ethanol oxidizing system
MUFA	monounsaturated fatty acids
NAD	nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NEC	nonesterified carnitine
NL	neutral lipids
PC	phosphotidylcholine
PCA	perchloric acid
PHCO	partially hydrogenated corn oil
PHFO	partially hydrogenated fish oil
PHHO	partially hydrogenated herring oil
PHLRSO	partially hydrogenated low erucic acid rapeseed oil
PHMO	partially hydrogenated marline oil
PHNCO	partially hydrogenated Norwegian capelin oil
PHOO	partially hydrogenated olive oil
PHPO	partially hydrogenated peanut oil
PHSF	partially hydrogenated soybean fat

PHSO	partially hydrogenated safflower oil
PHSBO	partially hydrogenated soybean oil
PHVO	partially hydrogenated vegetable oils
PL	phospholipids
PO	peanut oil
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
SFO	sunflowerseed oil
SSO	sunflowerseed oil
TFA	trans fatty acids
TC	total carnitine
TG	triglyceride
TL	total lipids
TLC	thin-layer chromatography
USFA	unsaturated fatty acids

CHAPTER I

INTRODUCTION

There has been a change in the type and amount of dietary fat consumed in the U.S. and the world. The leading change in consumption of visible fats is the shift from animal fats to the fats of vegetable origin (Enig et al. 1990, FAO 1994, Hudgins et al. 1991, Meydani et al. 1991). Elevated intake of the processed vegetable fats and oils has led to an increased availability of chemically altered fatty acids known as trans isomers. Trans fatty acids (TFA) are formed during the catalytic partial hydrogenation of vegetable oils to manufacture margarines, shortenings and cooking oils and also are synthesized by rumen microorganisms. Approximately 90-95 % of dietary TFA are provided by the partially hydrogenated vegetable oils and the remaining 5-10 % from ruminant fats in the average American diet (Emken 1984, Enig 1996). The principal TFA produced by the catalytic partial hydrogenation and microbial biohydrogenation are elaidic acid and vaccenic acid, respectively (Kris-Etherton and Nicolosi 1995, Senti 1985, Sommerfeld 1983). The average intake of TFA in the U.S. is estimated to be 8.1 to 13.3 g/day or 3-4% of total energy intake (Enig et al. 1990, Hunter and Applewhite 1991). Nevertheless, intake may be much higher in individuals who eat large amounts of foods prepared with such fats (Enig et al. 1990, Lichtenstein 1995).

The answers concerning the health implications and physiological effects of TFA are not yet clear (Katan 1998, Willett and Ascherio 1994). The concept that the effect of dietary TFA is not deleterious should be reevaluated. The least of their effect is to inhibit

the conversion of linoleic and α-linolenic acids to their major metabolites, arachidonic and docosahexaenoic acids, and thus increase the requirement for these essential fatty acids (Dupont et al. 1991, Sugano and Ikeda 1996, Zevenbergen et al. 1988).

Ethanol metabolism is profoundly affected by the type and amount of dietary lipids. Epidemiological and animal studies suggested that saturated fats were relatively protective against the process of alcoholic liver disease (ALD), whereas unsaturated fats promoted ALD (French 1993, Nanji and French 1986, Nanji et al. 1989, Nanji et al. 1994a). Rats fed saturated fatty acids (SFA) were found to have lower rates of ethanol metabolism than those fed unsaturated fatty acids (USFA) (Cha and Sachan 1994).

Dietary fat modifications have been shown to alter the absorption of ingested nutrients (Thomson and Rajotte 1983, Thomson et al. 1986, Bernard et al. 1987), which may involve the changes in the fatty acid composition of intestinal membranes. Information concerning the influences of feeding TFA on the intestinal uptake of nutrients and xenobiotics, however, is not complete.

Carnitine has been shown to ameliorate ethanol-induced fatty livers in rats (Berger and Sachan 1986;1991, Rhew and Sachan 1986, Sachan 1992) which can be explained by the competitive inhibition of alcohol dehydrogenase (ADH) by acetylcarnitine (Sachan and Cha 1994). When effects of SFA and USFA on ethanol metabolism were determined, higher blood ethanol concentrations (BEC) were associated with higher concentrations of carnitine fractions (Cha and Sachan 1994). Therefore, it was essential to determine the changes in carnitine fractions in animals fed cis and trans fatty acid isomers.

We used ethanol metabolism as a model to explore the physiological effect of

TFA. The main objective of these studies was to determine the effects of cis and trans fatty acids on BEC. Possible mechanisms responsible for the changes in BEC, such as hepatic ADH activity, plasma carnitine concentrations, urinary excretion of ethanol, and intestinal uptake and metabolism of ethanol, were investigated. Fatty acid composition of plasma and different tissues was also analyzed to examine the relative changes by the incorporation of TFA.

CHAPTER II

LITERATURE REVIEW

ETHANOL

Ethanol, ethyl alcohol or simply alcohol, is a colorless volatile liquid of a pungent odor which is produced principally by means of sugar or carbohydrate fermentation through the action of microorganisms such as bacteria and yeasts. It is a liquid of polar nature and is miscible with water. Because of its small size and weak charge, ethanol is able to pass freely through biological membranes in the same manner as other low molecular weight polar molecules such as water (Batt 1989, Kricka and Clark 1979).

In mammals, ethanol is normally present in cells at low concentration, on the order of 0.1 to 1 mmol/L, and it is derived from the fermentation activity of gastrointestinal flora and some due to endogenous production in different tissues, especially liver (Baraona et al. 1986, Krebs and Perkins 1970, McManus et al. 1960). However, the main source of ethanol in man is not its endogenous production but its direct consumption. Only in rare cases such as patients with a blind intestinal loop and those with an overproduction of the intestinal flora including *Candida* and other yeasts, can there be observed elevated levels of ethanol in blood. The fermentation of carbohydrates in intestine of these cases may even produce drunkenness.

Absorption of Ethanol

Once ethanol is ingested, it begins to be absorbed immediately through the mucous epithelium of the mouth and as vapor in the pulmonary alveoli (Batt 1989);

however, the amount of ethanol absorbed through these two pathways is practically negligible. For all practical purposes, the orally ingested alcohol is absorbed in the stomach by simple diffusion through the epithelium of the stomach and intestines at the same rate as water (Batt 1989, Watson 1989). Small proportions of ethanol (approximately 20 to 30%) are absorbed in the bloodstream through the gastric mucosa and the remaining large amount through the small intestine, principally duodenum and jejunum (Batt 1989, Cooke and Birchall 1969, Smith et al. 1992). Ethanol is rapidly carried directly to liver to be metabolized via the portal vein.

Once the ethanol is readily distributed throughout the body fluids, it crosses freely through the totality of the biological membranes (Watson 1989). Women, who have a lower percentage of body fluids (53% versus 61.8%) and a higher percentage of body fat (26.9% versus 14.7%) than men, reach higher levels of ethanol in blood even if both men and women ingest equivalent amounts of alcohol (Batt 1989, Watson 1989).

The absorption rate of ethanol depends mainly on the velocity with which it passes from the stomach to the small intestine, which in turn depends principally on the speed at which gastric emptying is carried out. In general, the presence of solid food retards gastric emptying and therefore, the speed of ethanol absorption (Horowitz et al. 1989). On the other hand, the concentration of the ingested ethanol also influences its absorption, so that high concentration of ethanol retard the emptying of food in the stomach (Horowitz et al. 1989, Roine et al. 1991). Various factors which influence to a greater or lesser extent the velocity of ethanol absorption are listed in **Table 2-1**.

TABLE 2-1

Factors affecting ethanol absorption [1]

Stomach emptying	Type of beverage
Food in the stomach	Protein deficiency
Ethanol concentration	Body temperature
Blood flow at the site of absorption	Physical exercise
Irritant properties of ethanol	Menstrual cycle
Rate of ingestion	

[1] Adapted from Kricka & Clark (1979).

Excretion of Ethanol

Small amount of ethanol (5 to 10%) are excreted unchanged in urine (0.2 to 2.0%), expired air (5%), and sweat (0.5%) (Agarwal and Goedde 1992). Urinary excretion of ethanol is a passive process and ethanol clearances of between 0.9 to 12.7 mL/minute have been reported by this route (Blackmore and Mason 1968). It was estimated that the total alcohol eliminated by the human body per hour is usually in the range of 100 to 300 mg/kg/hour, which is equivalent to 6 to 9 g alcohol/hour, for a healthy subject with an average body weight (Agarwal and Goedde 1992).

Metabolism of Ethanol

Ethanol is absorbed mainly from the small intestine, where it is channeled through the portal vein directly to liver before passing into the systemic circulation and the rest of the body (Goldstein 1983). The liver is the principal organ responsible for the oxidation and elimination of ingested alcohol (Hawkins and Kalant 1972). Hepatic vein

catheterization studies have shown that around 75% of a dose of ethanol is eliminated by hepatic metabolism (Winkler et al. 1969). Maximal hepatic metabolic capacity is approximately 2 mmol ethanol/minute (Tygstrup et al. 1974) as compared to a total extrahepatic metabolism which is estimated to be 0.4 mmol/minute.

In liver, there are three metabolic systems capable of carrying out ethanol oxidation. The first system is made up of a series of specialized enzymes known generically as alcohol dehydrogenases or ADH, which are found in the cytosol of the different tissues, principally hepatic of the human body. These enzymes promote the oxidation of ethanol into acetaldehyde, coupling this oxidation with the reduction of a nicotinamide adenine dinucleotide (NAD^+). The second oxidizing system is called microsomal ethanol oxidizing system (MEOS), which is located in the microsomes and requires participation of cytochrome P-450. The cytochromes P-450 couple ethanol and nicotinamide adenine dinucleotide phosphate oxidation (NADPH) to the reduction of an oxygen molecule to form hydrogen peroxide. The third system is found in hepatic peroxisomes, and in this system, the oxidation of a molecule of ethanol into acetaldehyde is coupled with the simultaneous decomposition of a hydrogen peroxide molecule in a reaction catalyzed by the enzyme called catalase. These three systems work simultaneously in the presence of ethanol, although with different activities and affinities.

Besides the three enzymatic systems described previously for ethanol oxidation, there exists a non-enzymatic ethanol oxidation in the microsomal system (Koop 1989). The presence of this non-enzymic pathway results from two factors. The first is the inherent degree of uncoupling of the microsomal electron transport system which results in the one- and two-electron reduction of oxygen to superoxide and hydrogen peroxide,

respectively (Black and Coon 1987, White and Coon 1980). The H_2O_2 formed in *in vitro* incubations can then participate in ethanol oxidation when the second factor is present, that being a reduced metal chelate (such as Cu, Fe, or Co) which facilitates a classical metal-dependent reduction of H_2O_2 (Fenton reaction) and can be formulated as follows:

$$M^{n+} + H_2O_2 \longrightarrow M^{(n+1)+} + OH^- + {}^{\cdot}OH$$

The typical promoters of this reaction *in vitro* appear to be iron chelates in a classical iron catalyzed Haber-Weiss reaction formulated as follows (Halliwell and Gutteridge 1986):

$$Fe^{3+} + O_2^- \longrightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + {}^{\cdot}OH$$

In microsomal reactions, the formation of reduced metal chelates such as iron-EDTA is facilitated by the ability of NADPH cytochrome P-450 reductase to reduce the complex (Feierman et al. 1985, Morehouse et al. 1984). Thus, in a microsomal incubation, if Fe^{3+} and EDTA are present, the system is poised to catalyze the iron-dependent oxidation of ethanol. Since NADPH and O_2 are required, this reaction would appear on the surface to be catalyzed by P-450, but in fact it is the microsomal electron transport chain which is supplying the reducing equivalents to both iron and molecular oxygen. In another word, the metal chelate catalyzes the oxidation. It was reported that biological iron complexes such as phosphate, carbohydrate, organic acid , and ferritin complexes can produce hydroxyl radical in a system containing hydrogen peroxide and superoxide (Halliwell and Gutteridge 1986). In addition, iron-EDTA was found to stimulate microsomal ethanol oxidation about threefold (Feierman et al. 1985). Therefore, this nonenzymic system may have a role *in vivo* in the metabolism of ethanol.

A non-oxidative metabolic pathway where ethanol forms fatty acid ethyl ester by

means of a fatty acyl ester synthetase also exists (Lange et al. 1981, Koop 1989). Even if its contribution to total ethanol metabolism is not significant (Koop 1989), its participation in the development of pathological alterations remains to be discussed in organs that lack high activity ethanol oxidating systems, such as pancreas, heart and brain, (Laposata and Lange 1986).

Alcohol Dehydrogenase (EC 1.1.1.1)

Alcohol dehydrogenase activity is carried out by three families of enzymes. Three protein families of different origins, structures and reaction mechanisms which exhibit this capacity have been described.

The first family is comprised of "short chain" alcohol dehydrogenases, which do not require a metallic ion as cofactor and which are found characteristically among insects (Persson et al. 1991). The second group is made up of the "medium and long chain" alcohol dehydrogenases, and includes the "classic" mammalian alcohol dehydrogenases. These enzymes require zinc atoms as cofactor, and are divided into tetramerics and dimerics. The latter conform the most diversified group in mammals and include about a dozen different isozymes classified into six distinct classes (Arnon 1995, Danielsson et al. 1994). The third family corresponds to the Fe-dependent alcohol dehydrogenases, which are characteristic of unicellular organisms such as *Zymomonas* and *Clostridium*. This group makes up the smallest and least studied family of alcohol dehydrogenases (Scopes 1983). In vertebrates, only the first two protein families are present, and only the "medium and long chain" family exhibits a significant alcohol dehydrogenase activity in these organisms, which will be referred to as the alcohol dehydrogenase system.

The alcohol dehydrogenase system in mammals is divided into six distinct classes with at least twenty different isozymes coded by more than seven distinct genes (Arnon 1995, Danielsson et al. 1994). These enzymes are amply distributed in different human tissues (Boleda et al. 1989), and all of them are characterized by being dimeric proteins with subunits of approximately 40kDa. Each subunit is characterized as containing two atoms of zinc bonded by cysteines that help stabilize the enzyme structure (Jelokova et al 1994), besides the fact that one of them forms part of the active site (Arnon 1995). **Table 2-2** shows the different classes of alcohol dehydrogenases described above as well as the distinct genes and subunits that they comprise.

All these enzymes use NAD^+ as electron-accepting coenzyme, to catalyze the oxidation of ethanol to acetaldehyde. This reaction mechanism has been described as bi-bi ordered type, where the NAD^+ and NADH should penetrate the coenzyme union site before the ethanol or acetaldehyde enters the catalytic domain (Brändèn et al. 1975). The reaction shows as follow:

$$CH_3CH_2OH + NAD^+ \xrightarrow{ADH} CH_3CHO + NADH + H^+$$

All ADH isozymes show Michaelis-Menten kinetics with respect to ethanol (Burnell and Bosron 1989), and only $\gamma\gamma$ isozymes seem to exhibit a negative cooperativity for ethanol. The dissociation of NADH is the rate-limiting step in these enzymes (Burnell and Bosron 1989, Fan and Plapp 1995).

"Medium and long chain" alcohol dehydrogenases are grouped into classes based on their structural differences. **Figure 2-1** shows the possible phylogenetic relationships among different ADH types. Of all the classes described, the first five have already been found in human tissues (Kedishvili et al. 1995, Seitz and Oneta 1998). The recently

TABLE 2-2

Alcohol dehydrogenases (ADH) polymorphisms in mammals [1]

Class	Gene Locus	Allele[a]	Subunit	Tissue distribution
I	ADH - 1	*ADH1*	α_1	Liver
I	ADH - 2	*ADH2*1*	β_1	Liver and lung
I		*ADH2*2*	β_2	Liver and lung
I		*ADH2*3*	β_3	Liver and lung
I	ADH - 3	*ADH3*1*	γ_1	Liver and stomach
I	ADH - 3	*ADH3*2*	γ_2	Liver and stomach
II	ADH - 4	*ADH4*	π	Liver, cornea, kidney and lung
III	ADH - 5	*ADH5*	χ	All tissues
IV	ADH - 7	*ADH*σ	σ	Liver, stomach, skin and cornea
IV	?	*ADH*μ	μ	Liver, stomach and skin
V	ADH - 6	*ADH6*	?	Liver and stomach
VI	?	?	?	Liver

[1] Modified from Arnon et al. (1995).

[a] Each gene locus has one allele, except for ADH-2 and ADH-3 which have 3 and 2 alleles, respectively. Alleles from the same gene locus are indicated with an asterisk.

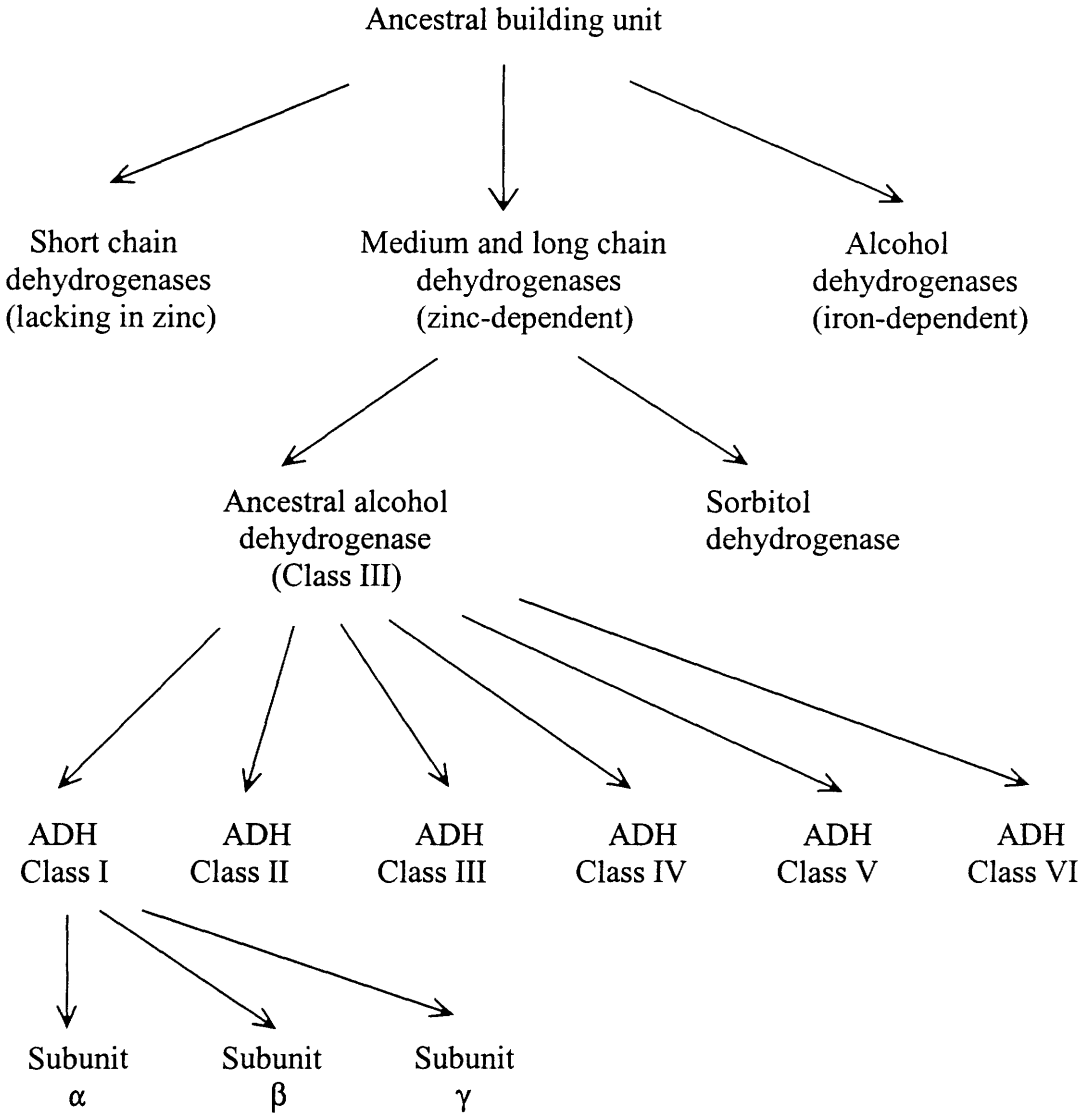


FIGURE 2-1 Phylogenetic relations among the different types of alcohol dehydrogenases.

described class VI has not yet been characterized. The most relevant characteristics of each class of mammalian alcohol dehydrogenase are briefly described below.

Class I: This is the most studied class in this enzyme family and includes the "classic" hepatic enzyme. It has three distinct gene loci (ADH-1, ADH-2, and ADH-3) found in human chromosome four, which encode for three types of subunits (α, β, and γ). The alleles for the β and γ subunits differ among themselves by a few amino acid residues (β_1, β_2, β_3, and γ_1, γ_2). These subunits can form homodimers or heterodimers contributing to the generation of a great polymorphism with many isoforms. Each isoform presents variants as to their kinetic properties (**Table 2-3**) which partially explains the great heterogeneity in the capacity for metabolizing ethanol by the human populations. For example, ADH-2 locus is commonly known as 'atypical' ADH that contains a variant β_2 subunit instead of the usual β_1 subunit. While about 5-10% of the English, 9-14% of the German, and 20% of the Swiss population possess the atypical ADH. The variant enzyme occurs in about 85% of Chinese, Japanese, and other Orientals (Agarwal and Goedde 1992).

All isozymes of this class are highly sensitive to pyrazole inhibition and show substrate inhibition ([ethanol] > 20 mmol/L) because an ethanol excess decreases the speed of dissociation of NADH from the enzyme (Danielsson et al. 1994, Kamlay and Shore 1983).

Class II: This class is made up of homodimetric isozyme $\pi\pi$, and is found in diverse organs such as liver, cornea, kidney and lung. It participates actively in the degradation of circulating epinephrine and norepinephrine (Mardh et al. 1986). This class exhibits a high affinity for ethanol, and is only slightly sensitive to pyrazole inhibition

TABLE 2-3

Kinetic properties of some ADH isozymes, with ethanol as substrate [1]

Isozyme	Kinetic constant			Optimal pH
	Km of NAD+ (μmol/L)	Km of ethanol (mmol/L)	Vmax (min^{-1})	
αα	13.0	4.2	27.0	10.5
$\beta_1\beta_1$	7.4	0.049	9.2	10.5
$\beta_2\beta_2$	180.0	0.94	400.0	8.5
$\beta_3\beta_3$	712.0	36.0	300.0	7.0
$\gamma_1\gamma_1$	7.9	1.0	87.0	10.5
$\gamma_2\gamma_2$	8.7	0.63	35.0	10.5
ππ	14.0	34.0	20.0	10.5
χχ		≥ 2000	?	

[1] Modified from Burnell & Bosron (1989).

(Bosron et al. 1979).

Class III: This class is made up of isozyme χχ, which is a homodimer encoded by the ADH-5 gene, and probably corresponds to the ancestral form which gave origin to the rest of the ADH (Arnon 1995, Danielsson et al. 1994). It possesses a great capacity to metabolize long chain alcohols; however, its capacity to oxidize ethanol is, on the other hand, very limited (Bosron et al. 1988). Class III ADH is broadly distributed in the different species and tissues (Arnon 1995), but it is the only ADH isozyme found in the brain (Beisswenger et al. 1985). Due to its poor capacity to oxidize ethanol (Km > 2.5 mol/L), the possibilities that ADH protects the brain against ethanol or its metabolites and

that the brain can generate energy from ADH-monitored ethanol metabolism for cerebral function is excluded. Therefore, it has been suggested that this class of enzyme plays special but as yet unknown physiological and biochemical roles (Beisswenger et al. 1985).

Class IV: this class is expressed preferably in the stomach (Danielsson et al. 1994), although it can be found in lesser quantities in other tissues such as liver, skin and cornea (Arnon 1995). It is made up of isozymes σσ and μμ, which are encoded by the ADHσ and ADHμ genes, located in the ADH-7 locus (Arnon 1995). It presents a very high Km for ethanol, and is thought to participate actively in the first-pass ethanol metabolism in stomach.

Class V: This class has been found in gastric epithelium, and is made up of a homodimer encoded by the ADH-6 gene (Arnon 1995). It possesses a very high Km for ethanol and is also thought to participate in the gastric first-pass metabolism of ethanol.

Class VI: This class is the newest of the ADH classes described in mammals (Zheng et al. 1993). It was identified for the first time in *Peromyscus maniculatus* (deer mouse), and is present in ADH-positive genotypes as well as in negative ADH (which lack the Class I "typical"alcohol dehydrogenase). It is expressed principally in liver and in minimum quantities in kidney. Its role in ethanol metabolism is still uncertain. No reports of its existence have been noted in humans, but it has been identified in rats (Höög et al. 1995).

Microsomal Ethanol Oxidizing System (MEOS)

The MEOS accounts for the major non-ADH pathway of ethanol oxidation in the liver. This system was investigated for the first time by Lieber's group at the late 60s

(Lieber and DeCarli 1968, Rubin et al. 1968) from an initial report by Orme-Johnson and Ziegler (1965), in which they described parallel increments in the capacity to metabolize drugs and ethanol with total activity of cytochrome P-450 in the smooth endoplasmic reticulum of hepatic cells. From these initial reports, the existence of a new cytochrome P-450 dependent pathway of ethanol oxidation was rapidly developed.

Currently, the presence of MEOS in the microsomes of different tissues is well recognized (Gonzalez 1992), and it is now known that this system involves the participation of various enzymes of the present cytochrome P-450 superfamily. The P-450 superfamily is composed of about 230 genes and pseudogenes distributed among thirty-six distinct families, of which at least ten are present in mammals (Degtyarenko and Archakov 1993, Gonzalez 1992). These families are defined basically as to their similarities in terms of amino acid sequences (Gonzalez 1992).

Cytochromes P-450 are divided into two main classes, from the functional point of view. The first class (which is comprised by mammalian classes CYP1, CYP2, CYP3 and CYP4) is involved in the metabolism of xenobiotic compounds. The second (the CYP7, CYP11, CYP17, CYP19, CYP21, and CYP27 families) is related to the synthesis of steroids and bile acids (Gonzalez 1992).

Different types of cytochrome P-450 are capable of catalyzing the ethanol oxidation, although the most efficient is cytochrome P-450 2E1 (Koop 1989). According to the nomenclature recommended by Nebert's group (Nebert et al. 1989, Nelson et al. 1993), cytochrome P-450 2E1 is also termed CYP2E1 or simply 2E1. This cytochrome was originally referred to as "3a" in rabbits and rats, and as "j" in humans, and these names are kept only for reference while reading papers published previously to the

classification proposed by Nebert et al. (1989).

Cytochrome 2E1 is the basic constituent of the MEOS system (Lieber 1994), which possesses activity to oxidize ethanol and other alcohols, such as butanol and pentanol (Morgan et al. 1982). This system holds a low affinity for ethanol, with a Km of 8 to 10 mmol/L (Lieber 1994), therefore, it is important participant in non-alcoholic individuals only when the concentrations of substrates are high in tissues.

The MEOS system catalyzes the oxidation from ethanol to acetaldehyde, coupling this reaction to the oxidation of an NADPH molecule and the reduction of an oxygen molecule to form hydrogen peroxide (Teschke and Gellert 1986).

$$CH_3CH_2OH + NADPH + H^+ + O_2 \xrightarrow{MEOS} CH_3CHO + NADP^+ + 2H_2O$$

The MEOS system is the only one whose activity is significantly induced (5 to 10 times) by the presence of ethanol or other molecules such as pyridine, acetone, and pyrazole (Koop and Tierney 1990, Lieber 1994). Although it appears that the enzyme concentration can be regulated by post-translational mechanisms such as mRNA stabilization, increased translation of existing mRNA, and inhibition of protein degradation (Koop and Tieney 1990), the molecular mechanism underlying 2E1 induction remains disputed (Lieber 1994).

Catalase (EC 1.11.1.6)

This enzyme is an oligomeric protein with 4 subunits (of 60 kDa each) arranged in tetrahedric form. Each subunit functions in an independent manner, and possesses as prosthetic groups a ferric protoporphyrin IX and a NADP+ molecule (Fita and Rudman 1985, Schonbaum and Chance 1976). Catalase was one of the first enzymes that was isolated in a highly purified form and its crystallization from liver extracts marked a

milestone in the history of biochemistry (Schonbaum and Chance 1976).

Catalase is localized in the peroxisomes and its main activity is the decomposition of hydrogen peroxide molecules:

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

In the particular case of ethanol, its oxidation can be coupled to the decomposition of a molecule of hydrogen peroxide, or even to the decomposition of organic peroxide, where:

$$HOOH + CH_3CH_2OH \longrightarrow CH_3CHO + 2H_2O$$
$$ROOH + CH_3CH_2OH \longrightarrow CH_3CHO + ROH + H_2O$$

R = [H, alkyl, acyl]

The main rate limiting factor in the oxidation of ethanol by catalase is the availability of hydrogen peroxides. It is generally accepted that under normal conditions, its contribution in ethanol metabolism is minimal (Lieber 1994).

Acetaldehyde Metabolism

Acetaldehyde, the principal metabolite generated in the first stage of hepatic ethanol catabolism, is produced mainly in the liver. It is metabolized through three metabolic pathways. The first of these is made up of an enzymatic system generically known as aldehyde dehydrogenases or ALDH (EC 1.2.1.3), which catalyze the oxidation of acetaldehyde to acetate in a reaction that requires NAD^+ as electron acceptor (Arnon 1995), where:

$$CH_3CHO + NAD^+ + H_2O \longrightarrow CH_3COO^- + NADH + 2H^+$$

This reaction like ADH-catalyzed ethanol oxidation, has a bi-bi ordered type mechanism, where the previous union of NAD^+ is indispensable for the acetaldehyde to penetrate the

active site (Farrès et al. 1995). The rate limiting step for ALDH is not the dissociation of NADH but the acyl group dissociation of the enzyme.

The second pathway is made up of aldehyde oxidase (EC 1.2.3.1). This enzyme is localized in the cytosol of the cell and has a molecular weight of 300 kDa. It catalyzes acetaldehyde oxidation to acetate in a reaction coupled with oxygen consumption and hydrogen peroxide formation. This enzyme has a Km for acetaldehyde of 1 mmol/L (Rajagopalan et al. 1964).

$$CH_3CHO + O_2 + H_2O \longrightarrow CH_3COO^- + H_2O_2 + H^+$$

The third pathway, described recently (Kunitoh et al. 1996), is named the Microsomal Acetaldehyde Oxidizing System, also called MAOS. This system requires the participation of cytochromes P-450, especially the CYP2E1. It carries out the oxidation of acetaldehyde into acetate in a reaction coupled to the oxidation of NADPH, in a reaction analogous to that achieved by the MEOS.

$$CH_3CHO + NADPH + O_2 + H_2O \longrightarrow CH_3COO^- + NADP^+ + H_2O_2 + 2H^+$$

The activity of this system is induced 2-fold in animals chronically treated with ethanol. However, this system is as yet poorly characterized and thus its role and physiological relevance are not fully recognized.

Interactions of Ethanol with Nutrients

Ethanol, generating 7.1 kcal/g, has profound effects on nutritional status (Feinman and Lieber 1992). Many ethanol-nutrient interactions are described in detail elsewhere (Feinman and Liber 1992, Lieber 1991). In general, an high intake of ethanol may cause primary malnutrition by substituting other nutrients in the diet because of the high energy and low nutrient contents of the alcoholic beverages or associated medical disorders. For

example, as alcohol consumption increases, the percentage of energy derived from protein, lipid, and carbohydrate decreases and the nutritional quality of the diet declines (Hillers and Massey 1985, Sherlock 1984). Concurrently, intake of vitamin A, ascorbate, thiamin, and folate may fall below their recommended daily allowances (Gruchow et al. 1985). In addition, ethanol is directly toxic to many tissues and therefore influences the nutrients by interfering with their intake, absorption, metabolism, and loss from the body. Ethanol was reported to enhance nitrogen loss in the urine of man and thus increasing protein requirements (Bunout et al. 1987).

Ethanol can affect nutrient entry to the body by interfering with digestive functions and intestinal absorption. The secondary alcoholic malnutrition syndrome is probably caused by a combination of factors, including the effect of alcohol on gastric emptying, intestinal motility, digestive functions of the pancreas and liver, cellular structure and function of the small intestine, and active transport of specific nutrients (Feinmen et al. 1992, Korsten et al. 1992, Stowell 1989). For single nutrients, ethanol seems to specifically impair the absorption of those that require an active mechanism for their transport across the intestinal border. Alcohol has been found to impair absorption of thiamin, folic acid, vitamin B12, amino acids, pyridoxine, glucose and some minerals (Feinmen and Lieber 1992, Lieber 1991, Thomson and Majumdar 1981).

TRANS FATTY ACIDS

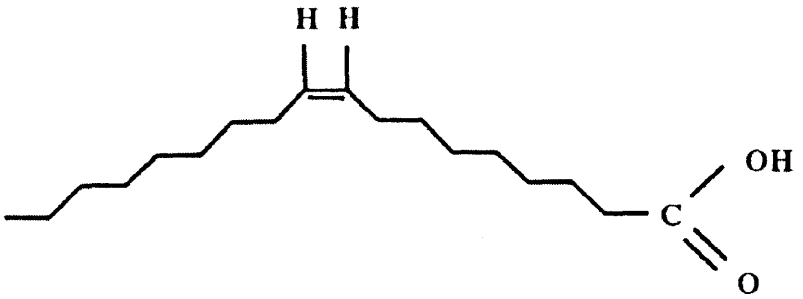
Unsaturated fatty acids are formed in living tissues by the removal of pairs of hydrogen atoms from adjacent methylene groups to produce at least one carbon-carbon double bond. Two geometrical configurations around that double bond are the hydrogen

atoms on the same side (cis) or on the opposite directions (trans). **Figure 2-2** shows the structure of cis and trans fatty acids. In addition, the double bond can be located in various locations along the length of the fatty acid molecule, which give rise to a number of positional isomers within each unsaturated fatty acid (Emken 1984).

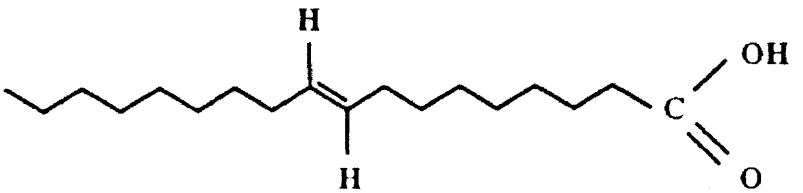
Dietary Sources of TFA

Trans fatty acids (TFA) are formed when vegetable oils are partially or fully hydrogenated to manufacture margarines, shortenings, salad oils, and cooking oils. Catalytic hydrogenation, discovered in 1897, is established by subjecting liquid oils to hydrogen gas under selected temperature, pressure, stirring speed, duration, and catalyst (Emken 1984, Katan and Mensink 1992). Food industry applied this process to convert liquid oils to semisolid fats with improved flavor and stability as well as lowered cost for commercial and household use (Dutton 1979). Food manufacturers favor such partially hydrogenated fats over fully hydrogenated fats, since the latter contains a high stearic acid content which makes them unpleasant to eat (Katan 1998). The fractions of trans isomers in hydrogenated vegetable oil varies widely with hydrogenation conditions. The proportion of TFA increase with the accelerated degree of hydrogenation (ACAN/AIN 1996). Most hydrogenated vegetable oils contain between 5% and 30% trans isomers (Enig et al. 1990) and most of the commercially produced trans isomers are monoenes (ACAN/AIN 1996). The trans-18:1 isomers represent about 90% of the total TFA. The 9t, 12c-18:2 and 9c, 12t-18:2 isomers are present in about equal amounts and stand for about 9% of the total TFA (Emken 1995).

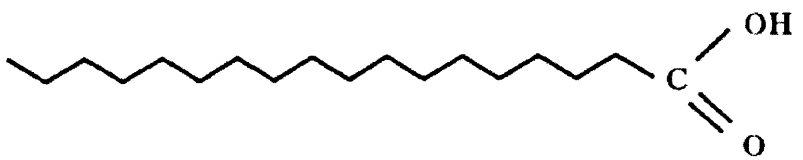
TFA are also synthesized in the rumen of ruminant animals. They are the end-products of anaerobic bacterial fermentation (Emken 1984, Lichtenstein 1997). Humans



Oleic acid (*cis* C18:1)



Elaidic acid (*trans* C18:1)



Stearic acid (C18:0)

Figure 2-2 Structure of oleic (cis), elaidic (trans), and stearic (saturated) acids.

consume them in the form of meat and dairy products. In general, butterfats contain 2 to 9% TFA and milk fats contain 2 to 5% TFA (Emken 1984, Katan and Mensink 1992).

About 90 to 95 % of dietary TFA are provided by partially hydrogenated vegetable oils and the remaining 5 to 10 % from ruminant fats in the average American diet (Emken 1984, Enig 1996). The principal TFA produced by the catalytic partial hydrogenation is elaidic acid (C18:1n9t), whereas microbial biohydrogenation in ruminants leads to the accumulation of mainly vaccenic acid (C18:1n7t) in dairy products and meats (Jenkins 1994, Kris-Etherton and Nicolosi 1995, Senti 1985, Sommerfeld 1983).

Dietary Intake of TFA

The actual intake of TFA can not be easily determined, since there is no readily available databases of fatty acids including TFA (Emken 1995, Enig 1996). In the U.S., the average intake of TFA is estimated to be 8.1 to 13.3 g per person per day, accounting for 3 to 4% of total energy intake as assessed by availability or disappearance data (Enig et al. 1990, Hunter and Applewhite 1991). Nonetheless, intake may be much higher in individuals who eat large amounts of foods prepared with such fats (Enig et al. 1990, Lichtenstein 1995), i.e., fast foods (hamburgers, fried chicken and fish, and french fries), potato chips, corn snacks, cheese snacks, doughnuts, cakes, cookies, pies and turnovers, etc. (Ali et al. 1996, Enig et al. 1983, Enig et al. 1990, Smith et al. 1985, Wootan et al. 1996). **Tables 2-4 to 2-7** show the trans fat contents of some popular foods. Recently, the trans fat content in margarines have largely been phased out (Katan 1995). However, shortenings and frying fats do remain high in trans fatty acids (Katan 1998).

Estimated intake of TFA based on food questionnaire data, 2.6 to 4.6 g per day,

TABLE 2-4

Trans fatty acids in french fries and fried chicken and fish[1]

	Calories (Kcal)	Total fat (g)	Saturated fat (g)	Trans fat (g)
French fries:				
Arby's (large)	490	20	4	3
Hardee's (large)	430	19	4	4
McDonald's (large)	470	19	4	4
Burger King (large)	470	22	6	7
Wendy's (Biggie)	470	20	5	7
Fried chicken and fish:				
Burger King fish sandwich	830	31	6	2
Burger King chicken sandwich	700	30	6	2
McDonald's chicken McNuggets (9 pieces)	500	28	6	3
Kentucky Fried Chicken original recipe chicken dinner	1160	52	12	7
Long John Silver's fish & more	1270	58	13	14
Red Lobster admiral's feast	2020	97	26	22

[1] Modified from Wootan et al. (1996).

TABLE 2-5

Trans fatty acids in margarines, fats, and oils (1 tablespoon)[1]

	Calories (Kcal)	Total fat (g)	Saturated fat (g)	Trans fat (g)
Butter	100	11	7	0
Beef tallow	120	13	6	0
Canola oil	120	14	1	0
Crisco, can	110	12	3	1
Fleischmann's lower fat margarine, tub	40	5	0	0
I can't believe it's not butter!, tub	90	10	2	1
Land O' Lakes country morning blend light, stick or tub	50	6	3	0
Land O' Lakes country morning blend margarine, tub	100	11	2	1
Land O' Lakes margarine, stick or tub	100	11	2	2
Land O' Lakes spread with sweet cream, stick	90	10	2	3
Lard	120	13	5	0
Olive oil	120	14	2	0
Parkay, stick	90	10	2	3
Promise, stick	90	10	2	2
Promise, tub	90	10	2	0
Promise ultra fat free, tub	5	0	0	0
Promise ultra 70% less fat, tub	30	4	0	0
Soybean oil	120	14	2	0

[1] Modified from Wootan et al. (1996).

TABLE 2-6

Trans fatty acids in baked goods[1]

	Calories (Kcal)	Total fat (g)	Saturated fat (g)	Trans fat (g)
Boston Market original chicken pot pie (1)	750	25	7	4
Cinnabon Cinnabon (1)	670	34	9	6
Dunkin' Donuts old fashioned cake donut (1)	310	19	5	6
Entenmann's homestyle apple pie (1/6)	310	10	3	3
Entenmann's rich frosted donut (1)	310	18	4	5
Kentucky Fried Chicken biscuit (1)	200	11	2	4
Kentucky Fried Chicken chicken pot pie (1)	830	31	9	8
Little Debbie swiss cake rolls (2)	300	12	3	4
McDonald's iced cheese danish (1)	360	19	5	4
Nabisco nilla wafers (8)	150	4	1	1
Nabisco oreos (3)	160	7	1	2
Pillsbury grands! Buttermilk biscuit (1)	230	11	3	4
Starbucks cholesterol free blueberry scone (1)	420	15	4	4

[1] Modified from Wootan et al. (1996).

TABLE 2-7

Trans fatty acids in frostings, crackers, and snacks[1]

	Calories (Kcal)	Total fat (g)	Saturated fat (g)	Trans fat (g)
Betty Crocker whipped deluxe chocolate frosting (2 tablespoons)	140	4	2	1
Keebler Club Partners Original Crackers (4)	70	3	1	1
Nabisco Ritz Crackers (5)	90	4	1	1
Nabisco Triscuits (7)	140	5	1	2
Nabisco Wheat Thins (16)	150	5	1	2
Orville Redenbacher's natural Popcorn, microwave (4 cups)	110	8	2	2
Pillsbury creamy supreme vanilla frosting (2 tablespoons)	150	8	2	2

[1] Modified from Wootan et al. (1996).

are much lower than that calculated from the availability or disappearance data (Emken 1995). The differences mainly due to availability data tend to overestimate actual intake whereas questionnaire, diet-record, and recall data generally underestimate intake (Mertz et al. 1991).

Because TFA are not synthesized by humans, analysis of human adipose tissue provides another method of determining dietary TFA intake. Adipose tissue reflects the long term average of dietary isomeric fatty acid content, since adipose tissue fatty acids have a relatively long half life. The cis and trans isomers have been detected in human tissue in varying amounts, probably reflecting their dietary levels. Adipose tissue fatty isomer profiles indicate that 90 to 95% of the TFA in this tissue comes from partially hydrogenated vegetable oils and fats. This information has been used to predict a dietary intake range from 11.1 to 27.6 g per person per day (Enig et al. 1990).

Metabolism of TFA

TFA tend to have a "straight" chain structure similar to saturated fatty acids (SFA), whereas cis fatty acids (CFA) have one or more kinks in them. The structural property of trans bonding is responsible for the higher melting point of TFA compared to CFA (Emken 1991). The melting points of oleic acid (C18:1n9), elaidic acid, and stearic acid (C18:0) are 13°C, 44°C, and 70°C, respectively. Animal studies with hydrogenated fats showed that all isomers, including those with melting points higher than body temperature, were absorbed as well as oleic acid (Wood 1979). Human studies with pure triglyceride containing deuterated cis and trans positional octadecenoic acid isomers are consistent with the animal studies (Emken 1984). Thus, melting point and geometry and position of double bond have no measurable effect on the absorption of any isomer.

However, Bernard et al. (1987) used the ^{14}C-labeled oleic and elaidic acids to study their absorption in rats and argued that the kinetics of absorption were different for the two fatty acid isomers: oleic acid was absorbed in greater amount and more quickly than elaidic acid.

Since the early work of Barbour (1933) and Sinclair (1935) revealed that TFA accumulated in the lipids isolated from rat tissues, many other reports have shown that TFA can be well incorporated into virtually every tissue and cell component examined. In animals fed a diet containing TFA, the trans isomers have been found in all the major organs and tissues (Atal et al. 1994, Decker and Mertz 1966, Hill et al. 1982, Moore et al. 1980, Pettersen and Opstvedt 1989;1992, Schrock and Connor 1975, Sgoutas et al. 1973, Sugano et al. 1984, Wood et al. 1977), in the animal's microsomes (Blomstrand et al. 1985, Hietanen et al. 1978, Kirstein et al. 1983, Mahfouz et al. 1984, Morgado et al. 1998, Sgoutas et al. 1973, Sugano et al. 1984, Svensson 1983), mitochondria (Decker and Mertz 1966, De Schrijver and Privett 1984, Hsu and Kummerow 1977, Ide et al. 1987, Pettersen and Opstvedt 1989;1992, Sgoutas et al. 1973, Sugano et al. 1984), erythrocyte (Decker and Mertz 1966, Moore et al. 1980), and various lipoprotein fractions (Egwim and Sgoutas 1972, Emken 1984, Kummerow 1974, Schrock and Connor 1975). Studies with animals have demonstrated that levels of TFA found in tissue lipids reflected levels of these geometrical and positional isomers found in the animal's diet (Ruttenberg et al. 1983, Wood 1979).

Decker and Mertz (1966) found that the patterns of tissue distribution of elaidic acid and other fatty acids was closely resembled in the depot fat than in the erythrocyte or mitochondrial lipid in rats fed elaidinized olive oil. Considerable incorporation of dietary

elaidic acid into different phospholipid (PL) fractions was reported in rat heart mitochondria (Hsu and Kummerow 1977). Nearly half of the total octadecenoic acid was trans isomer. There was little or no isomeric selectivity occurred during absorption, transport, activation, esterification, etc., of TFA in all the tissue triglycerides of rats (Wood 1979, Wood et al. 1977). In adipose tissue membrane PL like phosphatidylcholine, TFA behave like SFA with respect to their pattern of incorporation. The incorporation of TFA into this lipid class was accompanied by reduction of the saturated fatty acid content, especially that of stearic acid. In the storage triglyceride, the incorporation of TFA did not have any effect on the content of SFA. Thus, in triglyceride, TFA did not appear to act like SFA. Furthermore, TFA apparently were not readily incorporated into brain lipids to any extent (Wood 1979), whereas adipose tissue and liver generally contain higher levels than other tissues (Heckers et al. 1977). Pettersen and Opstvedt (1989;1992) also detected that 19 to 36% of TFA in maternal diets had no noticeable effect on the brain phosphatidylethanolamine composition but had slight to moderate effects on the fatty acid profile of other organs in the newborn or suckling piglets. Cook (1979), in contrast, reported that TFA were incorporated more readily than oleic acid into developing rat brain tissue lipids. Moore et al. (1980) observed that insignificant amounts of trans isomers were present in the tissues of rats after the animals were transferred from high trans diets to trans free diet for 8 weeks, with the exception of adipose tissue. The incorporation of TFA in the tissues of rat, rabbit, hamster, mouse and piglets are summarized in **Tables 2-8** to **2-10**.

By measuring the fat samples from human placental, maternal, fetal, and baby tissue for presence of TFA, Johnston et al. (1958) identified that the TFA present in

TABLE 2-8

Trans fatty acids incorporated in rat tissues[1]

Tissue	% TFAs						Diet and feeding time	Reference
	TL	PL	PC	PE	TG	CE		
Adipose (epididymal)	24.8						8 wt% elaidinized olive oil for 6 weeks	Decker and Mertz (1966)
(epididymal)	1) 39.8 2) 30.0						1) 20 wt% PHSF + 2 wt% corn oil and 2) 20 wt% PHSF for 60 days	Sgoutas et al. (1973)
(epididymal)	1) 10.0 2) 17.1	1) 7.9 2) 8.7					1) 4.2 wt% and 2) 8.5 wt% PHSO for 8 weeks	Ostlund-Lindovist et al. (1985)
					30.4		15 wt% hydrogenated fat diet for 12 weeks	Moore et al. (1980)
Erythrocyte	13.9						8 wt% elaidinized olive oil for 6 weeks	Decker and Mertz (1966)
		12.7					15 wt% hydrogenated fat diet for 12 weeks	Moore et al. (1980)
Heart		13.6			17.1		15 wt% hydrogenated fat diet for 12 weeks	Moore et al. (1980)
Heart mitochondria			1) 17.0 2) 21.6	1) 18.5 2) 28.8			22 wt% partially hydrogenated fat for 1) 1 week and 2) 6 weeks	Hsu and Kummerow (1977)

Continued

TABLE 2-8 (Continued)

Trans fatty acids incorporated in rat tissues[1]

Tissue	% TFAs						Diet and feeding time	Reference
	TL	PL	PC	PE	TG	CE		
Ileum (lower)	15.5						7.6 wt% PHCO + 2.4 wt% safflower oil for 35 days	Sugano et al. (1984)
Jejunum (upper)	17.2		10.1		20.4		7.6 wt% PHCO + 2.4 wt% safflower oil for 35 days	Sugano et al. (1984)
Liver			14.6	13.5	21.8	23.5	20 wt% PHSF + 2 wt% corn oil for 60 days	Sgoutas et al. (1973)
			29.5	23.6	48.4	32.6	15 wt % PHSO free fatty acids for 4 weeks	Wood et al. (1977)
		18.6			17.9	11.3	15 wt% hydrogenated fat diet for 12 weeks	Moore et al. (1980)
		13.3					20 wt% PHSBO for 31 weeks	Hill et al. (1982)
Liver cytosol					1) 26.5 2) 22.0	1) 30.5 2) 25.0	1) 20 wt% PHSF + 2 wt% corn oil and 2) 20 wt% PHSF for 60 days	Sgoutas et al. (1973)

Continued

TABLE 2-8 (Continued)

Trans fatty acids incorporated in rat tissues[1]

Tissue	% TFAs						Diet and feeding time	Reference
	TL	PL	PC	PE	TG	CE		
Liver microsome			1) 13.4	1) 14.1	1) 22.5 2) 18.0	1) 17.0 2) 14.2	1) 20 wt% PHSF + 2 wt% corn oil and 2) 20 wt% PHSF for 60 days	Sgoutas et al. (1973)
	1) 14.8 2) 11.0						1) 36 en% PHPO + 10 en% PO and 2) 36 en% PHMO + 10 en% PO for 13 or 16 weeks	Kirstein et al. (1983)
		1) 12.4 2) 7.7 3) 7.0					1) 20 wt% PHPO + safflower oil, 2) 20 wt% PHNCO + safflower oil, and 3) 20 wt% PHHO + safflower oil	Svensson (1983)
	13.1	12.4					12 wt% PHSBO and 3 wt% corn oil for 6 weeks	Mahfouz et al. (1984)
			10.3	9.9			7.6 wt% PHCO + 2.4 wt% safflower oil for 30 to 32 days	Sugano et al. (1984)
	1) 12.6 2) 8.3 3) 11.7						1) 16.5 wt% PHLRSO + 3.5 wt % SSO, 2) 16.5 wt% PHHO + 3.5 wt% SSO, and 3) 20 wt% PHLRSO for 10 weeks	Blomstrand et al. (1985)
	2.1 ± 0.2						15 wt% PHFO + 0.5 wt% sunflower oil for 40 days	Morgado et al. (1998)

Continued

TABLE 2-8 (Continued)

Trans fatty acids incorporated in rat tissues[1]

Tissue	% TFAs						Diet and feeding time	Reference
	TL	PL	PC	PE	TG	CE		
Liver mitochondria	12.9						8 wt% elaidinized olive oil for 6 weeks	Decker and Mertz (1966)
			1) 13.6 2) 15.0	1) 13.0 2) 13.0	1) 24.2 2) 20.0	1) 23.9 2) 19.5	1) 20 wt% PHSF + 2 wt% corn oil and 2) 20 wt% PHSF for 60 days	Sgoutas et al. (1973)
		14.0 ± 0.8					8 wt% PHSBO + 2% safflower oil for 4 weeks	De Schrijver and Privett (1984)
			9.8				15 wt % PHCO supplemented with safflower oil for 30 days	Ide et al. (1987)
Kidney		10.0			22.7		15 wt% hydrogenated fat diet for 12 weeks	Moore et al. (1980)
Plasma		16.9			26.1	5.9	15 wt% hydrogenated fat diet for 12 weeks	Moore et al. (1980)
Testes					24.3	8.6	15 wt% hydrogenated fat diet for 3 months	Moore et al. (1980)

[1] Abbreviation used: CE, cholesterol esters; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PHCO, partially hydrogenated corn oil; PHFO, partially hydrogenated fish oil; PHHO, partially hydrogenated herring oil; PHLRSO, partially hydrogenated low erucic acid rapeseed oil; PHMO, partially hydrogenated marline oil; PHNCO, partially hydrogenated Norwegian capelin oil; PHPO, partially hydrogenated peanut oil; PHSF, partially hydrogenated soybean fat; PHSO, partially hydrogenated safflower oil; PHSBO, partially hydrogenated soybean oil; PL, phospholipids; PO, peanut oil; SSO, sunflowerseed oil; TFAs, trans fatty acids; TG, triglycerides; TL, total lipids.

TABLE 2-9

Trans fatty acids incorporated in rabbit tissues[1]

Tissue	% TFAs				Diet and feeding time	Reference
	TL	PL	TG	CE		
Adipose (epididymal)	1) 8.9 ± 0.8 2) 19.2 ± 1.4				1) 3.2 wt% or 2) 6.0 wt% TFAs (mixture of partially hydrogenated safflower, soybean, and cottonseed oils) for 5 months	Ruttenberg et al. (1983)
Adipose (psoas)	21.7 ± 3.7				40 en% PHOO for 3 weeks	Schrock and Connor (1975)
Erythrocyte	1) 4.8 ± 0.3 2) 12.0 ± 0.7				1) 3.2 wt% or 2) 6.0 wt% TFAs for 5 months	Ruttenberg et al. (1983)
Liver microsome	1) 17.6 ± 0.8 2) 35.2 ± 3.0				1) 3.2 wt% or 2) 6.0 wt% TFAs for 5 months	Ruttenberg et al. (1983)
Liver mitochondria	1) 18.7 ± 2.0 2) 33.4 ± 2.4				1) 3.2 wt% or 2) 6.0 wt% TFAs for 5 months	Ruttenberg et al. (1983)
Plasma	1) 6.9 ± 0.2 2) 13.2 ± 1.3				1) 3.2 wt% or 2) 6.0 wt% TFAs for 5 months	Ruttenberg et al. (1983)
Serum		10.4 ± 2.8	15.4 ± 2.4	6.0 ± 1.0	40 en% PHOO for 3 weeks	Schrock and Connor (1975)

[1] Abbreviations used: CE, cholesterol esters; PHOO, partially hydrogenated olive oil; PL, phospholipids; TFAs, trans fatty acids; TG, triglycerides.

TABLE 2-10

Trans fatty acids incorporated in hamster, piglet, and mouse tissues[1]

Tissue	% TFAs						Diet and feeding time	Reference
	TL	PL	PE	NL	CE	TG		
Hamster								
Liver		5.3 ± 0.7			n.d.	4.4 ± 0.3	10 wt% PHCO +0.1 wt% cholesterol for 2 weeks	Hayashi et al. (1993)
Plasma		1.5 ± 0.5			n d.	2.7 ± 0.5		
Newborn Piglet								
Adipose (subcutaneous)	1) n.d. 2) 0.1						Piglets were bred from the mother sows fed 1) 14 wt% PHFO + 4 wt% SFO, or 2) 14 wt% PHSBO + 4 wt% SFO from 3 weeks of age through pregnancy.	Pattersen and Opstvedt (1989)
Brain			1) n.d. 2) n.d.					
Heart			1) 0.8 2) 1.0					
Liver mitochondria			1) 2.2 2) 2.6					
Plasma	1) 2.0 2) 2.1							

Continued

TABLE 2-10 (Continued)

Trans fatty acids incorporated in hamster, mouse and piglet tissues[1]

Tissue	% TFAs						Diet and feeding time	Reference
	TL	PL	PE	NL	CE	TG		
Sucking piglet								
Adipose (subcutaneous)	1) 8.9 2) 11.2						1) 14 wt% PHFO + 4 wt% SFO, or 2) 14 wt% PHSBO + 4 wt% SFO fed to mother sows from 3 weeks of age through pregnancy. Piglets were only allowed to suckle milk from mother sows for 3 weeks.	Pattersen and Opstvedt (1992)
Brain			1) n.d. 2) n.d.					
Heart			1) 1.9 2) 3.8					
Liver mitochondria			1) 6.3 2) 7.6					
Plasma	1) 5.0 2) 8.9							
Mouse								
Adipose (epididymal)		13.1		8.1			10 wt% mixture of shortening and corn and soybean oils for 8 months	Atal et al. (1994)

[1]Abbreviations used: CE, cholesterol esters; n.d., not detectable; PHCO, partially hydrogenated corn oil; PHFO, partially hydrogenated capelin oil; PHSBO, partially hydrogenated soybean oil; NL, neutral lipids; PE, phosphotidylcholine; PL, phospholipids; SFO, sunflowerseed oil; TFAs, trans fatty acids; TL, total lipids.

human tissue apparently arose solely from dietary fat. Levels of TFA in human tissue revealed that adipose tissue contained 2.4 to 12.2%; liver, 4.0 to 14.4%; heart, 4.6 to 9.3%, and aortic tissue, 2.3 to 8.8% (Johnston et al. 1957, Sgoutas and Kummerow 1970). Perkins and associates (1977) found human serum lipid samples had 1.9% trans-18:1 and 0.8% trans, trans-18:2. The corresponding erythrocytes contained 2.4% trans-18:1 and 0.7% trans, trans-18:2. Ohlrogge et al. (1982) reported that the pattern of trans isomer distribution in triglyceride of human tissues was similar to partially hydrogenated vegetable oils (PHVO). The distribution of PL of human tissues, however, reflected different pattern than PHVO. Higher levels of TFA, moreover, were found in triglyceride as compared to the PL (Ohlrogge 1982;1983). Garland et al. (1998) compared fatty acid content in adipose tissue with fatty acid intake calculated from both dietary records and questionnaires from 140 participants in the Nurses' Health Study. They confirmed that the amounts of TFA in adipose tissue reflect dietary intake. An estimate of TFA intake from partially hydrogenated vegetable fats correlated much more strongly with adipose TFA than did that from animal sources.

The incorporation of TFA in human tissues are summarized in **Table 2-11**. Generally, the level of the TFA found in animals or humans depends on the following factors:

1) the particular organ or tissue studied;

2) the PL and neutral lipid classes in the same tissue studied;

3) the amount of trans fat ingested;

4) the duration of time on the diet;

5) the form in which the trans fat is administered;

TABLE 2-11

Trans fatty acids in human tissues

Tissue	% TFA	Method	No. of subject	Reference
Adipose	2.4 - 12.2	IR	24	Johnston et al. (1957)
	1.5 - 6.8	IR	6 (F)	Johnston et al. (1958)
	5.3	GLC	231 (M)	Thomas et al. (1981)
	2.0 - 5.8	GLC	8 (3 F, 5 M)	Ohlrogge et al. (1981)
	4.7	GC	5 (M)	Adlof & Emken (1986)
	2.3 - 8.2	GC	115 (F)	London et al. (1991)
	1.6	GC	1388 (M)	Aro et al. (1995)
	2.8	GC	352 (M)	Roberts et al. (1995)
	1.3	GC	1058 (700 M, 358 F)	Bakker et al. (1997)
	1.2	GLC	698 (F)	Kohlmeier et al. (1997)
	1.8 - 7.4	GC	161 (F)	Petrek et al. (1997)
	6.1 ± 1.3	GC	141 (F)	Garland et al. (1998)
Aorta	2.3 - 8.8	IR	24	Johnston et al. (1957)
	1.0	GLC	17 (M)	Heckers et al. (1977)
	1.4 - 3.9	GLC	8 (3 F, 5 M)	Ohlrogge et al. (1981)
Atheroma	2.3 - 8.8	IR	24	Johnston et al. (1957)
Brain	0.2 - 0.9	GLC	8 (3 F, 5 M)	Ohlrogge et al. (1981)
	0.3	GC	4 (M)	Adlof & Emken (1986)
Heart	4.6 - 9.3	IR	24	Johnston et al. (1957)
	1.2 - 4.1	GLC	8 (3 F, 5 M)	Ohlrogge et al. (1981)

Continued

TABLE 2-11 (Continued)

Trans fatty acids in human tissues[1]

Tissue	% TFA	Method	No. of subject	Reference
Heart	2.0	GC	3 (M)	Adlof & Emken (1986)
Jejunum	0.6	GLC	17 (M)	Heckers et al. (1977)
Liver	4.0 - 14.4	IR	24	Johnston et al. (1957)
	1.1 - 2.5	GLC	8 (3 F, 5 M)	Ohlrogge et al. (1981)
	1.5	GC	6 (M)	Adlof & Emken (1986)
Kidney	1.5	GC	5 (M)	Adlof & Emken (1986)
Myocardium	0.9	GLC	23 (M)	Heckers et al. (1977)
Placenta	0.0 - 0.5	IR	6 (F)	Johnston et al. (1958)
Fetal liver	0	IR	5	Johnston et al. (1958)
Infant adipose	0.1 - 0.9	GLC	2	Ohlrogge et al. (1982)
Infant plasma PL	1.1 ± 0.1	GLC	29	Koletzko (1992)
Infant plasma TG	1.6 ± 0.1	GLC	29	Koletzko (1992)
Children plasma PL	1.8 ± 0.1	GC	31 (M)	Decsi & Koletzko (1995)

1Abbreviations used: F, females; GC, gas chromatography; GLC, gas-liquid chromatography; IR, infrared chromatography; M, males; PL, phospholipids; TFA, trans fatty acids; TG, triglycerides.

6) the animal species being fed.

Elaidic and oleic acids were shown to be catabolized to CO_2 to the same extent (Coots 1964, Ono and Fredrickson 1964). Similar rates of digestion, absorption and transport in the lymph as triglyceride were also reported in these studies. Munsch et al. (1969b) compared the metabolism of oleic and elaidic acids in the rat with respect to oxidation to CO_2 and their retention in the lipids of blood, brain, excreta, liver and carcass. In addition to the similar behavior already noted by previous workers in relation to ease of oxidation, they found that their retention pattern in the tissues was quite similar: liver and carcass lipids incorporated most of the ingested radioactivity over a time period of 3½ hours. However, these two isomers showed interesting differences in behavior regarding the distribution, turnover and desaturation (Munsch et al. 1969a). While elaidate was referentially localized in the PL, oleic acid was largely found in the triglyceride. The turnover rate of oleate was faster than elaidate in both the liver and plasma lipids.

A few *in vitro* studies showed that trans octadecenoic acids were oxidized at a slower rates than their cis isomers in the mitochondria isolated from rat hearts and livers (Ide et al. 1987, Lawson and Holman 1981, Lawson and Kummerow 1979) and in rat heart homogenates (Lanser et al. 1986). The cis monoenes were the preferred substrates and of the trans isomers, vaccenoly-CoA was oxidized more rapidly than elaidoyl-CoA (Lawson and Kummerow 1979). These results are not in accord with *in vivo* experiments, which showed no difference on mitochondrial respiratory function when rats received none and 8% of partially hydrogenated soy oil (De Schrijver and Privett 1984).

Interactions of TFA with Essential Fatty Acids (EFA) Metabolism

The influence of dietary TFA on linoleic acid metabolism and essential fatty acids (EFA) deficiency states have been examined extensively. Several studies have shown that the trans monoenes were substrates for desaturation and therefore competed with the cis acids for this activity (Hill et al. 1982, Mahfouz et al. 1980;1981, Rosenthal and Doloresco 1984, Rosenthal and Whitehurst 1983). When TFA were present in the experimental diets, noted decreases in the activities of Δ6-desaturase (De Schrijver and Privett 1982, Kurata and Privett 1980, Mhfouz et al. 1980, Shimp et al. 1982, Svensson 1983), Δ5-desaturase (Kurata and Privett 1980, Mahfouz et al. 1980, Svensson 1983), and Δ9-desaturase (Kurata and Privett 1980, Mahfouz et al. 1980) were shown in *in vitro* studies. *In vivo* studies further demonstrated that dietary TFA decreased desaturase activities. Examination of the tissue lipids revealed an increase in the ratio of C20:3n-9/C20:4n-6 (Hill et al. 1982, Kurata and Privett 1980, Watkins 1988), an indicator of EFA deficiency states. Elevated ratios of C18:2n-6/C20:4n-6 were also detected (De Schrijver and Privett 1982, Kurata and Privett 1980, Hill et al. 1982, Watkins 1988). Results of cultured human skin fibroblasts grown in media with various fatty acid isomers confirmed that both elaidate and linoelaidate are potent inhibitors of the Δ6- and Δ5-desaturase (Rosenthal and Doloresco 1984, Rosenthal and Whitehurst 1983). However, the linoelaidate-induced suppression of arachidonic acid can be readily overcome by increasing the concentration of available linoleate (Chern and Kinsella 1983). Consequently, TFA in the diet appear to increase the need for EFA. However, dietary TFA do not pose a serious problem in the presence of adequate levels of dietary linoleic

acid. On the other hand, in marginally EFA deficient states, the presence of dietary TFA could exacerbate the deficiency condition. It has been established that dietary TFA had no interference with mitochondrial function when dietary linoleate was provided no less than 2% of total calories (Zevenbergen et al. 1988).

The interactions of TFA with EFA may be of particular importance in early childhood. Metabolites of EFA need to be deposited in large amounts in growing tissues, especially the brain, and their availability during rapid growth is related to functional development. C22:6n3 status during the first months of life correlates with development of visual acuity both in preterm and full-term infants, and C20:4n6 status correlates with early human growth (Decsi and Koletzko 1994). Koletzko (1992) studied the possible untoward effects of TFA in a group of 29 stable premature infants, who have limited EFA stores and probably a low activity of enzymatic conversion. Plasma samples obtained on the fourth day of life were assayed for fatty acid composition. It was found that TFA in plasma sterol esters were not related to the precursor EFA - linoleic and α-linolenic (C18:3n3) acids, but significantly inverse correlated with their major metabolites arachidonic and docosahexaenoic (C22:6n3) acids. TFA were also significantly associated with the product/substrate ratios of n6 and n3 fatty acid desaturation and elongation. Likewise, in 53 healthy well-nourished children aged 1 to 15 years, significant inverse correlations of trans-18:1 and total TFA to the principal metabolite arachidonic acid was observed (Decsi and Koletzko 1995). There was also an inverse correlation of total TFA to the ratio of arachidonic to linoleic acid. Hence, the questions abut the physiological effects of TFA should be further emphasized.

Interactions of TFA with Membrane and Membrane-Bound Enzymes

Incorporation of TFA into membrane PL could have an influence on the physical properties of the membrane and the membrane bound enzymes. Several studies of membrane fluidity lead to the conclusion that TFA generally substitute for SFA in animal membranes with little change in membrane physical properties (Senti 1985). When the TFA became the predominant fatty acid in the membrane, the TFA could bring about decreased fluidity, associated changes in permeability and other metabolic parameters (Tsao and Lands 1980). Elaidic acid have been shown to lower the content of PL and microsomal cytochrome P-450 in rats fed at a dose of 200 mg/kg every other day for 4 weeks (Hietanen et al. 1978). Trans isomers were also found to inhibit the activities of Na^+, K^+-ATPase and adenylate cyclase in rat heart membrane (Alam et al. 1989). In contrast, in rabbits fed either 3.2% or 6% TFA for 5 months, Ruttenberg et al. (1983) were unable to demonstrate significant differences in activities of 5 hepatic enzymes including glucose-6-phosphatase (microsomal), FA synthetase (cytosolic), malate dehydrogenase, beta-hydroxybutyrate dehydrogenase, and monoamine oxidase (mitochondrial). Sugano et al. (1984) also reported no difference in the content of cytochrome P-450 in hepatic microsomes of rats fed cis- or trans-fat.

Other Health Concerns of TFA

Most studies with TFA have focused on these isomers and the risk of coronary heart disease (CHD). To date, no consensus conclusions have been reached. High intake of TFA appears to be associated with increased risk of coronary heart disease in epidemiologic studies. Willett and colleagues (1993) calculated the dietary intake of

TFA in 85,095 women who were followed for 8 years. They noted that increased intake of dietary TFA was directly related to risk of CHD after adjustment for age and other factors. The increased risk was linked to partially hydrogenated vegetable oils in margarine and other foods that contain vegetable shortening, including cookies, cake and white bread. New data in a follow-up report, which extended the study period to 14 years, confirmed the initial findings and concluded that TFA may be more dangerous for the heart than saturated fats (Hu et al. 1997).

Troisi et al. (1992) assessed the relationship of trans fats intake to fasting serum lipid concentrations in 748 men aged 43 to 85 years. They found that intake of trans fats was directly related to total and low-density-lipoprotein (LDL) cholesterol, and inversely related to high-density lipoprotein (HDL) cholesterol. Trans fats intake was also positively associated with the ratios of total to HDL cholesterol and LDL to HDL cholesterol. In the Seven Countries Study involving 12,763 middle-aged men, strong positive associations were observed between 25-year death rates from coronary heart disease and average intake of elaidic acid ® = 0.78, $P < 0.001$) (Kromhout et al. 1995). After observing 832 men from Framingham Heart Study for two decades, Gillman et al. (1997) reported that the risk ratio of CHD for each increment of 1 teaspoon per day of margarine was 0.98 for the first 10 years of follow-up and 1.10 for follow-up years 11 to 21. A significant positive association between the intake of TFA and the risk of coronary death was also found in 21,930 Finnish men aged 50 to 69 years (Pietinen et al. 1997). Men with an intake of 6.2 g TFA per day had a risk of CHD of 1.39 related to men with lower TFA intake.

In a case-control study, Thomas et al. (1983a;1983b) examined the depot fat taken from 136 cases died of ischaemic heart disease and from 95 controls died of unrelated causes. It was concluded by the authors that the cases consumed a higher proportion of hydrogenated fats rich in C16:1- and C18:1-trans fatty acids than did the controls (Thomas et al. 1983a;1983b). With the same experimental design, Ascherio et al. (1994) also reported that intake of TFA was directly related to risk of myocardial infarction (relative risk for highest compared with lowest quintile, 2.44) in 239 patients and 282 control subjects. In particular, intake of margarine, the major source of trans isomers, was significantly associated with risk of myocardial infarction.

TFA also manifest undesirable lipoprotein profiles in humans, e.g., raise serum total cholesterol or LDL cholesterol concentrations and may reduce high-density-lipoprotein (HDL) cholesterol concentration by comparison with their cis isomers. In a controlled trial, Mensink and Katan (1990) examined the effects of TFA on HDL and LDL cholesterol levels in 59 healthy subjects. Each subject received three diets for 3 weeks each, in a random order and without washout periods. The 3 study diets were rich in oleic, elaidic or a mixture of lauric, myristic and palmitic acids. It was found that HDL cholesterol was significantly lower ($P < 0.0001$) on the elaidic acid diet (1.25 mmol/L) than on the other two diets (1.42 mmol/L). LDL cholesterol was 3.04 mmol/L on the trans C18:1 diet, 2.67 mmol/L on the oleic acid diet, and 3.14 mmol/L on the SFA diet. Therefore, elaidic acid, in comparison with oleic acid, had unfavorable effects on plasma lipoproteins because HDL cholesterol was reduced and LDL cholesterol was elevated However, the trans C18:1 isomers in the trans diet accounted for 11% of total energy

intake, an amount 2 to 4 times higher than in a typical Western diet. In another study conducted in 56 healthy subjects, similar effects of elaidic acid on LDL and HDL cholesterols were confirmed (Zock and Katan 1992). The trans diet contained just under 8% total energy form elaidic acid was compared to diets in which energy derived from trans C18:1 was replaced by linoleic or stearic acids. LDL cholesterol levels rose from 2.83 mmol/L with linoleate to 3.00 mmol/L with stearic acid and to 3.07 mmol/L with TFA. In contrast, HDL cholesterol levels reduced by 0.06 mmol/L with stearic acid and by 0.10 mmol/L with TFA.

Nestel and co-workers (1992) studied the effects of trans C18:1 on plasma lipoprotein levels in a 27 mildly hypercholesterolemic men. Each subject consumed 3 test diets for 3 weeks each. The dietary fat provided 35% total energy. The fatty acid composition of the three diets were comparable; 4 to 5% of energy as trans C18:1 was exchanged exclusively for either oleic or palmitic acid. Compared with the oleic acid diet, the trans diet raised both total and LDL cholesterol levels by 0.36 mmol/L, but HDL cholesterol levels were unaffected. Palmitic acid raised total, LDL, and HDL cholesterol levels by 0.28, 0.26, and 0.10 mmol/L, respectively, as compared to the oleic acid diet. It was thus concluded that both TFA and palmitic acid raised cholesterol levels to about the same extent but that TFA, at about 7% energy, did not lower HDL cholesterol levels relative to oleic acid.

Lichtenstein et al. (1993) compared the effect s of a corn oil-enriched diet with those of a diet rich in partially hydrogenated corn oil in the form of margarine in 14 moderately hypercholesterolemic middle aged and elderly subjects. The margarine diet

contained about 3.7% more energy as trans monounsaturated fatty acids than the unhydrogenated corn oil diet, at the expense of cis mono- and polyunsaturated fatty acids. The margarine diet yielded significantly higher levels of total and LDL cholesterol than the unhydrogenated oil diet. Again, no change in HDL cholesterol levels was detected.

Wood et al. (1993) studied 38 healthy men with normal plasma lipid concentrations, whose diets were supplemented with butter, butter enriched with olive oil or sunflower oil, hard margarine (29% TFA), or soft margarine (no TFA). The dietary fat provided approximately 40% total energy. The overall changes were relatively small, with the largest difference in total cholesterol levels occurring between the butter and soft margarine diets (5.48 ± 0.85 vs. 4.86 ± 0.80 mmol/L, respectively). Soft margarine diet significantly reduced LDL cholesterol and apolipoprotein B as compared to all the other diets. Hard margarine caused a reduction in apolipoprotein A-1 and B levels, but did not change total or LDL cholesterol levels, relative to habitual diet. HDL cholesterol levels remained unchanged among all diets. Since the percentages of total saturated and unsaturated (including trans) were equal in the two margarine diets, it was assumed that the different lipid response was attributed to the TFA in the hard margarine.

Judd et al. (1994) carried out a large, well-controlled trial in 29 men and 29 women. Each individual consumed four different diets in random order for 6 weeks each. One diet was high in oleic acid, and two were enriched with different amounts of partially hydrogenated soybean oil; supplying 3 and 6% of energy as trans-18:1, respectively. In the fourth diet, 6% of energy was obtained from a mixture of SFA, which were substituted for oleic acid. Compared with levels on the oleic acid diet, LDL cholesterol

levels elevated by 0.2 mmol/L with the 3% trans-18:1 diet, and by 0.3 mmol/L with the SFA diet. HDL cholesterol levels were not lowered significantly on the 3% TFA diet, and they decreased by 0.04 mmol/L on the 6% TFA diet relative to the levels on the oleic acid diet. Changes in apolipoprotein A1 and B corresponded with changes in the lipoprotein cholesterols. Thus, compared with oleic acid, dietary TFA raise LDL cholesterol, but to a slightly lesser degree than saturates, and high amount of TFA could result in minor declines of HDL cholesterol. A possible dose-dependent effect of dietary TFA on lipoproteins has been notified by some researchers (Khosla and Hayes 1996, Zock and Mensink 1996).

The contradictory results regarding TFA and the risk of CHD have been reported by several researchers. The plasma cholesterol and triglyceride levels in the subjects consumed a diet rich in TFA were not different from the control group received a diet rich in cis isomers (Mattson et al. 1975). Hudgins et al. (1991) found no correlation between the adipose tissue concentrations of total TFA and clinical risk factors for CHD in 76 free-living males. Aro et al. (1995) compared the trans-18:1 content in adipose tissue samples of 671 men who had survived an acute myocardial infarction (AMI) with 717 controls in ten centers in eight European countries and Israel (the EURAMIC study). There was no association overall between high trans-18:1 intake and AMI, but the possibility that TFA had a significant impact on risk of CHD could not be excluded. In U. K., a population case-control study of sudden cardiac death in men was conducted to evaluate the effect of trans isomers of oleic acid and linoleic acid on the risk of CHD (Roberts et al. 1995). The percentages of TFA in adipose tissue samples were obtained

from 66 cases of sudden cardiac death were compared with those from 286 healthy age and sex matched controls. No overall association was found between trans isomers of oleic and linoleic acids combined and sudden cardiac death. However, elaidic acid was negatively associated with risk of sudden cardiac death, whereas no association with trans forms of linoleic acid was seen. Recently, Judd et al. (1998) compared the effect of butter and tub-type margarines on blood lipid profiles in a crossover feeding trial. Twenty-three male and 23 female normolipemic adults randomly consumed 3 different diets for 5 weeks each. The 3 dietary fat sources, including butter, margarine high in TFA (TFA-M), and margarine high in PUFA (PUFA-M) and free of TFA, were supplied as table spreads. These table spreads provided 8.3% of energy as fat. After consumption of TFA-M and PUFA-M, total cholesterol was 3.5% and 5.4% significantly lower ($P < 0.01$) compared to butter, respectively. Neither margarine differed from butter in its effect on HDL cholesterol or triglyceride. It was thus concluded that consumption of TFA-M or PUFA-M improved blood lipid profiles when compared with butter, with a greater improvement with PUFA-M than TFA-M.

TFA may also increase the concentration of lipoprotein (a) [Lp(a)], which is considered to be a risk factor for coronary heart disease. Nestel et al. (1992) found that the elaidic acid-rich diet led to significant elevations in the levels of Lp(a) compared to the other diets rich in palmitic or lauric/myristic/palmitic acids. The mean Lp(a) levels were not different between the diets rich in elaidic and oleic acids. When TFA were consumed at a dose of 11% of energy, median Lp(a) levels in the trans diet group were significantly higher than those in the SFA diet or in the oleic acid diet (Mensink et al.

1992). At 8% of energy as TFA, median Lp(a) levels in trans diet were higher than those in stearic and linoleic acid diets. Lichtenstein et al. (1993) did not detect an increase in Lp(a) levels on a diet containing partially hydrogenated corn oil (trans-18:1 at 4.16% of energy) compared with unmodified corn oil or the baseline saturated fat (trans-18:1 at 0.44 and 0.77% of energy, respectively) diet. In a Norwegian study by Almendingen et al. (1995), the Lp(a) levels were measured in 31 young men who consumed three diets rich in butterfat, partially hydrogenated soybean oil (PHSO-diet), and partially hydrogenated fish oil (PHFO-diet) for 19 to 21 days in a random order. It was found that when 8 to 9% of the dietary energy was obtained from TFA, median Lp(a) levels were significantly higher on the PHSO and PHFO diets compared to those on butterfat diet. In a Malaysian study with a crossover design, Lp(a) levels were also higher on a TFA rich diet (elaidic acid at 5.5% of energy) than on cis or saturated fat diets in twenty-seven healthy subjects (Sundram et al. 1997). Similar results were also reported by Aro et al. (1997) in a study including 80 healthy subjects. The baseline diet contained TFA and stearic acid at 0.8% and 3.6% of energy, respectively. Lp(a) levels significantly increased more with TFA at 8.7% of energy than with stearic acid at 9.3% of energy (30% compared to 10%, $P < 0.01$) compared to the baseline diet. However, in a double-blind study of 29 men and 29 women who received 4 controlled dits in random order for 6 weeks each, the trans diets had no adverse effect on Lp(a) levels compared to the oleic diet when all subjects were considered collectively (Clevidence et al. 1997). A subgroup with initially high levels of Lp(a) responded to the high trans diet (6.6% energy as TFA) with a slight increase (5%) in Lp(a) levels relative to the oleic (16.7% energy as oleic

acid) and moderate trans (3.8% energy as TFA) diets. Therefore, TFA appear to be one of the rare dietary factors that influence Lp(a) levels, especially at high intake. Judd et al. (1998) reported that Lp(a) concentrations were significantly lower after the butter diet than after the tub-type margarines diet. No apparent effect of dietary TFA on Lp(a), however, was evident while comparing margarines containing TFA or no TFA.

The inconsistent cardiovascular effects of TFA are mainly due to the different experimental designs (Kris-Etherton and Nicolosi 1995). In addition, the discrepancy could arrive from methodological difficulties in assessing dietary intake, the inadequacy of single plasma cholesterol measurements, and the absolute amount of fat consumed (Borlak and Welch 1994). The effect of other factors, such as genetic make up and differences in life style, dietary habits long before the study period, could also have influence on the association of TFA with CHD development (Borlak and Welch 1994).

In addition, TFA have been shown to increase the activity of serum cholesterylester transfer protein (CETP) in animal and man (Abbey and Nestel 1994, Lagrost 1992, Khosla et al. 1997, van Tol et al. 1995), inhibit both human and rat lecithin:cholesterol acyltransferase (LCAT) activities (Subbaiah et al. 1998), increase fat cell size in rats (Ostlund-Lindqvist et al. 1985), depress milk fat content in the lactating mice in the presence of adequate dietary linoleic acid (Teter et al. 1990), reduce fat accumulation in 3T3-L1 cells (Panigrahi and Sampugna 1993), and decrease epididymal and perirenal fat weight, epididymal fat cell number, and the triglyceride to polar lipid ratio in epididymal fat pads in mice (Atal et al. 1994), which might be explained by enhanced lipolysis and inhibited glucose utilization (Cromer et al. 1995).

Results on the potential link of TFA with cancer are also not consistent. Some data reveal that TFA are no more carcinogenic than their cis isomers (Ip 1997, Ip & Marshall 1996, Roberts 1995). Furthermore, in women with breast cancer, Petrek et al. (1997) found that the odds of having positive lymph nodes were significantly lower for women with a higher adipose tissue proportion of TFA. Nevertheless, in the EURAMIC study, the adipose concentrations of TFA were positively associated with breast cancer (Bakker et al. 1997, Kohlmeier et al. 1997) and colon cancer (Bakker et al. 1997).

DIETARY FATS AND ETHANOL METABOLISM

Studies show that the quantity and type of dietary lipids have a critical effect on ethanol metabolism. When rats were fed nutritionally adequate diets varying in fat content (5, 10, 20, and 43% of total calories) for 3 weeks, fatty liver developed only in rats fed a high fat diet (43%) plus ethanol (Jones and Greene 1966). The quantitative relationship between dietary fat content and hepatic lipid accumulation after ethanol intake was also accessed by Lieber and DeCarli (1970). They found that hepatic lipid accumulation produced by ethanol was increased by feeding a high fat diet starting at 25% of total calories in rats. Utilizing a continuous intragastric feeding rat model of chronic ethanol ingestion, Tsukamoto and colleagues investigated the role of the amount of dietary fat on ethanol-induced hepatic fibrosis in a series of studies. When the diet contained 5% of calories as corn oil, rats developed fatty liver and necrosis but no fibrosis in the liver (Tsukamoto et al. 1985). When corn oil was fed as 25% of calories, the rats had more severe fatty change, necrosis, and inflammation (Tsukamoto et al. 1986,

Tsukamoto and French 1985). As the dietary fat was further increased to 32% and 35% of total calories, the fibrogenic effects of ethanol was enhanced in the corn oil-fed rats (French et al. 1986). The rates of liver fibrosis in the ethanol-infused rats fed varying fat content (5, 25, 32 or 35% of total calories) diets were 0%, 59%, 67%, and 43%, respectively (French et al. 1986). Thus, high dietary fat was essential to induce hepatic fibrosis in rats fed ethanol.

Several ethanol-induced biochemical abnormalities also seem to depend on a high fat diet. The ethanol elimination rate after 1 month of ethanol feeding is faster when the rats were fed a high fat diet (Kanayama et al. 1984). MEOS exhibited greater activity in the rats fed the high fat diet and high fat diet worsened ethanol-pyrazole hepatitis compared with low fat diet (Takada et al. 1986). Free radical generation in the liver, as measured *in vivo*, was increased after ethanol ingestion when the diet was high in fat (Reinke et al. 1987).

In a chronic ethanol study carried out by Jones and Greene (1966), high hepatic content of fat was induced by a high fat diet (43%) and alcohol in rats regardless of the type of dietary fat (coconut and safflower oils). However, replacement of the long chain fatty acid in the dietary triglyceride with medium chain fatty acids reduced the capacity of ethanol to cause fatty liver in rats after 24 days of ethanol administration (Lieber et al. 1967). An epidemiological study involving 16 countries has been published in which per capita consumption of beef, pork, and alcohol has been related to liver cirrhosis (Nanji and French 1985). The correlation between cirrhotic mortality and ethanol or pork consumption was 0.64 and 0.40, respectively. There was no correlation between beef

consumption and cirrhotic mortality. When the product of pork and ethanol consumption was correlated with cirrhosis mortality, the correlation was highly significant ® = 0.98, $P < 0.001$). In another study, dietary saturated fats were relatively protective against the process of alcoholic liver disease (ALD), whereas unsaturated fats promoted ALD based on calculations of the deviation from expected mortality in 17 countries (Nanji and French 1986). These results suggested that the type of dietary fats had discrete effects on ethanol metabolism. When rats were pair-fed diets high in either beef fat, pork fat, corn oil, or fish oil with or without ethanol by intragastric tube feeding for up to 6 months, the liver pathology was very different among groups. Rats fed ethanol with tallow or saturated fats developed none of the features of ALD (Nanji & French 1989, Nanji et al. 1989;1994a;1994b, Takahashi et al. 1992), even their ethanol intake was significantly increased ($P < 0.01$) to achieve comparable BEC found in lard and corn oil groups (Nanji and French 1988). Animals fed lard and ethanol had minimal to moderate ALD (Nanji & French 1989, Nanji et al. 1989). Animals fed ethanol with corn oil developed severe ALD (Nanji et al. 1989, Nanji et al. 1994a;1994b;1995, Takahashi et al. 1992, Tsukamoto et al. 1986). Rats fed ethanol and fish oil had more severe alcoholic liver injury when compared to rats pair-fed corn oil and ethanol (Morimoto et al. 1994, Nanji et al. 1994c). Moreover, MEOS and CYP2E1 induction were significantly greater in the rats fed corn oil (25% of calories) and ethanol compared to those fed tallow and ethanol (Nanji et al. 1994b, Takahashi et al. 1992). The induction of CYP2E1 was even higher in the fish oil versus corn oil group when the dietary fat content was at 25% of total calories (Nanji et al. 1994c). However, when the dietary fat content was increased to 35% of total calories,

CYP2E1 induction was comparable between the fish and corn oil (Morimoto et al. 1994). Rats fed a diet enriched in SFA were found to have lower rates of ethanol clearance than those fed a diet rich in USFA (Cha and Sachan 1994). Review of dietary fats and ethanol metabolism is listed in **Table 2-12**.

CARNITINE AND ETHANOL METABOLISM

Carnitine, chemically known as β-hydroxyl-γ-trimethylammonium butyrate, is present in the body as free carnitine and as acylcarnitines. The acyl group in the acylcarnitines range from short-chain like acetyl to long-chain like palmitoyl. Acetyl-carnitine is the predominant acylcarnitine identified in rat tissues and in blood and urine (Choi and Bieber 1977).

The well recognized physiologic function of carnitine is the transport of fatty acyl-coenzyme A (CoA) across the inner mitochondrial membrane for β-oxidation (Carter et al. 1995, Feller and Rudman 1988). Without carnitine, most of the ingested fatty acids would not be fully metabolized for energy and the body would be forced to store the fatty acids as triglyceride in adipose tissue (Bieber 1988). This strategic role of carnitine in long-chain fatty acid metabolism has received much attention and has prompted several studies on its potential to help prevent the ethanol-induced liver diseases.

Carnitine was found to significantly diminish serum triglyceride in rats receiving ethanol (Hosein and Bexton 1975). The addition of carnitine to a diet containing 36% calories as ethanol significantly depressed ethanol-induced increases of total lipids, free

TABLE 2-12

Review of dietary fats and ethanol metabolism in animal studies[1]

Author (year)	Animal model	Fat source and level	Ethanol level and duration	Results
Jones and Greene (1966)	Male albino rats, weighing 63 to 73 g	Coconut or safflower oil at 5 and 43 en%	36 en% ethanol for 3 weeks, oral	Fatty livers developed only in rats fed 43 en% of dietary fat with ethanol regardless of the type of fat sources
Lieber et al. (1967)	Male Charles River rats, weighing 100 to 150 g	LCT (olive-corn oil mixture), or MCT at 41 en%	36 en% ethanol for 24 days, oral	Hepatic TG accumulation was significantly higher in the LCT group than in the MCT group
Lieber and DeCarli (1970)	Male S. D. rats, weighing 120 to 150 g	Mixture of ethyl linoleate, olive and corn oils at 2, 5, 10, 15, 25, 35, and 43 en %	36 en% ethanol for 24 days, oral	≥25 en% of dietary fat significantly increased hepatic lipid accumulation
Kanayama et al. (1984)	Male Wistar rats, weighing ca. 200 g each	Corn oil at 15 and 36 en%	36 en% ethanol for 4 weeks, oral	Ethanol metabolism was accelerated by the high fat diet (36 en%)
Tsukamoto et al. (1985)	Male Wistar rats, weighing 350 to 400 g	Corn oil at 5 en%	32 to 41.4 en% ethanol for 15 to 85 days, intragastric	Severe and progressing fatty liver developed in the alcoholic rats
Takada et al. (1986)	Male Wistar rats, weighing ca. 150 g each	Corn oil at 15 and 35 en%	36 en% ethanol for 12 weeks, oral	Alcohol-pyrazole hepatitis was accelerated by the high fat diet (35 en%)

Continued

TABLE 2-12 (Continued)

Review of dietary fats and ethanol metabolism in animal studies[1]

Author (year)	Fat source and level	Animal model	Ethanol level and duration	Results
Tsukamoto et al. (1986)	Male Wistar rats, weighing 350 to 400 g	Corn oil at 25 en%	32 to 47 en% ethanol for 30 to 120 days, intragastric	Alcohol-induced liver injury increased with raising dietary fat level
Reinke et al. (1987)	Female S. D. rats, weighing 140 to 150 g	Mixture of olive, corn, and safflower oils at 12 and 35 en%	36 en% ethanol for 2 weeks, oral	Level of cytochrome P-450 was increased by a high fat diet plus ethanol
Nanji and French (1989)	Male Charles River rats, weighing 220 to 355 g	Lard, tallow, or tallow supplemented with 2.5% linoleic acid at 25 en%	8 to 17 g/kg/day for 2 to 6 months, intragastric	With ethanol intake, ALD was none in the tallow group, moderate in the lard group, and most severe in the tallow plus linoleic acid group
Nanji et al. (1989)	Male Charles River rats, weighing 220 to 355 g	Corn oil, lard, or tallow at 25 en%	8 to 17 g/kg/day for 2 to 6 months, intragastric	With ethanol intake, ALD was none in the tallow group, moderate in the lard group, and most severe in the corn oil group
Takahashi et al. (1992)	Male Wistar rats, weighing 200 to 250 g	Corn oil or tallow at 25 en%	42 en% ethanol for 5 to 24 weeks, intragastric	Severe fatty change was observed in the corn oil and ethanol group

Continued

TABLE 2-12 (Continued)

Review of dietary fats and ethanol metabolism in animal studies[1]

Author (year)	Fat source and level	Animal model	Ethanol level and duration	Results
Cha and Sachan (1994)	Male S. D. rats, weighing 75 to 92 g	10 wt% corn oil (UFA) or coconut oil (SFA)	3 g/kg body weight ethanol solution (13%) administrated on days 30, 90, 105 and 120, oral	Ethanol metabolism is slower in the SFA group than in the UFA group
Nanji et al. (1994a)	Male Wistar rats, weighing 225 to 250 g	Corn oil or saturated fat at 25 en%	8 to 17 g/kg/day for 2 and 4 weeks, intragastric	Alcoholic liver injury was limited to rats fed the corn oil for 4 weeks
Nanji et al. (1994b)	Male Wistar rats, weighing 225 to 250 g	Corn oil or saturated fat at 25 en%	8 to 17 g/kg/day for 2 and 4 weeks, intragastric	Ethanol induced increase in CYP2E1 was greatest in rats fed the corn oil for 2 and 4 weeks
Nanji et al. (1994c)	Male Wistar rats, weighing 225 to 250 g	Corn or fish oil at 25 en%	8 to 17 g/kg/day for 4 weeks, intragastric	Alcoholic liver injury was more severe in the fish oil versus corn oil group; CYP2E1 was highest in the fish oil and ethanol group
Morimoto et al. (1994)	Male Wistar rats, weighing 210 to 240 g	Corn or fish oil at 35 en%	8 to 14 g/kg/day for 72 days	Alcoholic liver injury occurred in both groups; ethanol inducible CYP2E1 content was similar between the 2 groups

Continued

TABLE 2-12 (Continued)

Review of dietary fats and ethanol metabolism in animal studies[1]

Author (year)	Fat source and level	Animal model	Ethanol level and duration	Results
Nanji et al. (1995)	Male Wistar rats, weighing 225 to 250 g	Fish oil at 35 en% and ethanol for 6 weeks, then fish or palm oil without ethanol for 2 weeks	8 to 16 g/kg/day for 6 weeks, intragastric	By 6 weeks, all rats fed fish oil and ethanol developed alcoholic liver injury. Rats then fed palm oil and dextrose for 2 more weeks showed near normal liver histology.

[1]Abbrevations used: LCT, long-chain triglycerides; MCT, medium-chain triglycerides; S. D., Sprague-Dawley; TG, triglycerides.

and esterified cholesterol, triglyceride and phospholipids in the liver samples of rats in a dose-dependent manner (Rhew and Sachan 1986, Sachan et al. 1984, Sachan and Rhew 1983). Under both acute and chronic doses of ethanol, dietary carnitine administration also reduced hepatic lipids and raised blood ethanol concentrations (BEC). The increase in BEC was dependent on the level of the supplemented carnitine (Berger & Sachan 1986;1991, Sachan 1992, Sachan and Berger 1987). Similar results were reported by Smith et al. (1994) that carnitine delayed ethanol metabolism and expanded the half-life of ethanol in broilers. It is believed that carnitine supplementation lowered oxidation of ethanol in the rats and decreased the burden of ethanol-derived metabolites on the liver. However, the *ex vivo* activities of ethanol-metabolizing enzymes were not significantly affected by supplementary carnitine (Mynatt and Sachan 1992, Sachan 1992). In addition, rats fed SFA diet had significantly higher plasma carnitine concentrations of different types as compared to those fed USFA diet (Cha and Sachan 1994). These results support that carnitine offers protection to the animal model against ethanol-induced lipid abnormalities by retarding ethanol metabolism.

In contrast to the results from animal studies, the carnitine mediated rise in BEC was not observed in a randomized double-blind and crossover human study (Adamo et al. 1988). The BEC were compared in 15 healthy men receiving ethanol (0.5 g/kg) in the form of white wine and a concomitant intravenous of L-carnitine (3 g/100 mL of saline solution) or saline solution. The plasma acetate concentrations, however, were significantly decreased ($P < 0.05$) by L-carnitine administration following ethanol ingestion. Besides, the urinary acetylcarnitine concentrations were significantly

increased ($P < 0.02$) following administration of ethanol plus carnitine, but not when L-carnitine was administrated alone. Therefore, the investigators suggested that L-carnitine supplementation might trap excess acetyls derived both from free acetate, formed by ethanol oxidation, and from acetyl coenzyme A, accumulated as a result of the ethanol-induced decrease in the Krebs cycle flux.

In the isolated rat hepatocytes, the rates of ethanol oxidation were reduced by adding acetylcarnitine and L-carnitine to the media as compared to those without acetylcarnitine or L-carnitine (Cha and Sachan 1995). The maximal inhibition of ethanol oxidation in the hepatocytes was clearly evident at 100-fold lower concentration of acetylcarnitine than carnitine. Therefore, the inhibition of ethanol oxidation by carnitine had to be mediated by the formation of acetylcarnitine in the isolated hepatocytes as well as in the intact animal (Cha and Sachan 1995). It has been found that the activity of ADH, not MEOS, was significantly inhibited by acetylcarnitine at NAD^+:acetylcarnitine ≤ 1. The inhibition is of a competitive nature where acetylcarnitine competes with NAD^+ (Sachan and Cha 1994).

DIETARY FATTY ACIDS AND SMALL INTESTINE

Absorption of almost all major dietary products take place in the small intestine (Castro 1981). Experimental evidence demonstrate that dietary fat modifications alter the absorption of ingested nutrients (Thomson and Rajotte 1983, Thomson et al. 1986;1989), which may be due to the changes in the fatty acid composition and fluidity of intestinal cell membranes.

Information concerning the influences of feeding TFA on the intestinal uptake of nutrients is not complete. Sugano et al. (1984) found that the cholesterol absorption rate was markedly lower in rats fed trans-fat than in those fed cis-fat. The trans C18:1 content was relatively higher in the intestinal epithelial cells than in the serum of these animals. An *in vitro* study carried out by Manteca et al. (1994) demonstrated that elaidic acid increased the intestinal uptake of cholesterol as compared to oleic acid. On the other hand, Thomson et al. (1994) observed that feeding TFA to rats for 2 weeks had no influence on the jejunal or ileal uptake of glucose, fatty acids (lauric, stearic, oleic, linoleic, and linolenic acids) or cholesterol. The effects of TFA feeding on many other nutrients and xenobiotics, especially ethanol, pesticides, and herbicides, are unknown.

RESEARCH OBJECTIVES

The review of the literature indicates that both the quantity and the saturation degree of fatty acid alter ethanol metabolism. There is, however, no published data of the effects of various fatty acid geometric and positional isomers on ethanol metabolism. Use of margarines and shortenings in place of butter and other saturated fats has become an increasingly popular dietary practice. These fats are usually utilized in preparation of convenient and snack foods which are frequently eaten with alcoholic beverages. Therefore, the overall objective of this study was to determine the effects of geometric isomers (cis and trans) of unsaturated fatty acids on the bioavailability and metabolism of ethanol and compare these results to those of the SFA. As a result, the following questions were proposed to address the overall objective:

1. Will TFA alter blood ethanol clearance? If so, would the effects be similar to those of saturated or cis fatty acids?

8. Can the alteration of BEC by TFA be explained by changes in hepatic ADH activity?

9. Can the alteration of BEC by TFA be explained by changes in acylcarnitine profiles?

10. Can the alteration of BEC by TFA be explained by changes in urinary excretion of ethanol?

11. Can the alteration of BEC by TFA be explained by changes in the intestinal transport of ethanol?

12. Can the alteration of BEC by TFA be explained by changes in fatty acid composition?

CHAPTER III

EFFECTS OF SATURATED, CIS, AND TRANS FATTY ACIDS ON BLOOD ETHANOL CONCENTRATIONS IN RATS

ABSTRACT

Two experiments were conducted to determine the effects of different types of fatty acids on blood ethanol clearance in 5-week-old male Sprague-Dawley rats. In Experiment 1, 18 rats were randomly assigned to one of the modified AIN[76] diets containing a predominance of saturated fatty acids (SFA), cis fatty acids (CFA), or trans fatty acid (TFA). Various edible fats, oils and one fatty acid were mixed to generate the diets of comparable fatty acid profiles. After 2, 4, and 6 weeks of dietary treatment, an oral dose of ethanol (3 g/kg body weight) was given to the animals. Serial tail vein blood samples were collected from 15 to 360 minutes post-ethanol administration and assayed for blood ethanol concentration (BEC). After 2 weeks of feeding, the BEC of SFA and CFA groups were higher than those of TFA group. However, these differences in BEC were not significant after 4 and 6 weeks of feeding the diets. Experiment 2 was undertaken to confirm the changes in BEC by feeding CFA and TFA diets for 2 weeks in 24 rats. Compared with rats fed the CFA diet, animals fed the TFA diet had an enhanced blood ethanol clearance as observed in the first experiment. Based on the BEC data, it is concluded that rats fed TFA for 2 weeks accelerated blood ethanol clearance compared with CFA. In addition, liver triglycerides were significantly lowered in the TFA group, whereas the plasma triglycerides were not affected by the dietary fatty acids.

INTRODUCTION

Ethanol-induced liver injury proceeds through three main stages, namely fatty liver, hepatitis, and finally cirrhosis (Sherlock 1995). Lipid is one of dietary factors known to affect the metabolism of ethanol and thus the pathogenesis of alcoholic liver disease (ALD). To investigate the relationship between the dietary fat and mortality rate of cirrhosis, Nanji and French (1985) observed a highly significant correlation ® = 0.98, $P < 0.001$) between cirrhosis mortality rate and the product of ethanol and pork consumption in 16 countries. Another epidemiological study in 17 countries indicated that saturated fats were relatively protective against the alcoholic cirrhosis, whereas polyunsaturated fats promoted alcoholic cirrhosis (Nanji and French 1986). The epidemiological correlations were further confirmed in animal studies. Using a continuously intragastric ethanol feeding model, Nanji and French (1988) detected that rats fed tallow needed significantly higher ($P < 0.01$) intake of ethanol to achieve comparable BEC than that found in the lard and corn oil groups. While rats fed ethanol and beef fat developed no hepatic damage, those fed ethanol and lard exhibited minimal to moderate ALD, and those fed ethanol with corn oil had all of the features of ALD (Nanji et al. 1989). It was suggested that the severity of ALD was related to the amount of linoleic acid in the dietary fat source (tallow 0.7%, lard, 2.5%, and corn oil 56.6%) (Nanji et al. 1989). When rats were fed ethanol and tallow supplemented with linoleic acid (2.5%), the pathological changes of ALD (fatty liver, necrosis, and inflamation) were more severe than those in the group fed lard and ethanol (Nanji and French 1989). Therefore, linoleic acid was proposed to be essential for the development of ALD in the rat model (Nanji and French 1989, Nanji et al. 1989).

To study the effects of saturated fatty acids (SFA) and unsaturated fatty acids (USFA) diets on ethanol pharmacokinetics after acute ethanol treatment, rats were fed diets containing 10% (w/w) coconut or corn oil (Cha and Sachan 1994). Feeding of the USFA diet consistently accelerated blood ethanol clearance compared to feeding the SFA diet to rats for 30, 90, 105, and 120 days.

USFA contain both cis fatty acids (CFA) and trans fatty acids (TFA). TFA resemble SFA in chemical structure and hypercholesterolemic effect (Katan and Mensink 1992). To our knowledge, there are no data in the literature on the effect of dietary TFA on ethanol metabolism. Therefore, the objective of the present study was to study the relative effects of diets rich in SFA, CFA, or TFA on blood ethanol clearance as indicated by the BEC curves.

MATERIALS AND METHODS

Animals. The experimental protocol was approved by the University of Tennessee Committee on Humane Care and Use of Laboratory Animals. After 1 week of acclimation with Purina Rodent Chow diet (Ralston Purina Co., St. Louis, MO), male Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN), approximately 5 weeks of age, were randomly divided into different dietary groups. Each rat was individually housed in a stainless steel wire bottom cage in a temperature controlled room ($23 \pm 2\,^{\circ}C$). Lighting was controlled automatically for 6:00 to 18:00. All animals had free access to food and water through the 6-week study. Body weights were measured weekly and food intakes were recorded on a daily basis.

Diets. The rats were fed modified AIN[76] diets enriched with 10% (w/w) SFA, CFA or

TFA. Various edible fats, oils, and one fatty acid were mixed to generate the diets of comparable fatty acid compositions. All diets were prepared weekly and stored at -20°C. To prevent oxidation, fresh food was provided daily to the animals. The fatty acid compositions of the different diets were analyzed in a Hewlet-Packard Model 5890 series II chromatograph equipped with a flame ionization detector and a DB23 fused silica capillary column (0.25 mm I.D. x 30 m x 0.25 μ film, J & W Scientific, Folsom, CA). In Experiment 1 (**Table 3-1**), the fat source of the SFA diet included coconut and corn oils. Butter, corn oil, and olive oil were combined for the CFA diet. Margarine and free elaidic acid were the source of the TFA diet. The dietary fatty acid composition data of the first experiment are shown in **Table 3-2**. In Experiment 2 (**Table 3-3**), the fat sources of the CFA diet consisted of butter, corn oil, and olive oil. Margarine, free elaidic acid, and butter were used as the fat sources of the TFA diet. **Table 3-4** presents the dietary fatty acid compositions used in the second experiment.

Experimental design. In Experiment 1, 18 male Sprague-Dawley rats were randomly divided into 3 dietary groups (n = 6): SFA, CFA, and TFA. After 2, 4, and 6 weeks of dietary treatment, the rats given a single oral dose of 3 g ethanol per kg body weight as 13% aqueous solution (v/v) via a gastric canula. Tail vein blood samples (20 μL each) were collected at 15, 30, 60, 120, 180, 240, 300, and 360 minutes after ethanol administration for determination of BEC . At the end of the 6-week study period, blood samples were collected by cardiac puncture under Metaphane anesthesia. Following the blood collection, liver (perfused) was removed, frozen in liquid nitrogen, and stored at -80°C until assayed for triglyceride.

TABLE 3-1

Composition of the experimental diets used in Experiment 1[1]

Ingredient	Diet		
	SFA	CFA	TFA
		g/kg	
Casein	200	200	200
DL-Methionine	3	3	3
Corn starch	150	150	150
Sucrose	450	450	450
Cellulose	50	50	50
Vitamin mix[2]	10	10	10
Mineral mix[2]	35	35	35
Coconut oil[a]	65	-	-
Corn oil[a]	35	37	-
Olive oil[a]	-	58	-
Butter[3a]	-	6.14	-
Margarine[4a]	-	-	111.8
Elaidic acid[5a]	-	-	10
Choline bitartrate	2	2	2

[1] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid.

[2] AIN 1977.

[3] Butter: 113 g of butter contains 92 g of fat; therefore, there is about 81% fat in butter.

[4] Margarine: 113 g of butter contains 91 g of fat; therefore, there is about 80% fat in butter.

[5] Elaidic acid (Sigma, St. Louis, MO).

[a] Differences presented in fat sources are due to their compositions of fatty acids which are balanced between the three experimental diets.

TABLE 3-2

Fatty acid composition of the experimental diets used in Experiment 1 (mole%)[1,2]

Fatty acid	Dietary group		
	SFA	CFA	TFA
C10:0	4.6	0.6	0.7
C12:0	34.1	0.2	0.0
C14:0	13.1	0.6	0.1
C16:0	10.6	17.3	12.4
C16:1	0.0	1.5	0.1
C18:0	4.1	3.1	8.6
cis C18:1 (n-9)	12.3	43.2	25.5
trans C18:1 (n-9)	0.1	0.0	19.0
C18:2 (n-6)	20.7	32.6	29.4
C18:3 (n-3)	0.4	0.9	4.2
Σ SFA	66.5	21.8	21.8
Σ MUFA	12.4	44.7	44.6
Σ PUFA	21.1	33.5	33.6
P:S	0.3	1.5	1.5
S:M:P	2/0.4/0.6	½/1.5	½/1.5

[1] Diets were analyzed for fatty acid compositions in a Hewlet-Packard Model 5890 series II gas chromatograph equipped with a flame ionization detector and a DB23 fused silica capillary column (0.25 mm I.D. x 30 m x 0.25 μ film, J & W Scientific, Folsom, CA).

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; P/S, PUFA:SFA ratio; S/M/P, SFA:MUFA:PUFA ratio.

TABLE 3-3

Composition of the experimental diets used in Experiment 2[1]

	Dietary group	
Ingredient	CFA	TFA
	g/kg	
Casein	200	200
DL-Methionine	3	3
Corn starch	150	150
Sucrose	450	450
Cellulose	50	50
Vitamin mix	10	10
Mineral mix	35	35
Corn oil[a]	52	-
Olive oil[a]	37	-
Butter[2a]	14	1.27
Margarine[3a]	-	110.73
Elaidic acid[a]	-	12
Choline bitartrate	2	2

[1] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid.

[2] Butter: 113g of butter contains 92g of fat; therefore, there is about 81% fat in butter.

[3] Margarine: 113 g of margarine contains 91g of fat; therefore, there is about 80% fat in margarine.

[a] Differences presented in fat sources are due to their compositions of fatty acids which are balanced between the three experimental diets.

TABLE 3-4

Fatty acid composition of the experimental diets used in Experiment 2 (mole%) [1,2]

	Dietary group	
Fatty acid	CFA	TFA
C10:0	0.2	0.0
C12:0	0.4	0.0
C14:0	1.3	0.2
C16:0	14.0	12.2
C16:1	0.5	0.1
C18:0	4.0	7.4
cis C18:1 (n-9)	44.5	26.1
trans C18:1 (n-9)	0.1	19.2
C18:2 (n-6)	33.1	33.1
C18:3 (n-3)	0.9	0.7
Σ SFA	20.6	20.6
Σ MUFA	45.4	45.5
Σ PUFA	34.0	33.9
P/S	1.7	1.7
S/M/P	1/2.2/1.7	1/2.2/1.7

[1] Diets were analyzed for fatty acid compositions in a Hewlet-Packard Model 5890 series II gas chromatograph equipped with a flame ionization detector and a DB23 fused silica capillary column (0.25 mm I.D. x 30 m x 0.25 μ film, J & W Scientific, Folsom, CA).

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; P/S, PUFA:SFA ratio; S/M/P, SFA:MUFA:PUFA ratio.

Two groups (n = 12) of male Sprague-Dawley rats were fed CFA or TFA diets for 2 weeks in Experiment 2. Tail vein blood samples were collected at 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, and 360 minutes after ethanol administration for BEC assay. At the end of the 2-week experimental period, the liver was perfused, removed, weighed, and stored at -80°C.

Determination of BEC. The modified enzymatic method of Bernt and Gutmann (1974) was used for the determination of BEC. Briefly, a blood sample (20 μL) was taken from the tail vein and immediately diluted with 980 μL of cold saline in a micro-centrifuge tube. The dilution was rapidly mixed and kept on ice until assayed. A total of 2.5 mL of reaction mixture containing 71 mmol/L pyrophsophate buffer (pH 8.7), 71 mmol/L semicarbazide, 20 mmol/L glycine, 0.96 mmol/L NAD^+, and ADH was prepared for each test sample. Ethanol standards were prepared by diluting the working standard, 0 to 100 μL, with cold normal saline. A 0.1 mL aliquot of supernatant from each centrifuged standard or sample was mixed with the reaction mixture and then incubated for 25 minutes in a 37°C water bath. After cooling to room temperature, the absorbance of each sample was determined by a spectrophotometer at 340 nm. The BEC was expressed as mmol/L.

Determination of triglyceride. The triglyceride in the plasma or liver homogenate was assayed by the method of Giegel et al. (1975). The triglyceride content in 200 μL of plasma or liver homogenate was extracted with 5 mL of n-nonane and isopropanol (2.0:3.5 v/v) mixture and acidified with 1 mL of 40 mmol/L sulfuric acid. A series of standards was made using 0 to 600 μL of triolein standard. Standards and samples were then centrifuged at 1500 x g for 10 minutes at 4°C. A 500 μL aliquot of the clear upper

phase from each extraction was mixed with 500 μL of transesterifying reagent (KOH in isopropanol, 100 nmol/L). After standing at room temperature for 5 minutes, 500 μL of oxidizing reagent (18 mmol/L sodium periodate in 2 mol/L acetic acid) was added to each tube, and the mixture was allowed to stand for an additional 5 minutes at room temperature. Once the two-phase system was developed, 6 mL of working color reagent (6 mol/L ammonium acetate, pH 6.0) was added to each tube and vortexed. All tubes were incubated in a water bath at 60°C for 10 minutes for color development. The absorbance was measured at 415 nm against a water blank. The triglyceride levels were expressed as mg triglyceride/dL plasma or mg triglyceride/g wet liver.

Statistics. All data were expressed as the group means ± SEM. The statistical significance was evaluated by student's t test when there were 2 dietary fatty acid groups. ANOVA was used to detect the statistical significance among the 3 dietary fatty acid groups and Duncan's multiple range test was used for multiple comparisons. The level of statistical significance was set at $P < 0.05$. All statistical analyses were performed using SAS for Unix system (version 6, SAS Institute, Cary, NC). In addition, the UNIVARIATE procedure of SAS was applied to check the outliers of BEC.

RESULTS

Weight gain, food intake, and liver weight of the rats. In Experiments 1 and 2, the weight gains and liver weights were not significantly different between rats fed different types of dietary fatty acids (**Table 3-5 and 3-6**). In Experiment 1, the TFA group had slightly but significantly greater ($P < 0.05$) food intake than the SFA and CFA groups, but the feed efficiencies were virtually the same in the 3 dietary groups (**Table 3-5**). The

TABLE 3-5

Body weight, food intake, liver weight, and triglyceride in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Parameter	Dietary group		
	SFA	CFA	TFA
Initial body weight (g)	152.0 ± 12.6[a]	149.2 ± 15.0[a]	148.5 ± 15.9[a]
Weight gain (g)	173.2 ± 14.4[a]	166.7 ± 13.3[a]	183.2 ± 10.3[a]
Food intake (g)	666.3 ± 18.7[b]	648.0 ± 8.6[b]	720.8 ± 13.4[a]
Feed efficiency[3] (%)	25.9 ± 1.8[a]	25.7 ± 1.9[a]	25.5 ± 1.6[a]
Liver weight (g)	12.7 ± 0.6[a]	12.4 ± 0.4[a]	13.1 ± 0.5[a]
(% body weight)	3.9 ± 0.1[a]	3.9 ± 0.1[a]	3.9 ± 0.1[a]
Hepatic triglyceride (mg/g liver)	4.0 ± 0.5[a]	3.0 ± 0.3[a]	2.3 ± 0.2[b]
Plasma triglyceride (mg/dL plasma)	81.4 ± 15.1[a]	63.2 ± 8.4[a]	71.2 ± 9.4[a]

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid.

[3] Feed efficiency was calculated as the amount of weight gained in grams per gram of experimental diets.

TABLE 3-6

Body weight, food intake, and liver weight in rats fed cis or trans fatty acids diet for 2 weeks from Experiment 2 [1,2]

Parameter	Dietary group	
	CFA	TFA
Initial body weight (g)	131.3 ± 1.8[a]	131.4 ± 2.0[a]
Weight gain (g)	112.1 ± 3.3[a]	108.8 ± 3.7[a]
Food intake (g)	293.9 ± 5.5[a]	290.0 ± 7.4[a]
Liver weight (g)	15.4 ± 0.5[a]	15.8 ± 0.6[a]
(% body weight)	6.3 ± 0.1[a]	6.6 ± 0.2[a]

[1] Values are group means ± SEM (n=12) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid.

food intake was not significantly different between the 2 dietary groups in Experiment 2 (**Table 3-6**).

BEC and pharmacokinetics. Time-dependent changes of BEC in rats fed different fatty acid diets for 2, 4, and 6 weeks from Experiment 1 are shown in **Figure 3-1**. The measured BEC values are present in **Appendix I, Tables I-1 to I-3**. When outliers for BEC were indicated by the UNIVARIATE procedure of SAS, the whole set of BEC data for that specific animal was excluded. Thus, the sample size was 5, 6, or 5 for BEC measurement in rats after 2, 4, and 6 weeks of feeding, respectively. The BEC was significantly higher in the SFA and CFA groups than in the TFA group ($P < 0.05$) at certain time points after 2 weeks of dietary treatment. However, these differences in BEC

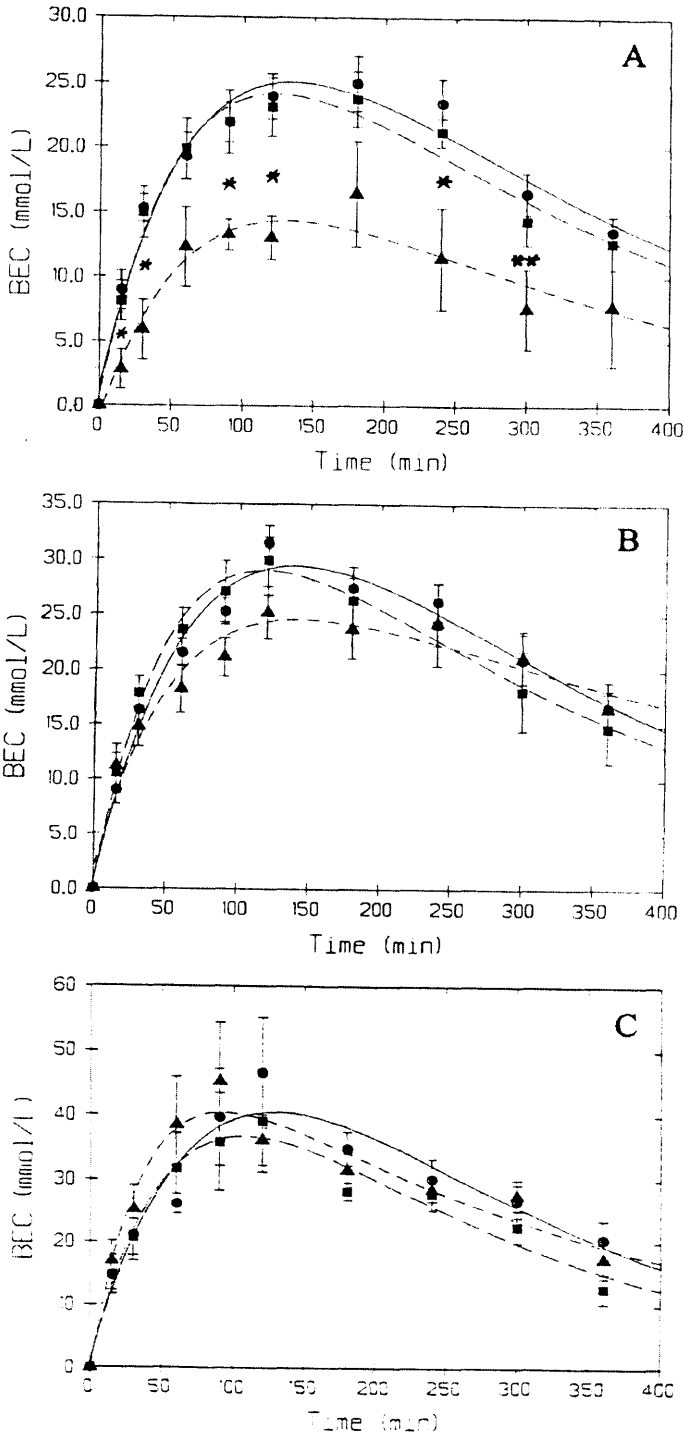


FIGURE 3-1 Blood ethanol concentrations (BEC, mmol/L) in rats fed saturated (●), cis (■), or trans (▲) fatty acids diet for 2 (A), 4 (B), and 6 (C) weeks from Experiment 1. Values are group means ± SEM (n = 5, 6, and 5 for A, B, and C, respectively) and asterisks indicate time points of statistically significant difference by ANOVA and Duncan's tests at $P < 0.05$.

were not significant after 4 and 6 weeks of dietary treatment. The pharmacokinetic parameters calculated from the BEC data (**Figure 3-1A**) after 2 weeks of dietary treatment are shown in **Table 3-7**. Compared to the TFA group, the SFA and CFA groups had higher (67 to 74%) peak BEC, greater (23 to 55%) zero time BEC (C_0), larger (72 to 82%) area under the curve (AUC), and longer (17 to 25%) half-life (T ½). The absorption rate was 20% higher in the CFA group than in the TFA group. The elimination rate was 39 to 41% and volume of distribution (Vd) was 20 to 33% lower in the SFA and CFA groups than in the TFA group. The peak time showed the least difference among the 3 dietary groups. The pharmacokinetic parameters calculated form the BEC data after 4 and 6 weeks of dietary treatment (**Figure 3-1B and C**, respectively) are presented in **Tables 3-8 and 3-9**.

In Experiment 2, the time-dependent changes of BEC were significantly higher in the CFA group than in the TFA group at certain time points after 2 weeks of dietary treatment ($P < 0.05$) (**Figure 3-2**). The numerical BEC values are presented in **Appendix I, Table I-4**. The pharmacokinetic parameters calculated from the BEC data (**Figure 3-2**) after 2 weeks of dietary treatment are shown in **Table 3-10**. The peak BEC was 31%, peak time; 45%, elimination rate; 54%, zero time BEC (C_0); 193%, and area under the curve (AUC); 32% higher in the CFA groups than in the TFA group. The absorption rate was 58%, volume of distribution (Vd); 65%, and half life of ethanol (T½); 36% lower in the CFA group than in the TFA group.

Liver and plasma triglyceride. The liver triglyceride concentrations were significantly higher ($P < 0.05$) in the SFA and CFA groups than in the TFA group in Experiment 1. There was no significant difference among the plasma triglyceride concentrations of the 3

TABLE 3-7

Pharmacokinetics of ethanol in rats fed saturated, cis or trans fatty acids diet for 2 weeks from Experiment 1 [1,2]

Pharmacokinetic parameters	Dietary group		
	SFA	CFA	TFA
Peak BEC (mmol/L)	25.0 (74%↑)	24.1 (67%↑)	14.4
Peak time (min)	134.4 (3%↑)	123.1 (6%↓)	130.9
Absorption rate (μmol/L/min)	11.8 (2%↓)	14.4 (20%↑)	12.0
Elimination rate (μmol/L/min)	4.1 (13%↓)	3.9 (17%↓)	4.7
C_0 (mmol/L)	66.9 (55%↑)	53.2 (23%↑)	43.1
Vd (L/kg)	1.0 (33%↓)	1.2 (20%↓)	1.5
AUC (mol/L/m)	7.1 (82%↑)	6.7 (72%↑)	3.9
T ½ (hour)	2.8 (17%↑)	3.0 (25%↑)	2.4

[1] The values of pharmacokinetic parameters were calculated from the mean blood ethanol concentrations (BEC) values of each group (n = 5), with the difference as compared to the TFA group in parentheses.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; C_0, zero time BEC; Vd, volume of distribution; AUC, area under the curve; T ½, half life.

TABLE 3-8

Pharmacokinetics of ethanol in rats fed saturated, cis or trans fatty acids diet for 4 weeks from Experiment 1 [1,2]

Pharmacokinetic parameters	Dietary group		
	SFA	CFA	TFA
Peak BEC (mmol/L)	29.4 (20%↑)	29.0 (18%↑)	24.6
Peak time (min)	139.6 (2%↓)	117.9 (17%↓)	142.6
Absorption rate (μmol/L/min)	10.8 (35%↓)	15.9 (4%↓)	16.6
Elimination rate (μmol/L/min)	4.3 (126%↑)	3.7 (95%↑)	1.9
C_0 (mmol/L)	90.1 (146%↑)	58.9 (61%↑)	36.6
Vd (L/kg)	0.7 (60%↓)	1.1 (39%↓)	1.8
AUC (mol/L/m)	8.3 (12%↑)	8.0 (8%↑)	7.4
T ½ (hour)	2.7 (55%↓)	3.1 (48%↓)	6.0

[1] The values of pharmacokinetic parameters were calculated from the mean blood ethanol concentrations (BEC) values of each group (n = 6), with the difference as compared to the TFA group in parentheses.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; C_0, zero time BEC; Vd, volume of distribution; AUC, area under the curve; T ½, half life.

TABLE 3-9

Pharmacokinetics of ethanol in rats fed saturated, cis or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Pharmacokinetic parameters	Dietary group		
	SFA	CFA	TFA
Peak BEC (mmol/L)	40.5 (0.2%↑)	36.7 (9%↓)	40.4
Peak time (min)	129.7 (40%↑)	107.6 (16%↑)	92.5
Absorption rate (μmol/L/min)	9.2 (64%↓)	15.4 (40%↓)	25.6
Elimination rate (μmol/L/min)	6.3 (91%↑)	5.0 (52%↑)	3.3
C_0 (mmol/L)	296.1 (373%↑)	92.6 (48%↑)	62.6
Vd (L/kg)	0.2 (80%↓)	0.7 (30%↓)	1.0
AUC (mol/L/m)	11.0 (2%↑)	9.6 (11%↓)	10.8
T ½ (hour)	1.8 (48%↓)	2.3 (34%↓)	3.5

[1] The values of pharmacokinetic parameters were calculated from the mean blood ethanol concentrations (BEC) values of each group (n = 5), with the difference as compared to the TFA group in parentheses.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; C_0, zero time BEC; Vd, volume of distribution; AUC, area under the curve; T ½, half life.

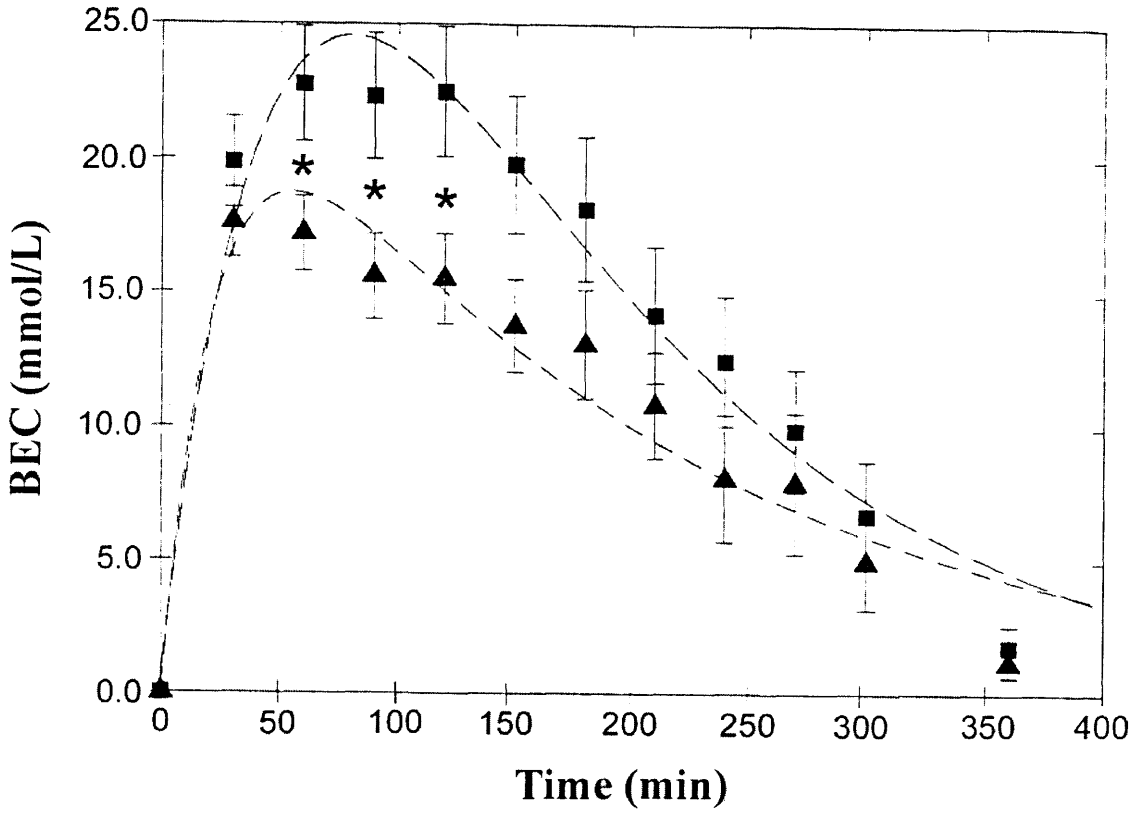


Figure 3-2 Blood ethanol concentrations (BEC, mmol/L) in rats fed cis (■) or trans (▲) fatty acids diet for 2 weeks from Experiment 2. Values are group means ± SEM (n = 12) and asterisks indicate time points of significant differences between the 2 groups by student's t test at $P < 0.05$.

TABLE 3-10

Pharmacokinetics of ethanol in rats fed cis or trans fatty acids diet for 2 weeks from Experiment 2 [1,2]

Pharmacokinetic parameters	Dietary group	
	CFA	TFA
Peak BEC (mmol/L)	24.6 (31%↑)	18.8
Peak time (min)	80.5 (45%↑)	55.5
Absorption rate (μmol/L/min)	18.0 (58%↓)	43.2
Elimination rate (μmol/L/min)	8.0 (54%↑)	5.2
C_0 (mmol/L)	83.4 (193%↑)	28.5
Vd (L/kg)	0.8 (65%↓)	2.3
AUC (mol/L/min)	5.3 (32%↑)	4.0
T ½ (hour)	1.4 (36%↓)	2.2

[1] The values of pharmacokinetic parameters were calculated from the mean blood ethanol concentrations (BEC) values of each group (n = 12), with the difference as compared to the TFA group in parentheses.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid; C_0, zero time BEC; Vd, volume of distribution; AUC, area under the curve; T ½, half life.

dietary groups (**Table 3-5**).

DISCUSSION

In Experiment 1, Sprague-Dawley rats were fed diets enriched in saturated, cis, or trans fatty acids. The proportion of individual dietary fatty acid could not be exactly matched, but the saturation of dietary fats was well controlled between the CFA and TFA diets (**Table 3-2**). Stick margarine was used as the major fat source for the TFA diet because of its high TFA content (Enig et al. 1983; 1990, Smith et al. 1985, Wootan et al. 1996), especially octadecenoic fatty acids (Kris-Etherton and Nicolosi 1995, Senti 1985). Since the composition of food fats is changing, the TFA content is decreasing (Katan 1995). The TFA content was 9 mole% in the margarine used in the present study, which is lower than the reported values of 11 to 30% (Slover et al. 1985). In order to mimic the average TFA intake in humans, free elaidic acid was added to the diet to increase the TFA content to 19 mole%. The absorption of elaidic acid as free fatty acid was demonstrated to be equal to its triglyceride form in rats (Clement et al. 1965). Experimental diets were prepared in the same manner for Experiment 2. The content of elaidic acid in margarine used for the second experiment was lower than that for the first experiment. Thus, more elaidic acid was added to the TFA diet in Experiment 2 to maintain the desirable level of this TFA (**Table 3-4**).

Among the 3 dietary groups in Experiment 1, even though the food consumption was higher in rats on the TFA diet, their body weight gains and liver weights were similar to those of rats on the SFA and CFA diet. Sugano et al. (1984) have also reported significantly greater food intake by rats fed trans fat than by those fed cis fat without

showing a difference in weight gains and relative liver weights.

It is estimated that the dose of 3 g ethanol/kg body weight in a rat is equivalent to about 210 g or 266 ml of absolute ethanol, approximately the amount in 5 portions (3.5 ounces) of 50% ethanol ingested by a man of 70 kg. However, more importantly, this specific dose (3 g/kg body weight) produced peak BEC from 0.1 to 0.2% in rats. These levels are physiologically similar to humans who are legally drunk (0.1%). Moreover, the LD_{50} (lethal dose at which 50% of animals will die) for ethanol is 13.7 g/kg body weight; thus, this dose (3 g/kg body weight) at less than one fourth of the LD_{50} avoids the life threat to the experimental animals throughout the study.

In Experiment 1, the blood ethanol clearance was accelerated by the TFA diet compared to the SFA and CFA diets after 2 weeks of dietary treatment (**Figure 3-1A**). The pharmacokinetic data revealed that the TFA diet caused smaller AUC, greater V_0, and shortened T½ of ethanol compared to the SFA and CFA diets (**Table 3-7**). This effect of TFA was most likely due to a relatively faster metabolism of ethanol; however, low absorption of ethanol in the gastrointestinal tract and rapid elimination of ethanol in the urine remained possibilities. The shorter T½ of ethanol in rats fed the TFA diet could be expected to increase the burden of ethanol-metabolites on these livers. Thus, it appeared that TFA was responsible for the faster blood ethanol clearance observed in the present study. The fact that TFA acted unlike SFA and that the CFA was similar to SFA with regard to ethanol metabolism is contrary to the expectation. For example, the hypercholesterolemic effect of TFA have been shown to resemble SFA (Judd et al. 1994, Lichtenstein et al. 1993, Mensink and Katan 1990, Troisi et al. 1992).

As the animals grew older, the effect of the TFA diet was minimized or reversed

(**Figure 3-1B,C**), as indicated by the pharmacokinetic data (**Tables 3-8 and 3-9**). The lack of sustained effect of TFA on ethanol metabolism is surprising because such inconsistency was not seen in rats fed SFA or USFA diet for 30, 90, 105 and 120 days (Cha and Sachan 1994). These results were perhaps a consequence of unknown effects of sources used in the diet preparations. In the present study, the fat source of CFA was from a mixture of corn and olive oils and that of TFA was from the combination of margarine and free elaidic acid. In the study of Cha and Sachan (1994), corn oil was used as the sole source of USFA. In addition, Hsu and Kummerow (1977) demonstrated that TFA decreased the oxidation rates of acylcarnitines, glutamate, and pyruvate in the heart mitochondria of rats after 1 week of feeding. The significance, however, disappeared after 6 weeks of feeding. Chiang and Lu (1996) reported that TFA significantly increased plasma total and low density lipoprotein cholesterol levels in rats after 2 weeks of feeding and the significant difference was not found after 4 weeks of feeding. Therefore, it is possible that undefined metabolic adaption compromised the dietary fatty acid related changes in BEC.

Experiment 2 confirmed the results of the first experiment that the time-dependent BEC were higher in the CFA group compared to the TFA group with increased number of animals (**Figure 3-2**). There were 6 rats in the first experiment and 12 rats in the second experiment. Pharmacokinetic data based on the BEC curves suggested that the CFA group had lower V_0 and higher AUC than the TFA group (**Table 3-10**). These results were consistent with the observations of the first experiment (**Table 3-7**). However, the theoretical absorption and elimination rates and half-lives were opposite to the earlier data (**Table 3-7**). The reasons for the inconsistency of the 3 parameters were

not immediately understood. In Experiment 1, only a single dose of ethanol was given to the rats to measure the BEC. However, 2 doses of ethanol were administrated to the rats 2 days apart to determine the urinary ethanol concentrations and BEC in Experiment 2. This difference might have some unexplainable effect in the expression of the pharmacokinetic data. Furthermore, there were only 2 data points in the absorption phase of the BEC curve from which pharmacokinetic program extrapolated to draw a line and calculate absorption slope. Therefore, absorption parameters can not be entirely reliable and other pharmacokinetic parameters presented in **Table 3-10** should be interpreted with caution.

The plasma triglyceride concentrations of the TFA group were not statistically different from the SFA and CFA groups in Experiment 1 (**Table 3-3**). However, the liver triglyceride content was lower in rats fed the TFA diet than in those fed the CFA diet (**Table 3-3**), which is in agreement with the observation of other investigators (Chiang and Yu 1996, Sugano et al. 1984). Therefore, it would appear that the TFA diet will offer protection against ethanol-induced fatty liver. The basis for this result may be in the fact that TFA impair fatty acid synthesis as they depress the activities of liver acetyl CoA carboxylase and fatty acid synthetase in rats (Egwim and Sgoutas 1972).

In summary, the results from the 2 experiments presented here demonstrate that TFA caused lower BEC which is due to a combination of higher volume of distribution, decreased rate of absorption, and enhanced rate of elimination of ethanol. It is note worthy that TFA did not act like SFA regarding ethanol metabolism and that TFA lowered liver triglycerides without affecting plasma triglycerides.

CHAPTER IV

EFFECTS OF SATURATED, CIS AND TRANS FATTY ACIDS ON HEPATIC ADH ACTIVITY, MITOCHONDRIAL CPT-1 ACTIVITY, AND CARNITINE CONCENTRATIONS IN RATS

ABSTRACT

It has been shown that rats fed the trans fatty acid (TFA) diet for 2 weeks had lower blood ethanol concentrations (BEC) as well as steeper absorption and elimination slopes than those fed either saturated fatty acids (SFA) or cis fatty acids (CFA) diet. In addition, TFA did not behave like SFA on blood ethanol clearance. Therefore, this study was conducted to further examine the possible mechanisms of dietary fat related changes on blood ethanol clearance in young male Sprague Dawley rats. It was found that TFA enhanced blood ethanol clearance in comparison to SFA or CFA which could not be explained on the basis of differences in the hepatic alcohol dehydrogenase (ADH) activity after either 2 or 6 weeks of dietary treatment. The excretion of ethanol in urine was relatively higher in the TFA group than in the CFA group during the first 4 hours after ethanol administration and may partially account for the differences in BEC between the CFA and TFA groups. In addition, changes in the plasma carnitines which changed parallel with BEC contributed to higher BEC in the SFA and CFA groups than in the TFA group.

INTRODUCTION

Trans fatty acids (TFA), the geometrical isomers of the cis fatty acids (CFA), are chiefly produced during the partial hydrogenation of vegetable oils (Emken 1984, Enig 1996). Such chemical hydrogenation is applied to manufacture margarines, shortenings, salad oils, and cooking oils with improved flavor, increased shelf-life, and lowered cost (Dutton 1979). TFA are also synthesized in the rumen of ruminant animals by microbial biohydrogenation (Emken 1984, Sachan and Davis 1969). About 90 to 95% of dietary TFA in the U. S. diet are provided by the partially hydrogenated vegetable oils and the remaining 5 to 10% from ruminant fats (Emken 1984, Enig 1996). The principal TFA produced by the chemical partial hydrogenation and microbial biohydrogenation are elaidic acid and vaccenic acid, respectively (Kris-Etherton and Nicolosi 1995, Senti 1985, Sommerfeld 1983). In the average American diet, the estimated intake of TFA is 8.1 to 13.3 g per person per day, accounting for 3 to 4% of total energy intake, as assessed by availability or disappearance data (Enig et al. 1990, Hunter and Applewhite 1991).

We have previously shown that feeding the TFA diet to rats for 2 weeks significantly decreased blood ethanol concentrations (BEC) as compared to the feeding of either SFA or CFA diets (Chapter 3). Based on the time-dependent changes in the BEC curves, the TFA group had steeper absorption and elimination slopes and consequently a higher blood ethanol clearance than the SFA or CFA groups. In addition, TFA and SFA did not behave alike regarding blood ethanol clearance. The mechanisms responsible for these changes were not clear. Thus, the objectives of this study were to explore the possible mechanisms behind the TFA-mediated acceleration of blood ethanol clearance. The changes in hepatic alcohol dehydrogenase (ADH) activity, mitochondrial carnitine

palmitoyl trans ferase-I (CPT-I) activity, carnitine concentrations, and urinary ethanol excretion were determined in rats fed SFA, CFA, or TFA diet for either 2 or 6 weeks.

MATERIALS AND METHODS

Animals and diets. The experimental protocol was approved by the University of Tennessee Committee on Humane Care and Use of Laboratory Animals. The details for the use of animals and preparation of experimental diets are described in the materials and methods section of Chapter 3. Briefly, 18 male Sprague-Dawley rats were randomly assigned to 3 dietary groups for 6 weeks of dietary treatment in Experiment 1. Twenty-four rats with similar characteristics were fed CFA or TFA diets for 2 weeks in Experiment 2. All animals were allowed free access to food and water. When urine samples were collected, the rats were placed in the individual metabolic cages for 24 hours. Food consumption was recorded daily and body weight was monitored weekly for each animal.

Rats were fed modified AIN[76] diets enriched with 10% (w/w) SFA, CFA, or TFA in Experiments 1 and 2 (**Tables 3-1 and 3-3** in Chapter 3). Various edible fats, oils and one fatty acid were mixed to generate the diets of comparable fatty acid content (**Tables 3-2 and 3-4** in Chapter 3). The composition of dietary fatty acids was analyzed in a Hewlet-Packard Model 5890 series II chromatograph equipped with a flame ionization detector and a DB23 fused silica capillary column (0.25 mm I.D. x 30 m x 0.25 μ film, J & W Scientific, Folsom, CA). All diets were prepared weekly and stored at -20 °C. Fresh food was provided daily to the animals.

Sampling. In Experiment 1, the animals were restrained for 3 to 5 minutes in a folded

towel or a plastic cone (DeCapicone) for tail vein blood sampling. Tail vein blood (500 μl) was collected for carnitine determination before and after 2, 4, and 6 weeks of dietary treatment. Twenty-four-hour urine samples were collected in a urine collector containing 1 mL of 0.2 mol/L HCl before and after 2, 4, and 6 weeks of dietary treatment for carnitine determination. At the end of the study period, blood samples were collected by cardiac puncture under Metaphane anesthesia. Following the blood collection, brain, heart, liver (perfused), skeletal muscle, and small intestine were removed, frozen in liquid nitrogen, and stored at -80°C until assayed for carnitine. A portion of liver was also processed at the same day for the ADH and CPT-I assays.

In Experiment 2, urine samples were collected at 2, 4, 6, 8, and 24 hours after giving ethanol and analyzed for urinary ethanol concentrations (UEC). Blood and urine samples for ethanol concentration determinations were collected at a 2-day interval to avoid the possible stress effect on the biochemical markers. At the end of the study, half of the rats of each dietary group were given an oral dose of ethanol (3 g/kg body weight) 2 hours prior to killing. Then, animals were anesthetized by Metaphane and blood samples were collected by cardiac puncture. After blood collection, the liver was perfused, removed, and processed immediately for ADH determination in 100,000 x g supernatant fractions. In Experiments 1 and 2, the diets were not taken away from the rats to preserve normal ethanol oxidation and maximal ADH activity (Lumeng et al. 1979).

Preparation of tissue homogenate. Pieces of frozen tissue cut from similar sites were weighed and homogenized with cold saline in a teflon/glass homogenizer yielding a weight by volume (w/v) homogenate. For the carnitine assay, the w/v ratio of brain and

heart was 1:9; liver, 1:6; muscle, 1:15; and small intestine, 1:4. These homogenates were prepared at 0 to 5°C and stored at -80°C until ready for assay.

Preparation of enzyme. In Experiment 1, the CPT-I activity was assayed in the intact hepatic mitochondria. The activity of ADH was assayed in the 100,000 x g supernatant fraction of the liver. Liver tissue was first minced by cutting with scissors in cold 0.25 mol/L sucrose and then homogenized using a teflon/glass homogenizer. The homogenate was then centrifuged at 600 x g for 10 minutes at 4°C. The supernatant containing the mitochondria was transferred to another centrifuge tube and then centrifuged at 12,000 x g for 10 minutes at 4°C. The 12,000 x g pellet was then resuspended in 0.25 mol/L sucrose and centrifuged again at 12,000 x g for 10 minutes at 4°C. The pellet was resuspended as before and used for determination of CPT-I. The 12,000 x g supernatant was centrifuged at 100,000 x g for 1 hour at 4°C in an ultracentrifuge. The 100,000 x g supernatant was used for ADH analysis. Protein concentration was determinated by the Biuret method (Layne 1957).

In Experiment 2, the liver was first minced by cutting with scissors in cold 150 mmol/L KCl in 10 mmol/L phosphate buffer (pH 7.4). The liver was then homogenized in 3 volumes of KCl-phosphate buffer (pH 7.4) by 4 strokes in a teflon/glass homogenizer. The homogenate was centrifuged at 600 x g for 10 minutes at 4°C. The 600 x g supernatant was transferred to another centrifuge tube and centrifuged at 10,000 x g for 10 minutes at 4°C. The 10,000 x g supernatant was centrifuged at 100,000 x g for 1 hour at 4°C in an ultracentrifuge. The 100,000 x g supernatant was used for ADH analysis. Protein concentration was determinated by the method of Bradford (1976). The activities of ADH in the stomach and small intestine were not determined because of the

difficulties in preparing the homogenates and cytosolic fractions from these tissues.

Determination of UEC. The modified enzymatic method of Bernt and Gutmann (1974) was used for the determination of ethanol concentrations in the urine samples. Briefly, urine samples (100 μL) were diluted (2 to 30 times) or undiluted and kept on ice until assayed. A total of 2.5 mL of reaction mixture containing 71 mmol/L pyrophosphate buffer (pH 8.7), 71 mmol/L semicarbazide, 20 mmol/L glycine, 0.96 mmol/L NAD^+, and ADH was prepared for each test sample. Ethanol standards were prepared by diluting the working standard, 0 to 100 μL, with cold saline. A 0.1 mL aliquot of supernatant from each centrifuged standard or sample was mixed with the reaction mixture and then incubated for 25 minutes in a 37°C water bath. After cooling to room temperature, the absorbance of each sample was determined by a spectrophotometer at 340 nm. The UEC was expressed as mg ethanol/mg creatinine, mg ethanol/mL urine, or mg total ethanol/collection period of time.

Determination of ADH activity. The modified method of Bergmeyer et al. (1974) was used to measure the activity of ADH. Each reaction was performed in a total volume of 3 mL at 37°C. The reaction mixture included 55 mmol/L pyrophosphate buffer (pH 7.4), 20 mmol/L ethanol, 2 mmol/L NAD^+, 1.1 mmol/L reduced glutathione, 55 mmol/L semicarbazide, 1.6 mmol/L glycine, and liver homogenate. The reaction mixture lacking ethanol was pre-incubated for 3 minutes at 37°C in a temperature controlled Beckman model 34 spectrophotometer. The reaction was initiated by adding ethanol and change in absorbance at 340 nm was recorded for 5 minutes. Each sample was read against a blank with distilled water replacing ethanol. The activity of ADH was expressed as nmol NADH per minute per mg protein.

Determination of CPT-I activity. CPT-I activity was estimated by the method of Bremer (1981). The mitochondrial suspension was adjusted to 10 mg protein/mL. Each assay mixture containing 82 mol/L sucrose, 70 mol/L KCl, 1 mol/L EGTA, 70 mol/L imidazole, 1 μg antimycin A, 2 mg BSA, 0.5 mol/L L-carnitine (0.4 mCi/mmol of L-[methyl-^{3}H] carnitine), 40 μmol/L palmitoyl CoA, and liver mitochondria in a total volume of 0.5 mL. For determination of malonyl CoA inhibition, the concentrations of malonyl CoA were 0, 10, 25, and 50 μmol/L. For estimation of the apparent Km of palmitoyl CoA, concentrations were 10, 50, 100, and 200 μmol/L and for the Km of carnitine, concentrations were 0.1, 0.5, 1, and 2 mmol/L. The CPT-I activity was expressed as nmol/min per mg protein.

Determination of carnitine. Plasma, urine, and tissue carnitine concentrations were determined by a radioisotopic method by Cederblad and Lindstedt (1972) as modified by Sachan et al. (1984). Total carnitine (TC) in plasma, urine, and tissue was determined by adding up measured free carnitine (NEC or nonesterified carnitine), acid soluble acylcarnitine (ASAC or short-chain carnitine), and acid insoluble acylcarnitine (AIAC or long-chain carnitine).

Carnitine standards were prepared by making 0 to 24 nmol/μL of carnitine concentrations. For each test sample, 20 to 100 μL of plasma, urine, and tissue homogenate was added to 200 μL of 0.6 mol/L perchloric acid (PCA) contained in a test tube. Enough distilled water was added to make a desired final volume of 400 μL. All vortexed standards and samples were centrifuged at 1500 x g for 10 minutes at 4°C. The 1500 x g supernatant was immediately separated from the pellet. Aliquots of 150 μL and 100 μL of PCA-supernatant were transferred to phenol red tubes for NEC and ASAC

determinations, respectively. The supernatant liquids were completely drained off from the tubes containing the pellets. The pellets were used for AIAC determination.

The PCA-supernatant of each NEC tube was mixed with about 50 μL of 1 mol/L $KHCO_3$ and then placed on ice for 30 minutes. PCA-supernatant of each ASAC tube was hydrolyzed by adding 100 μL of 0.5 mol/L KOH, incubated in a shaking water bath at 37°C for 30 minutes, neutralized with 25 μL PCA-MOPS-II solution, and then placed on ice for 30 minutes. The pellet of each AIAC tube was washed twice with 200 μL of 0.6 mol/L PCA and inverted to drain. A drop of phenol red indicator was added to the AIAC tube. The pellet was then hydrolyzed by adding 200 μL of 0.5 mol/L KOH, incubated in a water bath at 65°C for 60 minutes, neutralized with 50 μL PCA-MOPS-I solution, and placed on ice for 30 minutes.

The NEC, ASAC, and AIAC tubes were centrifuged at 1500 x g for 10 minutes at 4°C. A 100 μL aliquot of each of the three fractions was transferred to a microcentrifuge tube and 400 μL of reagent mixture, including 1 mol/L MOPS, 0.1 mol/L EGTA, 0.1 mol/L $Na_2S_4O_6$, 0.1 mol/L ^{14}C-acetyl CoA solution (^{14}C-acetyl CoA and acetyl-CoA, 2:1 v/v), and distilled water was added. Carnitine acetyltransferase (CAT, 20 μL, 1 unit) was then added. Each tube was capped, gently mixed by tapping, and placed in a shaking incubator water bath at 37°C for 30 minutes. At the end of the incubation period, 200 μL of incubation mixture was transferred onto a mini column filled with anion exchange resin. The column was then rinsed down with 2 aliquots of 500 μL distilled water into a scintillation vial. Five mL of scintillation fluid was added to each scintillation vial and the vial was capped and mixed by swirling . The radioactivity of all vials was determined in a Beckman LS-3801 scintillation counter. Inverse slope of standards was applied to

determine the μmol carnitine per L plasma, μmol carnitine per 24-hour urine, or μmol carnitine per g of tissue.

Determination of creatinine. Creatinine assays in urine were performed by the Jaffe reaction according to the methods of Taussky (1954) as modified by Henry (1967). Three mL of a dilute urine sample was mixed with 2 mL of alkaline-picrate solution (0.04 mol/L picric acid and 0.75 mol/L NaOH, 1:1, v/v). A series of standard were made using 7.5 to 45 μg of creatinine. After standing at room temperature for 20 minutes, the absorbance was measured at 520 nm against a water blank. The creatinine levels were expressed as μg creatinine/mL urine.

Statistics. All data were expressed as the group means ± SEM. The statistical significance was evaluated by student's t test when there were 2 dietary FA groups. ANOVA was used to detect the statistical significance among 3 dietary FA groups and Duncan's multiple range test was used for multiple comparisons. The level of statistical significance was set at $P < 0.05$. All statistical analyses were performed using SAS for Unix system (version 6, SAS Institute, Cary, NC).

RESULTS

ADH and CPT-I activities. In Experiment 1, hepatic ADH and mitochondrial CPT-I activities were not significantly different among the 3 dietary groups after 6 weeks of dietary treatment, although there was a trend that the TFA group had lower activities of ADH and CPT-I than the SFA and C FA groups (**Table 4-1**). In Experiment 2, hepatic ADH activity was not significantly different between the 2 dietary groups after 2 weeks of feeding (**Table 4-2**).

TABLE 4-1

Activities of hepatic alcohol dehydrogenase (ADH) and mitochondrial carnitine palmitoyl transferase (CPT-1) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Parameter	Dietary group		
	SFA	CFA	TFA
Hepatic ADH (nmol/min/mg)	11.9 ± 1.4[a]	12.0 ± 1.4[a]	11.3 ± 1.2[a]
Mitochondrial CPT-1 (nmol/min/mg)	4.4 ± 0.3[a]	4.9 ± 0.3[a]	4.2 ± 0.3[a]

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; ADH, alcohol dehydrogenase; CPT-I, carnitine palmitoyl transferase-I.

Urinary ethanol concentrations (UEC). The UEC are expressed in 3 units: mg ethanol/mg creatinine, mg ethanol/mL urine, or mg ethanol/collection period (**Figure 4-1**). There was no significant difference in the UEC between the 2 dietary groups regardless the units of expression. The numerical UEC values are presented in **Appendix I, Tables I-4 to I-7**.

Plasma, urinary, and tissue carnitine. In Experiment 1, plasma concentrations of ASAC and AIAC were significantly higher ($P < 0.05$ and $P < 0.01$, respectively) in the SFA and CFA groups than the TFA group after 2 weeks of dietary treatment (**Figure 4-2B**). However, there was no significant difference for NEC and TC concentrations among the 3 dietary groups. Similar trends of plasma carnitine concentrations were found after 4 and 6 weeks of feeding. However, significant differences were observed in

TABLE 4-2

Activity of hepatic alcohol dehydrogenase (ADH) in rats fed cis or trans fatty acids diet for 2 weeks from Experiment 2 [1,2]

Parameter	Dietary group	
	CFA	TFA
Hepatic ADH activity (nmol/min/mg)		
no EtOH[a]	20.3 ± 0.7[a]	19.8 ± 1.9[a]
with EtOH[a]	16.9 ± 1.3[a]	19.9 ± 1.2[a]
Combined	18.6 ± 0.8[a]	19.9 ± 1.1[a]

[1] Values are group means ± SEM (n=12), except for those with or without ethanol treatment (n = 6), and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid; ADH, alcohol dehydrogenase; EtOH, ethanol.

[a] With EtOH, animals received an oral dose (3 g/kg body weight) of ethanol (13%, v/v) 2 hours before being killed; no EtOH, animals received no ethanol before being killed.

AIAC after 4 weeks ($P < 0.04$) (**Figure 4-2C**) and ASAC after 6 weeks ($P < 0.02$) (**Figure 4-2D**). There was no difference in urine carnitine concentrations at any of the collection periods (**Figure 4-3**). It appeared that both plasma and urinary carnitine concentrations were increased with the age of the rats (**Figures 4-2 and 4-3**). The plasma and urinary carnitine concentrations are presented in **Appendix II, Tables II-1 and II-2**. The plasma carnitine concentrations in rats after 2 weeks of dietary treatment from Experiment 2 are presented in **Table 4-3**. Although the different fractions of carnitine concentrations were lower in the TFA group than in the CFA group, the differences were not statistically significant.

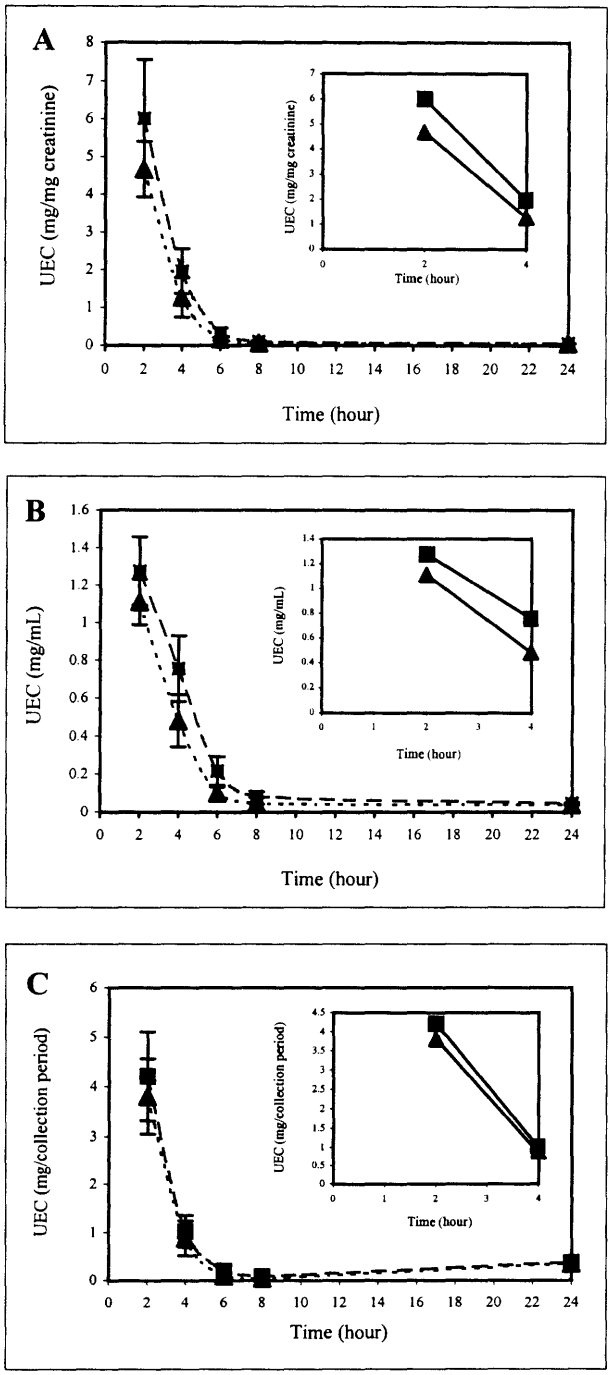


Figure 4-1 Time-dependent changes of urinary ethanol concentrations (UEC) expressed as mg ethanol/mg creatinine (A), mg ethanol/mL urine (B), and mg ethanol/collection period in rats fed cis (■) or trans (▲) fatty acid diet for 2 weeks from Experiment 2. All values are the group means ± SEM (n = 12).

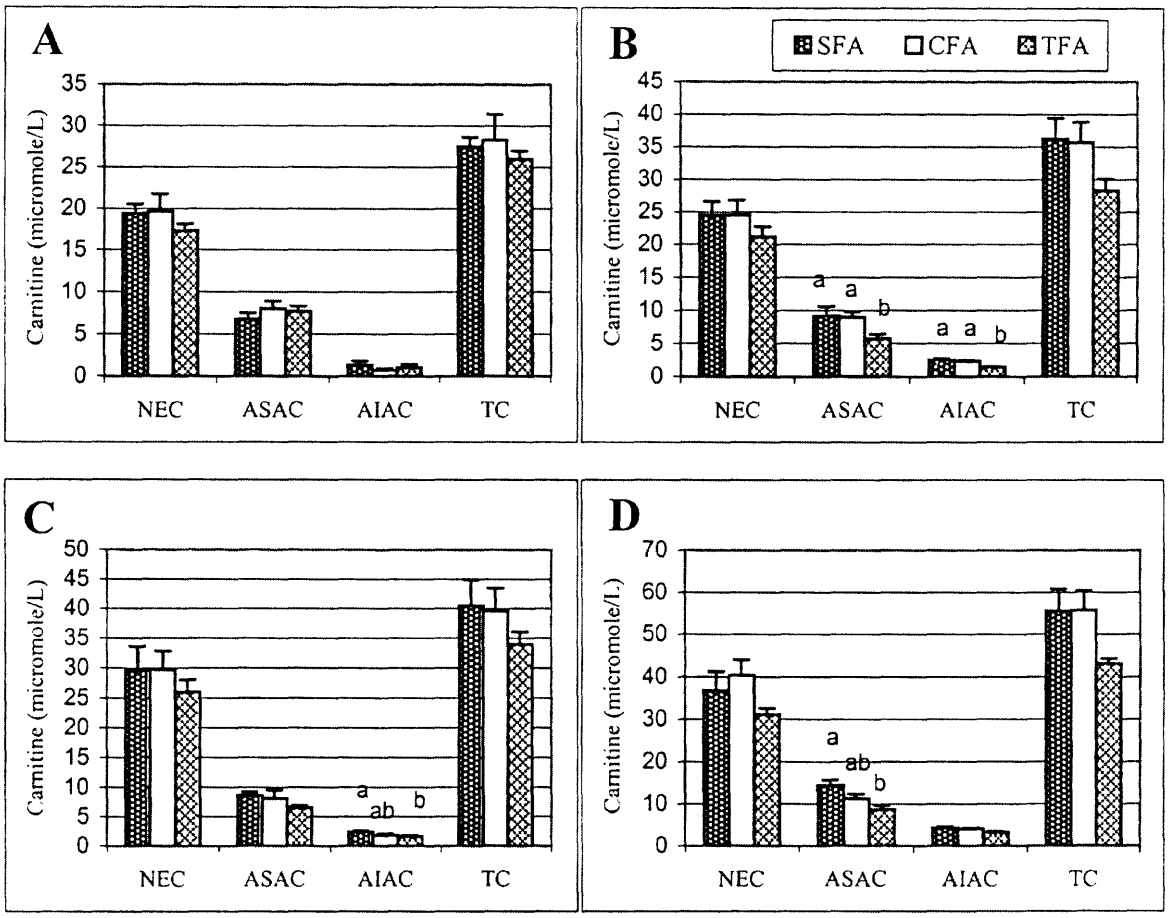


FIGURE 4-2 Plasma concentrations of carnitine (μmol/L) in rats fed saturated, cis, or trans fatty acids diet for 0 (A), 2 (B), 4 (C), and 6 (D) weeks from Experiment 1. SFA = saturated fatty acid, CFA = cis fatty acid, TFA = trans fatty acid, NEC = non-esterified carnitine, ASAC = acid-soluble acyl carnitine, AIAC = acid-insoluble acyl carnitine, TC = total carnitine. All values are the group means ± SEM (n = 6). Different letters indicate significant differences by ANOVA and Duncan's tests at $P < 0.05$.

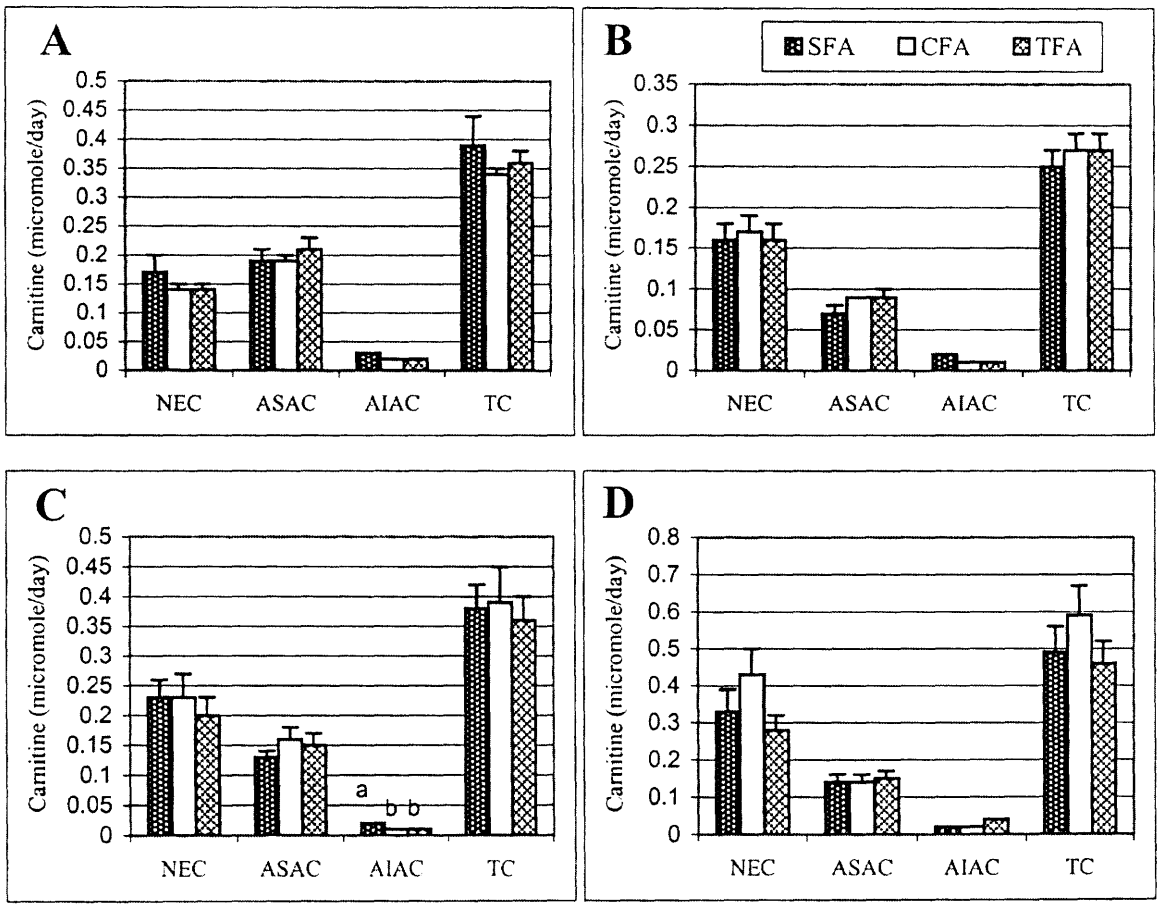


FIGURE 4-3 Urinary excretion carnitine (μmol/day) in rats fed saturated, cis, or trans fatty acids diet for 0 (A), 2 (B), 4 (C), and 6 (D) weeks from Experiment 1. SFA = saturated fatty acids, CFA = cis fatty acids, TFA = trans fatty acid, NEC = non-esterified carnitine, ASAC = acid-soluble acyl carnitine, AIAC = acid-insoluble acyl carnitine, TC = total carnitine. All values are the group means ± SEM (n = 6). Different letters indicate significant differences by ANOVA and Duncan's tests at $P < 0.05$.

TABLE 4-3

Plasma carnitine concentrations (μmol/L) in rats fed cis or trans fatty acids diet for 2 weeks from Experiment 2 [1,2]

	Dietary group	
	CFA	TFA
No EtOH[a]:		
NEC	24.6 ± 1.2[a]	23.5 ± 1.7[a]
ASAC	7.8 ± 0.7[a]	7.7 ± 1.1[a]
AIAC	1.7 ± 0.3[a]	1.3 ± 0.6[a]
TC	34.2 ± 1.2[a]	32.7 ± 2.4[a]
With EtOH[a]:		
NEC	20.6 ± 1.4[a]	19.6 ± 0.7[a]
ASAC	11.2 ± 1.0[a]	8.6 ± 1.2[a]
AIAC	1.8 ± 0.5[a]	1.7 ± 0.4[a]
TC	33.6 ± 1.2[a]	29.9 ± 1.2[a]
Combined:		
NEC	22.6 ± 1.1[a]	21.6 ± 1.0[a]
ASAC	9.5 ± 0.8[a]	8.3 ± 0.8[a]
AIAC	1.8 ± 0.3[a]	1.6 ± 0.4[a]
TC	33.9 ± 0.8[a]	31.3 ± 1.4[a]

[1] Values are group means ± SEM (n = 12), except for those with or without ethanol treatment (n = 6), and those with the same superscript letters in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid; NEC, nonesterified carnitine; ASAC, acid-soluble acylcarnitine; AIAC, acid-insoluble acylcarnitine; TC, total carnitine.

[a] With ethanol, animals received an oral dose (3 g/kg body weight) of ethanol (13%, v/v) 2 hours before being killed; no ethanol, animals received no ethanol before being killed.

The distributions of the various fractions of carnitine in the brain, heart, liver, and small intestine were affected by dietary fats in Experiment 1 (**Table 4-4**). In the brain samples, the NEC and AIAC concentrations were significantly higher in the SFA group ($P < 0.03$ and $P < 0.0001$, respectively), while the ASAC concentrations were significantly lower in SFA group than the TFA group ($P < 0.02$). There was no significant change between the CFA and TFA groups. In the heart samples, the SFA group also had significantly higher NEC concentrations than the CFA and TFA groups ($P < 0.0001$); however, the concentrations of other carnitine fractions was not significantly different among the 3 dietary groups. The TFA group had significantly higher liver ASAC levels than the CFA group ($P < 0.0009$) and significantly lower liver AIAC levels than the other 2 groups ($P < 0.05$). No statistical difference was found in the muscle carnitine concentrations. In the small intestine samples, the NEC levels were significantly higher in the SFA group than in the CFA and TFA groups ($P < 0.0001$), while the AIAC levels were higher in the CFA group than in the TFA group ($P < 0.03$).

DISCUSSION

In the previous study as described in Chapter 3, rats fed the TFA diet for 2 weeks had consistently lower blood ethanol concentrations (BEC) compared to those fed the SFA or CFA diets after an acute ethanol administration (3 g/kg body weight). The changes in BEC modulated by dietary fatty acids may arise from changes in ethanol metabolism. However, it was also possible that CFA and TFA differently altered the speed of ethanol transport or the movement of ethanol in the digestive tract rather than its metabolism after absorption.. Therefore, in the present study, the activities of ADH and

TABLE 4-4

Tissue carnitine concentrations (nmol/g) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

	Dietary group		
	SFA	CFA	TFA
Brain:			
NEC	77.7 ± 5.7[a]	66.2 ± 3.9[ab]	59.0 ± 2.5[b]
ASAC	6.7 ± 1.2[b]	12.8 ± 1.4[a]	15.8 ± 2.8[a]
AIAC	13.2 ± 1.1[a]	6.0 ± 0.5[b]	7.6 ± 1.0[b]
TC	97.6 ± 6.1[a]	85.1 ± 4.5[a]	82.3 ± 2.9[a]
Heart:			
NEC	727.2 ± 25.9[a]	594.6 ± 24.6[b]	534.8 ± 21.4[b]
ASAC	280.9 ± 30.6[a]	323.4 ± 20.4[a]	316.5 ± 21.3[a]
AIAC	11.9 ± 3.2[a]	11.1 ± 2.1[a]	11.6 ± 2.9[a]
TC	986.8 ± 36.2[a]	929.1 ± 29.8[a]	862.9 ± 20.1[a]
Liver:			
NEC	148.3 ± 12.9[a]	146.8 ± 3.6[a]	125.1 ± 7.5[a]
ASAC	7.5 ± 1.7[b]	9.1 ± 0.8[b]	19.3 ± 2.7[a]
AIAC	3.8 ± 0.5[ab]	5.0 ± 0.1[a]	3.5 ± 0.5[b]
TC	159.6 ± 13.8[a]	161.0 ± 4.0[a]	147.9 ± 10.2[a]
Skeletal muscle:			
NEC	474.1 ± 20.2[a]	423.9 ± 41.4[a]	391.5 ± 27.6[a]
ASAC	192.2 ± 17.6[a]	177.9 ± 18.8[a]	188.4 ± 17.8[a]
AIAC	44.2 ± 6.9[a]	38.0 ± 7.2[a]	29.0 ± 1.8[a]
TC	710.5 ± 29.8[a]	639.9 ± 50.9[a]	609.0 ± 24.0[a]

Continued

TABLE 4-4 (Continued)

Tissue carnitine concentrations (nmol/g) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

	Dietary group		
	SFA	CFA	TFA
Small intestine:			
NEC	122.7 ± 2.9[a]	113.3 ± 2.0[b]	101.6 ± 2.3[c]
ASAC	69.0 ± 6.4[a]	64.7 ± 4.0[a]	69.4 ± 2.9[a]
AIAC	7.0 ± 0.7[ab]	8.0 ± 0.5[a]	5.4 ± 0.6[b]
TC	198.8 ± 8.5[a]	185.9 ± 5.1[ab]	176.4 ± 1.3[b]

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; NEC, nonesterified carnitine; ASAC, acid-soluble acylcarnitine; AIAC, acid-insoluble acylcarnitine; TC, total carnitine.

CPT-I as well as the concentrations of carnitine were determined to provide a mechanistic explanations for the differences in BEC.

In general, ethanol is almost completely metabolized by oxidative metabolism (Li 1977). Under physiological conditions, ADH is the major enzyme catalyzing the oxidation of ethanol to acetaldehyde in the liver (Lieber 1994). The changes in ethanol metabolism parallel alterations in enzyme activity. For example, Sachan and Mynatt (1993) showed in rats that a lysine-deficient wheat gluten diet delayed ethanol metabolism and produced higher BEC than did a casein-based diet because of decreased hepatic ADH activity. In Experiment 1, the hepatic ADH activity was slightly but not

significantly lower in the TFA group than in the SFA and CFA groups after 6 weeks of feeding (**Table 4-1**). However, it is important to remember that the ADH activity was measured after 6 weeks of dietary treatment and the ethanol clearance was not different among the 3 dietary groups after 4 weeks of feeding. In Experiment 2, the activity of liver ADH was not statistically different after 2 weeks of feeding (**Table 4-2**). Analogous results were reported by Cha and Sachan (1994) that hepatic ADH activities were identical between the SFA and USFA groups. Therefore, the changes in BEC could not be explained by the changes in hepatic ADH activity. Other metabolisms of ethanol such as the extrahepatic ADH activities and non-ADH dependent ethanol oxidation may explain the changes in BEC.

The presence of ADH activity in the gastrointestinal tract is well established. Mistilis and Garske (1969) have clearly showed that the activity of ADH in the stomach and small intestine significantly increased with administration of single or repeated doses of alcohol and the effect lasted up to 72 hours. Carter and Isselbacher (1971) reported that, in male rats, the ADH specific activity of the gastric slices (0.48 μmol/hour/mg protein) was about 3 times higher than in the liver slices (0.11 μmol/hour/mg protein), while that of the small intestinal slices (0.15 μmol/hour/mg protein) was comparable to that of the liver slices. In 1985, Julkunen et al. (1985a) demonstrated that the BEC was lower in rats after intragastric compared to intravenous ethanol administration, which suggested the incomplete bioavailability of orally ingested alcohol and the existence of first-pass metabolism of ethanol. Julkunen's group (1985b) again reported that the AUC of BEC was significantly lower after intragastric administration than after administration via intraduodenal, intravenous, and intraportal routes and thus the stomach was most

likely the site of first-pass metabolism of ethanol. The metabolism of ethanol in the digestive tract will cause a decline in systemic bioavailability of ethanol (first-pass metabolism) and may explain some of the differences in BEC observed with various diets. The determination of ADH activities in the small intestine and stomach was intended in Experiment 2. However, the assay was not performed due to the difficulties in preparing the homogenates and subcellular fractions.

The rate of gastric emptying is one of the factors that influence the absorption and first-pass metabolism of ethanol (Kricka and Clark 1979, Seitz and Oneta 1998). A delayed gastric emptying rate leads to prolonged exposure of alcohol to gastric ADH and results in an increased gastric metabolism of alcohol. Delayed gastric emptying would also stall the delivery of ethanol to the small intestine. A smaller rate of ethanol absorption from the small intestine could optimize hepatic metabolism of alcohol and could contribute to an increased hepatic first-pass metabolism of ethanol (Pedrosa et al. 1996). Delayed gastric emptying has been shown in the fed versus fasted state which was associated with an increased first-pass metabolism (Di Padova et al. 1987). No food was taken away from the animals before they were killed in the present study so as to preserve maximal ADH activity (Lumeng et al. 1979). Therefore, the BEC may have been indeed modulated by metabolism in the stomachs and possibly in the small intestines of the TFA group more than the SFA or CFA group.

The microsomal ethanol oxidizing system (MEOS) is induced following chronic and/or highly concentrated alcohol intake. The ethanol-inducible cytochrome P450 2E1 (CYP2E1) is a major component of the MEOS (Lieber 1994). Studies have shown that the level of CYP2E1 was modulated by the quantity and degree of saturation of dietary

lipids without administration of ethanol (Yoo et al. 1990;1991;1992). Microsomes from rats fed a diet containing 20 wt% corn oil or an amount of linoleic acid equivalent to the 20% corn oil diet showed 2- to 3-fold increases in the level of CYP2E1 protein and its catalytic activity over those from the rats fed the fat-free diet (Yoo et al. 1990;1991; 1992). Furthermore, at moderate fat levels (5 and 20 wt%), rats fed corn oil and menhaden oil diets produced significantly higher ($P < 0.05$) CYP2E1 activity than those fed lard and olive oil diets (Yoo et al. 1991). In the intragastric feeding rat model (chronic ethanol treatment), the extent of CYP2E1 was also dependent on the type of dietary fat (Nanji et al. 1994b;1994c, Takashi et al. 1992). Rats fed corn oil and ethanol had 2- to 6-fold higher levels of CYP2E1 protein and enzyme activity compared with animals fed saturated fat and ethanol (Nanji et al. 1994b, Takashi et al. 1992). The CYP2E1 protein level in rats fed fish oil and ethanol was even higher ($P < 0.01$) than that in rats fed corn oil and ethanol (Nanji et al. 1994c). However, chronic ethanol ingestion is not required for induction of CYP2E1; significant increases in this enzyme can be observed after a single dose of ethanol (Koop 1992). Therefore, although the present study was designed to study the acute ethanol treatment, CYP2E1 might be induced by the administration of a single dose of ethanol after 2 weeks of dietary treatment. Whether the dietary fat compositions in the present study can influence CYP2E1 is unknown and needs to be investigated.

The excretion of ethanol from the urine must be considered as another possible factor which may affect the BEC. Thus, the time-dependent changes in UEC were determined in Experiment 2. There was no difference in the UEC between the 2 dietary groups (**Figure 4-1**). However, as the slopes between UEC (mg/mL) at 2 and 4 hours

were calculated by the simple linear regression, the TFA group had a steeper slope (17% higher) than the CFA group (-0.63 and -0.52, respectively) (**Figure 4-1B**). Therefore, the urinary excretion of ethanol was involved in the dietary fatty acids related changes in the BEC; it was faster in the TFA group than in the CFA group.

It has been shown that dietary carnitine raised BEC in a dose-dependent manner following both acute and chronic treatment of ethanol in rats (Berger and Sachan 1986;1991, Sachan 1992, Sachan and Berger 1987). Carnitine also delayed ethanol oxidation and thus expanded the half-life of ethanol in broilers (Smith et al. 1994). In the isolated rat hepatocytes, the rate of ethanol oxidation was reduced by acetylcarnitine and L-carnitine as compared to that without carnitine (Cha and Sachan 1995). All these results clearly indicated that carnitine retarded ethanol metabolism. Furthermore, the BEC was positively related to plasma NEC and TC of rats fed the SFA and USFA diets as reported by Cha and Sachan (1994). In Experiment 1 of the present study, the TFA group had significantly lower concentrations of plasma ASAC ($P < 0.05$) and AIAC ($P < 0.01$) than the SFA and CFA groups after 2 weeks of dietary treatment (**Figure 4-2B**). Plasma AIAC concentrations remained significantly lower ($P < 0.04$) in the trans FA group than in the SFA group after 4 weeks of feeding (**Figure 4-2C**). After 6 weeks of feeding, plasma ASAC concentration was significantly lower ($P < 0.02$) in the TFA group than in the SFA group (**Figure 4-2D**). In Experiment 2, plasma carnitine concentrations were lower in the TFA group than in the CFA group (**Table 4-3**), which was consistent with the earlier results. Therefore, faster rate of ethanol metabolism in the TFA group and relatively slower rates of ethanol metabolism in the SFA and CFA groups may be mediated at least in part through carnitine.

In addition to the liver, the measured tissue carnitine concentrations were within the reported range of tissue carnitine concentrations in rats (Bøhmer and Mølstad 1980). The low carnitine concentrations of the liver might be because the liver was perfused; therefore, the liver carnitine content was not contaminated with plasma carnitine. The liver carnitine content might also be diluted by extra perfused solution (saline) retained in the liver. Data of tissue carnitine concentrations (**Table 4-4**) indicate that the changes in carnitine created by dietary fats were not consistent. Except for the liver and skeletal muscle, the NEC concentrations were significantly elevated with the increased degree of saturation of dietary fats. The ASAC concentrations in the brain decreased with increased degree of saturation of dietary fats. However, in the liver, the ASAC concentrations was affected by the geometry of dietary fatty acids; the concentrations of ASAC were significantly higher in the TFA group than in the SFA and CFA groups ($P < 0.001$). The AIAC concentrations were significantly increased in the brain ($P < 0.0001$) of rats fed the SFA diet but were significantly lowered in the liver ($P < 0.05$) and small intestine ($P < 0.03$) of rats fed the TFA diet. Only the TC concentrations of the small intestine were significantly modulated by the dietary lipids; it was higher in the SFA group than in the TFA group ($P < 0.05$). The relation of the changes in tissue carnitine distributions to ethanol metabolism is not clear. Since the liver is the primary organ of ethanol metabolism, the changes in hepatic carnitine may influence ethanol metabolism. However, the liver carnitine profile was opposite of that seen in plasma, especially with regard to the ASAC concentrations. The ASAC concentrations in the liver or plasma may not be equated with acetyl carnitine even though acetyl carnitine is part of ASAC. Therefore, determination of acetyl carnitine is one of the logical ways to assess the role of

endogenous acetyl carnitine and relate it to ethanol metabolism.

CPT-I, the outer form of carnitine palmitoyltransferase, is regarded as a key enzyme regulating hepatic and extrahepatic mitochondrial FA oxidation (Saggerson 1986, Schulz 1991). The activity of hepatic CPT-I has been shown to be inhibited by both short-term and prolonged ethanol feeding in animal studies (Castro 1991, Guzmán and Geelen 1988, Guzmán et al. 1987). Consequently, CPT-I could play a critical role in the ethanol-induced inhibition of hepatic FA oxidation leading to triglyceride accumulation in the liver (Guzman et al. 1987). The CPT-I activity has been reported to be modulated by the dietary lipids. Feeding olive, safflower, and menhaden oils to rats resulted in higher CPT-I activities in the liver (Power et al. 1994), heart, and skeletal muscle (Power and Newsholme 1997) than feeding hydrogenated coconut oil. In the present study, hepatic CPT-I activity was not affected by the TFA diet (**Table 4-1**) which is in agreement with the results of other studies. Ide et al. (1987) reported that short- and long-term TFA (elaidic acid) feeding had only a marginal effect on the activities of liver mitochondrial fatty acid oxidation enzymes, including carnitine acyltransferase, in different strains of rats. In primary rat hepatocyte cultures, brassidic acid (trans C22:1) had no effect on CPT-I activities compared to erucic (cis C22:1) or oleic acids (Christiansen et al. 1985). Feeding conjugated linoleic acid, a positional and geometric isomer of conjugated dienoic derivatives of linoleic acid, had no effect on CPT-I activity in the liver of mice (Park et al. 1997). It has been noted that freezing/thawing of mitochondria inactivated CPT-I but not CPT-II, the carnitine palmitoyltransferase present in the inner mitochondrial membrane (McGarry and Brown 1997). In Experiment 1, the mitochondria were prepared freshly (not frozen) for CPT-I assay and thus the

measurement should be representative of the CPT-I activity.

In summary, the hepatic ADH activity was not significantly affected by the dietary fatty acids after 2 or 6 weeks of dietary treatment. Therefore, the dietary fatty acids related changes in BEC must be explained by other mechanisms like MEOS and by the extrahepatic ADH activity. The TFA group appeared to eliminate more ethanol per mL of urine than the CFA group during the first 4 hours after ethanol administration and may partially be the reason for the differences in BEC. Further, the parallel changes in the BEC and plasma carnitines may also contributed to higher BEC in rats fed the SFA and CFA diets compared to those fed the TFA diet.

CHAPTER V

EFFECTS OF SATURATED, CIS, AND TRANS FATTY ACIDS ON INTESTINAL UPTAKE AND METABOLISM OF ETHANOL IN RATS

ABSTRACT

The rats fed trans fatty acid (TFA) for 2 weeks maintained lower blood ethanol than those fed saturated fatty acids (SFA) or cis fatty acids (CFA) which may have been due to differences in the absorption of ethanol from the gastrointestinal tract. This experiments was designed to determine the differences in ethanol uptake and metabolism in the small intestines of 18 male Sprague-Dawley rats fed dietary SFA, CFA, or TFA. The uptake and/or metabolism of ethanol was measured in everted intestinal sacs. Ethanol transport expressed as the ratio of serosal to mucosal ethanol concentrations and the *in vitro* absorption rate of ethanol was not significantly affected by dietary fatty acids. However, when the amount of ethanol metabolized to CO_2 during the transport process was taken into account, uptake and metabolism of ethanol by the proximal segments of the small intestine were significantly higher in the rats fed the TFA and CFA than in those fed SFA. Because ethanol oxidation was not significantly different between the CFA and TFA group, some other mechanism must be responsible for the differences in BEC of animals fed CFA and TFA. Nonetheless, it is recognized that ethanol metabolism in extrahepatic tissues can not be ignored due to the effects of SFA and unsaturated fatty acids on ethanol pharmacokinetics.

INTRODUCTION

The manipulation of dietary fatty acids alters the fatty acid composition of the intestinal membranes and thus influences the absorption of ingested nutrients (Thomson and Rajotte 1983, Thomson et al. 1986). Sugano et al. (1984) found that the cholesterol absorption rate was markedly reduced in the enterocytes of rats fed trans fat than in those fed cis fat. Manteca et al. (1994) have shown an increased intestinal uptake of cholesterol in rats fed a diet supplemented with elaidic acid. However, Thomson et al. (1994) determined that feeding TFA to rats for 2 weeks had no influence on the jejunal or ileal uptake of glucose, fatty acids (lauric, stearic, oleic, linoleic, and linolenic acids), and cholesterol. In addition, it was noticed that SFA, compared to polyunsaturated fatty acids (PUFA) prevented the acute ethanol-induced inhibition of the jejunal and ileal uptake of hexoses and lipids in rats (Thomson et al. 1991). These reports and the slower rate of ethanol absorption in rats fed the TFA diet observed earlier (Chapter 3), added strength to the hypothesis that ethanol transport and/or metabolism may be modulated at the small intestinal level in these animals. A search of the literature revealed that there is no data on the response of the small intestine to TFA feeding regarding ethanol uptake and metabolism in rats. Thus, the objective of this study was to determine the uptake and metabolism of ethanol in the small intestine of rats fed SFA, CFA, or TFA diets for 2 weeks using the *in vitro* everted intestinal sacs.

MATERIALS AND METHODS

Animals. The experimental protocol was approved by the University of Tennessee Committee on Humane Care and Use of Laboratory Animals. Male Sprague-Dawley rats

of approximately 4 weeks of age were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN). Each rat was individually housed in a stainless steel wire bottom cage in a temperature controlled room (23 ± 2 °C) with a 12-hour light-dark cycle. After 1 week of acclimation with Purina Rodent chow diet (Ralston, Purina Co., St. Louis, MO), rats were randomly assigned to different groups for 2 weeks of dietary treatment. All animals were allowed free access to food and water. When urine samples were collected, the rats were placed in the individual metabolic cages for 24 hours. Food consumption was recorded daily and body weight was monitored weekly for each animal.

Diet. Rats were fed modified AIN[76] diets enriched with 10% (w/w) SFA, CFA, or TFA (**Table 5-1**). Various edible fats and oils and one fatty acid were mixed to generate the diets of comparable fatty acid composition. The fat sources of the SFA and CFA diets consisted of butter, corn oil, and olive oil. Margarine, free elaidic acid, and butter were used as the fat sources of the TFA diet. The dietary fats were adjusted to have a similar level of total poly-unsaturated fatty acids (PUFA). **Table 5-2** presents the fatty acid compositions of the different diets as determined in a Hewlet-Packard (Model 5890 series II) chromatograph equipped with a flame ionization detector and a DB23 fused silica capillary column (0.25 mm I.D. x 30 m x 0.25 μ film, J & W Scientific, Folsom, CA). All diets were prepared weekly and stored at -20 °C. The fresh diets were given every morning and leftovers were discarded.

Experimental design. The 18 rats were randomly assigned to one of the 3 diets (n = 6): SFA, CFA, or TFA and fed the respective diets for 2 weeks. The food was withheld overnight prior to their being killed by cervical dislocation. The small intestine was processed immediately to determine the uptake and metabolism of ethanol by the everted

TABLE 5-1

Composition of the experimental diets[1]

Ingredient	Diet		
	SFA	CFA	TFA
		g/kg	
Casein	200	200	200
DL-Methionine	3	3	3
Corn starch	150	150	150
Sucrose	450	450	450
Cellulose	50	50	50
Vitamin mix	10	10	10
Mineral mix	35	35	35
Corn oil[a]	58	52	-
Olive oil[a]	5	37	-
Butter[2a]	47.1	14	1.27
Margarine[3a]	-	-	110.73
Elaidic acid[a]	-	-	12
Choline bitartrate	2	2	2

[1] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid.

[2] Butter: 113g of butter contains 92g of fat; therefore, there is about 81% fat in butter.

[3] Margarine: 113 g of margarine contains 91g of fat; therefore, there is about 80% fat in margarine.

[a] Differences presented in fat sources are due to their compositions of fatty acids which are balanced between the three experimental diets.

TABLE 5-2

Fatty acid composition of the experimental diets (mole%) [1,2]

	Dietary group		
Fatty acid	SFA	CFA	TFA
C10:0	0.8	0.2	0.0
C12:0	1.2	0.4	0.0
C14:0	4.3	1.3	0.2
C16:0	19.5	14.0	12.2
C16:1	0.6	0.5	0.1
C18:0	7.3	4.0	7.4
cis C18:1 (n-9)	29.3	44.5	26.1
trans C18:1 (n-9)	0.1	0.1	19.2
C18:2 (n-6)	34.5	33.1	33.1
C18:3 (n-3)	0.8	0.9	0.7
Σ SFA	34.0	20.6	20.6
Σ MUFA	30.4	45.4	45.5
Σ PUFA	35.6	34.0	33.9
P/S	1.0	1.7	1.7
S/M/P	1/0.9/1	1/2.2/1.7	1/2.2/1.7

[1] Diets were analyzed for fatty acid compositions in a Hewlet-Packard Model 5890 series II gas chromatograph equipped with a flame ionization detector and a DB23 fused silica capillary column (0.25 mm I.D. x 30 m x 0.25 μ film, J & W Scientific, Folsom, CA).

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; P/S, PUFA:SFA ratio; S/M/P, SFA:MUFA:PUFA ratio.

sacs of small intestine.

Preparation of everted intestine segments. The everted intestinal sac was prepared according to the procedure described by Wilson and Wiseman (1954). After the animal was killed by cervical dislocation, the abdomen was opened and the entire small intestine was washed out with cold saline. The whole small intestine was removed by cutting across the beginning of duodenum and the end of ileum. The mesentery was manually stripped from the intestine and the intestine was rinsed gently with 10 to 20 mL of cold saline. To evert the entire gut, one end of the entire intestinal segment was tied with cotton thread. A plastic rod was then used to push the tied end of the gut into the gut lumen until it appeared at the opening of the intestinal segment. The eversion was completed by rolling the whole intestinal segment on the rod. The everted intestine was then slipped off the rod, cut into 4 (2 proximal and 2 distal) segments, and placed in cold saline at room temperature in a flat porcelain dish.

Determination of ethanol uptake by everted intestinal sacs. Two proximal and 2 distal small intestinal sacs, approximately 6 cm in length, were prepared from each animal. Using a blunt needle, 1 mL of oxygenated Krebs-Ringer bicarbonate (KRB) buffer containing 0.3% glucose (serosal medium) was injected into the intestinal segment with one end tied. The open end of the intestinal segment was then closed off with a ligature. The everted sacs were then placed in a 25 mL Erlenmeyer flask containing 5 mL KRB buffer with 0.3% glucose and 1-^{14}C ethanol (specific activity, 10932 cpm/mmol) or unlabeled ethanol (mucosal medium). The flask and its content were flushed with a mixture of O_2:CO_2 (95:5), sealed with rubber stopper, and incubated at 37°C for 20 minutes in a Dubnoff metabolic shaking incubator at 96 cycles/minute. At the end of the

incubation period, the sacs were removed and the serosal medium was drained into a test tube. The radioactivities of ^{14}C from serosal media, mucosal media, and intestinal sac homogenates were determined in a Beckman LS-3801 liquid scintillation counter. The concentrations of ethanol of the mucosal or serosal medium were also assayed by the modified enzymatic method of Bernt and Gutmann (1974) as described in the materials and methods section of Chapter 3. The ratio of ^{14}C radioactivities or the ethanol concentrations in the serosal and mucosal media were used as indices of uptake function.

Determination of $^{14}CO_2$ in mucosal media. Determination of $^{14}CO_2$ content in mucosal medium was carried out in 25 mL Warburg flasks. Two mL of mucosal medium was used for the determination of CO_2 derived from ethanol. The center well contained 0.2 mL of 100% ethanolamine and the side arm contained 0.2 mL of 60% perchloric acid (PCA). The flask was sealed with a rubber stopper. Any possible ongoing ethanol oxidation was stopped by tipping PCA from the side arm into the mucosal medium. The flask and its contents were then incubated for 60 minutes at 37°C in a Dubnoff metabolic shaking water bath to allow CO_2 absorption into the ethanolamine. After the incubation, the flask was allowed to equilibrate in chilled ice water for 30 minutes. Ethanolamine was transferred from center well to a scintillation vial. Residual ethanolamine was washed 3 times with scintillation fluid (200 μL). After adding 4 mL of scintillation fluid to the vial, the radioactivity was counted in a Beckman LS 3801 liquid scintillation counter.

Statistics. All data were expressed as the group means ± SEM. The statistical significance was evaluated by ANOVA among 3 dietary groups and Duncan's multiple range test was used for multiple comparisons. All statistical analyses were performed

using SAS for Unix system (version 6, SAS Institute, Cary, NC) and the significance level was set at $P < 0.05$.

RESULTS

Weight gain and food intake. There were no differences in the weight gain and food intake among the 3 dietary groups (**Table 5-3**).

Ethanol uptake and/or metabolism by everted intestinal sacs. The effect of different dietary fatty acids on the intestinal uptake and/or metabolism of ethanol carried out in the everted intestinal sacs model is presented in **Tables 5-4 to 5-7.** Two methods were applied to measure the ethanol concentrations: ^{14}C radioactivity counting and the enzymatic method. At first, ethanol transport across everted intestinal sacs was expressed

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TABLE 5-3

Body weight and food intake in rats fed saturated, cis or trans fatty acids diets for 2 weeks [1,2]

Parameter	Dietary group		
	SFA	CFA	TFA
Initial body weight (g)	187.8 ± 6.4[a]	186.6 ± 5.7[a]	186.7 ± 6.1[a]
Weight gain (g)	65.2 ± 3.0[a]	64.2 ± 7.3[a]	70.7 ± 5.4[a]
Food intake (g)	219.8 ± 7.0[a]	212.7 ± 10.7[a]	220.1 ± 4.6[a]

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid.

TABLE 5-4

Ethanol transport across everted intestinal sacs as determined by the specific activity of 1-^{14}C-ethanol in rats fed saturated, cis or trans fatty acids diet for 2 weeks [1,2]

Segment	Medium	Dietary group		
		SFA	CFA	TFA
		μmol ethanol/mg tissue/20 minutes		
Proximal	Serosal	3.53 ± 0.25	5.48 ± 0.52	5.01 ± 0.72
	Mucosal	22.16 ± 1.81	34.10 ± 1.73	35.04 ± 1.90
	Serosal/mucosal	0.16 ± 0.01[a]	0.16 ± 0.01[a]	0.14 ± 0.02[a]
Distal	Serosal	2.75 ± 0.52	5.02 ± 1.33	4.89 ± 0.78
	Mucosal	18.53 ± 2.69	36.06 ± 6.57	31.15 ± 4.28
	Serosal/mucosal	0.15 ± 0.01[a]	0.14 ± 0.02[a]	0.15 ± 0.01[a]
Proximal and distal	Serosal	3.14 ± 0.30	5.25 ± 0.69	4.95 ± 0.51
	Mucosal	20.34 ± 1.64	35.08 ± 3.25	33.10 ± 2.31
	Serosal/mucosal	0.15 ± 0.01[a]	0.15 ± 0.01[a]	0.15 ± 0.01[a]

[1] Values are mean ± SEM (n = 3, 2 proximal and 2 distal segments from each rat) and those bearing the same superscript letters in a row are not significantly different at $P < 0.05$. Substrate used was 1-^{14}C-ethanol. Concentrations were determined by specific activity of ethanol (10932 cpm/mmol).

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid.

TABLE 5-5

Ethanol transport across everted intestinal sacs as determined by enzymatic ethanol assay in 3 rats fed saturated, cis or trans fatty acids diet for 2 weeks [1,2]

Segment	Medium	Dietary group		
		SFA	CFA	TFA
		μmol ethanol/mg tissue/20 min		
Proximal	Serosal	2.75 ± 0.42	4.32 ± 0.56	3.44 ± 0.52
	Mucosal	16.68 ± 2.08	24.47 ± 2.00	23.48 ± 1.58
	Serosal/mucosal	0.16 ± 0.01[a]	0.17 ± 0.01[a]	0.15 ± 0.02[a]
Distal	Serosal	2.11 ± 0.63	3.83 ± 1.24	3.64 ± 0.62
	Mucosal	12.50 ± 2.35	25.83 ± 5.90	21.91 ± 3.31
	Serosal/mucosal	0.16 ± 0.02[a]	0.15 ± 0.02[a]	0.16 ± 0.01[a]
Proximal and distal	Serosal	2.43 ± 0.37	4.07 ± 0.65	3.54 ± 0.38
	Mucosal	14.59 ± 1.62	25.15 ± 2.98	22.69 ± 1.76
	Serosal/mucosal	0.16 ± 0.01[a]	0.16 ± 0.01[a]	0.15 ± 0.01[a]

[1] Values are mean ± SEM (n = 3, 2 proximal and 2 distal segments from each rat) and those bearing the same superscript letters in a row are not significantly different at $P < 0.05$. Substrate used was 1-^{14}C-ethanol. Ethanol concentrations were determined by enzymatic method.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid.

TABLE 5-6

Ethanol transport across everted intestinal sacs as determined by enzymatic ethanol assay in 6 rats fed saturated, cis or trans fatty acids diet for 2 weeks [1,2]

Segment	Medium	Dietary group		
		SFA	CFA	TFA
		μmol ethanol/mg tissue/20 min		
Proximal	Serosal	4.00 ± 0.43	4.89 ± 0.35	4.16 ± 0.44
	Mucosal	21.12 ± 1.79	27.13 ± 1.38	27.66 ± 2.25
	Serosal/mucosal	0.18 ± 0.01[a]	0.18 ± 0.01[a]	0.15 ± 0.01[a]
Distal	Serosal	4.01 ± 0.68	4.68 ± 0.69	4.68 ± 0.65
	Mucosal	20.55 ± 3.02	31.16 ± 5.05	24.58 ± 2.00
	Serosal/mucosal	0.18 ± 0.01[a]	0.16 ± 0.02[a]	0.18 ± 0.01[a]
Proximal and distal	Serosal	4.00 ± 0.38	4.79 ± 0.38	4.42 ± 0.39
	Mucosal	20.85 ± 1.72	29.14 ± 2.59	26.12 ± 1.50
	Serosal/mucosal	0.18 ± 0.01[a]	0.17 ± 0.01[a]	0.17 ± 0.01[a]

[1] Values are mean ± SEM (n = 6, 2 proximal and 2 distal segments from each rat) and those bearing the same superscript letters in a row are not significantly different at $P < 0.05$. Half of the intestinal sacs were incubated with 1-^{14}C-ethanol and the other half in nonradioactive ethanol. Ethanol concentrations were determined by enzymatic method.

[2] Abbreviations used: SFA, saturated fatty acids; Cis FA, cis fatty acids; Trans FA, trans fatty acid.

TABLE 5-7

Ethanol uptake and metabolism by everted intestinal sacs in rat fed saturated, cis or trans fatty acids diet for 2 weeks[1,2]

Segment	Ethanol equivalent in	Dietary group		
		SFA	CFA	TFA
		μmol ethanol/mg tissue/20 min		
Proximal	Serosal medium	3.53 ± 0.25	5.48 ± 0.52	5.01 ± 0.72
	Intestinal sac	0.32 ± 0.08	0.38 ± 0.08	0.34 ± 0.06
	CO_2	1.05 ± 0.07	1.41 ± 0.10	1.32 ± 0.14
	Total	4.90 ± 0.29[b]	7.27 ± 0.50[a]	6.68 ± 0.80[a]
Distal	Serosal medium	2.75 ± 0.52	5.02 ± 1.33	4.89 ± 0.78
	Intestinal sac	0.44 ± 0.09	0.40 ± 0.10	0.42 ± 0.11
	CO_2	0.70 ± 0.09	1.49 ± 0.37	0.95 ± 0.19
	Total	3.89 ± 0.59[a]	6.91 ± 1.62[a]	6.26 ± 0.82[a]
Proximal and distal	Serosal medium	3.14 ± 0.30	5.25 ± 0.69	4.95 ± 0.51
	Intestinal sac	0.38 ± 0.06	0.39 ± 0.06	0.38 ± 0.06
	CO_2	0.87 ± 0.08	1.45 ± 0.18	1.14 ± 0.12
	Total	4.39 ± 0.35[b]	7.09 ± 0.81[a]	6.47 ± 0.55[a]

[1] Values are mean ± SEM (n = 3, 2 proximal and 2 distal segments from each rat) and those bearing the same superscript letters in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid.

as the ratio of the ethanol concentrations inside and outside the sacs. The ratios were not different in any of the segments or in the proximal plus distal segments as determined by either method (**Tables 5-4 and 5-5**). The ethanol concentrations determined by the enzymatic method (**Table 5-5**) were generally lower than those measured by radioactive counting (**Table 5-4**), but the ratios of ethanol concentrations were similar between the 2 methods. Therefore, the intestinal segments from 3 rats were incubated with 1-^{14}C ethanol and those of the remaining 3 rats were incubated with unlabeled ethanol in each dietary group. The ratios of ethanol concentrations assayed by the enzymatic method were then calculated for 6 rats from each dietary fatty acid group. The index of ethanol absorption was also similar among the 3 groups (**Table 5-6**).

The ethanol uptake and metabolism was also expressed as the sum of ethanol concentrations in the serosal medium and the intestinal sac plus the amount of ethanol metabolized to CO_2 (μmole ethanol/mg tissue within 20 minutes of incubation) (**Table 5-7**). Both the CFA and TFA groups had significantly higher ethanol concentrations than the SFA group in proximal ($P < 0.01$) and proximal plus distal segments ($P < 0.02$). No difference was found in the distal segments among the 3 dietary groups.

DISCUSSION

Generally, ethanol can be absorbed into the body through the whole GI tract from the mouth to the rectum, but the main sites for ethanol absorption are the duodenum and jejunum (Butt 1989). Everted sacs of rat small intestine were used to understand the mechanism of dietary fatty acid-mediated differences in blood ethanol clearance. The everted intestinal sac technique was developed by Wilson and Wiseman (1954) in which

only the mucosa is directly in contact with oxygenated incubation medium, while the serosal fluid is isolated inside the closed sac and thus can be sampled independently from the mucosal medium. Consequently, the ratio of the substrate concentration in the serosal medium (inside the sac) to the concentration of the substrate in the mucosal medium (outside) is used as an indicator of transport function.

In the present study, the ethanol concentrations were determined by the specific activity of the ^{14}C and by the enzymatic method. The results indicated that ethanol concentrations were comparable between the 2 methods (**Table 5-4 and 5-5**). The ethanol transport across the everted intestinal sacs, determined by either method, was not affected by dietary fatty acids (**Table 5-4 to 5-6**).

Because a significant amount of the substrate may be retained and/or metabolized in the tissue, the disappearance of a solute from the mucosal fluid does not necessarily constitute absorption in the everted intestinal sacs model (Foulkes 1996). It is well established that ethanol is absorbed from the digestive tract and metabolized by ADH at this site (Boleda et al. 1989, Carter and Isselbacher 1971). Therefore, the absorption of ethanol was expressed as the sum of ethanol concentrations in serosal and intestinal sac plus the amount of ethanol metabolized to CO_2 (**Table 5-7**). Ethanol uptake and metabolism of the CFA and TFA groups was significantly higher than that of the SFA group in the proximal ($P < 0.01$) and proximal plus distal segments ($P < 0.03$). Therefore, it appeared that the degree of saturation and not the geometry of the double bond affected the uptake and metabolism of ethanol in the everted intestinal sacs model. The ethanol absorption, however, can be affected by other factors such as blood flow and gastric emptying. These factors cannot be imitated in the everted intestinal sac model. In

addition, with a simplified system like the everted intestinal sac, extrapolation back to the *in vivo* situation introduces considerable uncertainty. As a result, other approaches may be needed to study the effects of dietary fatty acids on ethanol absorption.

In summary, the everted sacs of the small intestine were able to convert 1-^{14}C-ethanol to $^{14}CO_2$. The uptake and metabolism of ethanol was higher in the CFA and TFA groups than in the SFA group in this model. Because there was no difference in the ethanol oxidation and uptake between the CFA and TFA groups, these data do not explain the differences in BEC. Therefore, additional studies are needed to understand the mechanism responsible for dietary fatty acid mediated differences in BEC of the rats.

CHAPTER VI

EFFECTS OF SATURATED, CIS, AND TRANS FATTY ACIDS ON TISSUE, PLASMA, AND MICROSOMAL FATTY ACID COMPOSITION IN RATS

ABSTRACT

The effect of feeding dietary saturated fatty acids (SFA), cis fatty acids (CFA), and trans fatty acid (TFA) on tissue fatty acid composition were determined in male Sprague-Dawley rats. In general, the fatty acid profiles of various tissues were highly reflective of the fatty acid content of the diets. In Experiment 1, the phospholipids (PL) fraction of the liver and small intestine of rats fed the TFA diet for 6 weeks had a higher percentage of linoleic acid and lower percentages of palmitic, stearic, and arachidonic acids than those fed the SFA and CFA diets. In Experiment 2, after 2 weeks of dietary treatment, elaidic acid was present in the plasma and liver microsomes of the TFA group only. Compared with rats fed the CFA diet, the fatty acid composition in the liver microsomes of rats fed the TFA diet was characterized by a) a significant decrease in palmitic, stearic, and oleic acids and an increase in linoleic acid, and b) an elevated percentage of total MUFA and a reduced proportion of total SFA regardless of the lipid fractions. These fatty acid changes were similar to those in plasma. The fatty acid composition data supports the suggestion that TFA and perhaps elaidic acid in particular, interfered with the conversion of linoleic acid to arachidonic acid, which has been shown to protect liver from alcohol injury.

INTRODUCTION

Trans fatty acids (TFA), formed during the biological and industrial hydrogenation of unsaturated fats, are found in ruminant fats, margarines, shortenings, and commercial bakeries (Emken 1984, Sachan and Davis 1969). A primary TFA produced by the chemically partial hydrogenation of vegetable oils is elaidic acid (Kris-Etherton and Nicolosi 1995). It has been suggested that TFA resemble SFA in their metabolic action (Beare-Rogers 1983, Dupont et al. 1991).

Dietary fatty acids have been shown to modify the fatty acid composition of cell membranes, which in turn affects their stability, permeability, transport properties, and other biological functions (Clandinin et al. 1991). It has been shown that exposure to ethanol affected the composition and metabolism of fatty acids in the liver (French et al. 1970, Nanji et al. 1993). A common finding of these studies was that the levels of polyunsaturated fatty acids (PUFA), especially arachidonic acid, were decreased in the liver. The decreased concentrations of arachidonic acid were accompanied by an increase in linoleic acid in some studies (Nanji et al. 1993). The increased linoleic acid and decreased arachidonic acid concentrations were also observed in the tissues of animals fed TFA (Atal et al. 1994, Ostlund-Lindqvist et al. 1985). It is not known if the tissue fatty acid composition relates to blood ethanol concentrations. Therefore, the objective of this study was to determine the relative effects of diets rich in saturated, cis or trans fatty acids on the changes in tissue fatty acid compositions and relate it to blood ethanol clearance.

MATERIALS AND METHODS

Animals, diets, and experimental design. The experimental protocol was approved by the University of Tennessee Committee on Humane Care and Use of Laboratory Animals. The details of treating animals, preparing experimental diets, and experimental design are described in the materials and methods section in Chapter 3.

Sampling. At the end of the 6-week study period of Experiment 1, adipose tissues (epididymal, perirenal, and inguinal fat pads), liver (perfused), skeletal muscle, and small intestine were removed, frozen in liquid nitrogen, and stored at -80°C until assayed for fatty acid composition. In Experiment 2, at the end of the 2-week experimental period, blood samples were collected by cardiac puncture. After blood collection, the liver was removed and processed immediately for microsome preparation. Blood samples were centrifuged at 1500 x g for 10 minutes at 4°C and the plasma samples were frozen at -80°C until assayed for fatty acid profile.

Preparation of microsome from liver. In Experiment 2, the liver was first minced by cutting with scissors in cold 150 mmol/L KCl in 10 mmol/L phosphate buffer (pH 7.4). Each liver sample was then homogenized in 3 volumes of KCl-phosphate buffer (pH 7.4) by 4 strokes in a teflon/glass homogenizer. The homogenate was centrifuged at 600 x g for 10 minutes at 4°C. The 600 x g supernatant was transferred to another centrifuge tube and centrifuged at 10,000 x g for 10 minutes at 4°C. The 10,000 x g supernatant was centrifuged at 100,000 x g for 1 hour at 4°C in an ultracentrifuge. The 100,000 x g pellet (microsomes) was suspended in KCl-phosphate buffer (pH 7.4) and used for determination of fatty acid composition. Protein concentration was determinated by the method of Bradford (1976).

Determination of fatty acid composition. Total lipids from all homogenized tissues were extracted by the Bligh and Dyer (1959) procedure. The lipids from each tissue homogenate (100 mg/mL) were extracted by a mixture of chloroform and methanol (1:2, v/v). The lipids extracted into the chloroform phase were removed after a centrifuge at 900 x g for 3 minutes. The mixture of tissue homogenate and extraction reagent was re-extracted with chloroform. The combined chloroform fractions were dried under a stream of nitrogen and resuspended in a small volume of chloroform for thin-layer chromatography (TLC) separation.

The phospholipids (PL) of liver, skeletal muscle, small intestine, and hepatic microsomes were chromatographed on HP-K silica gel TLC plate (Whatman, Maidstone, England) with a mixture of chloroform and methanol (8:1, v/v) as a moving solvent. The PL bands (at the origin) were scraped into screw-cap test tubes and suspended in toluene. Each PL sample was then saponified with 0.5 mol/L of KOH in methanol at 86°C for 8 minutes and acidified with 0.7 mol/L HCl in methanol. The fatty acids were twice extracted with hexane, evaporated, and methylated with diazomethane.

Fatty acid methyl esters (FAME) were resuspended in hexane and analyzed using a Hewlett-Packard Model 5890 series II gas chromatograph equipped with a flame ionization detector and a DB23 fused silica capillary column (0.25 mm I. D. x 30 m x 0.25 μ film) (J & W Scientific, Folsom, CA). Separation of FAME was achieved by temperature programming from 160 to 250°C per minute with hydrogen as the carrier gas. The internal standard, pentadecanoic acid (C15:0) methyl ester (100 μg) was added to each sample prior to the saponification process. Unknown FAME peaks were identified by comparison of retention times with those of known standards and the

individual FAME was quantified as area percentage compared with the internal standards. The protocol of the fatty acid assay is present in Appendix III.

Statistics. All data were expressed as the group means ± SEM. The ANOVA and Duncan's multiple range test as well as the student's t test were utilized in Experiments 1 and 2, respectively. The level of statistical significance was set at $P < 0.05$. All statistical analyses were performed using SAS for Unix system (version 6, SAS Institute, Cary, NC).

RESULTS

Tissue fatty acid compositions in Experiment 1. The fatty acid composition of various tissues from Experiment 1 are shown in **Tables 6-1 to 6-9**. As expected, the fatty acid composition of various tissues were modified according to the fatty acid content of the diets. Significant percentages of elaidic acid, derived from the exogenous dietary source, were found only in rats fed the TFA diet. Trace amounts of elaidic acid were detected in some tissues and lipid fractions from rats fed the SFA and CFA diets. Relative to the proportions distributed in the diets, more monounsaturated fatty acids (MUFA) were present in the adipose fats isolated from rats fed the TFA diet due to the increased percentages of palmitoleic and elaidic acids in the adipose tissues. Because of the comparable fatty acid content of the CFA and TFA diets, the main comparisons of tissue fatty acid composition were made between these 2 dietary groups.

Adipose tissue. The effects of dietary fats on the fatty acid composition of epididymal, perirenal and inguinal adipose tissues are presented in **Tables 6-1 to 6-3**. Compared to the CFA diet, the TFA diet resulted in significantly higher ($P < 0.05$)

percentages of palmitoleic and elaidic acids and a significantly lower ($P < 0.05$) proportions of oleic acid in the 3 different types of adipose tissue. The percentage of arachidonic acid was significantly lower ($P < 0.05$) in the epididymal and inguinal fat pads of the TFA group compared to the CFA group (**Tables 6-1 and 6-3**). Rats fed the TFA diet had a significantly higher ($P < 0.05$) percentage of stearic acid and a significantly lower ($P < 0.05$) percentage of palmitic acid in the epididymal fat pads than those fed the CFA diet (**Table 6-1**). A significantly lower ($P < 0.05$) percentage of linoleic acid was found in the perirenal adipose fat of the TFA group compared to that of the CFA group (**Table 6-2**). The deposition of elaidic acid significantly increased the percentage of total MUFA in the 3 types of adipose tissues.

Liver. The fatty acid composition of PL and NL of the liver are shown in **Tables 6-4 and 6-5**. In the PL fraction, the incorporation of elaidic acid was accompanied by a significant reduction ($P < 0.05$) in the proportions of palmitic and stearic acids (**Table 6-4**). By contrast, in the NL fraction, the TFA group showed a significantly lower ($P < 0.05$) level of oleic acid (**Table 6-5**). Significantly higher ($P < 0.05$) percentages of palmitoleic, oleic, elaidic, and linoleic acids and a significantly lower ($P < 0.05$) percentage of arachidonic acid were found in the PL of rats fed the TFA versus the CFA diet (**Table 6-4**). Rats fed the TFA diet had significantly higher ($P < 0.05$) proportions of palmitoleic and elaidic acids and significantly lower ($P < 0.05$) proportions of oleic and arachidonic acids than those fed the CFA diet in the NL fraction (**Table 6-5**). Reduced total SFA and increased total MUFA proportions of the PL fraction were observed in the TFA group compared to the CFA group (**Table 6-4**). The proportions of total SFA, MUFA, and PUFA were not affected by the dietary fats in the NL fraction (**Table 6-5**).

TABLE 6-1

Fatty acid composition of epididymal adipose tissue (mole%) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Fatty acid	Dietary group		
	SFA	CFA	TFA
C10:0	0.6 ± 0.1^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}
C12:0	11.6 ± 0.7^{a}	0.3 ± 0.1^{b}	0.2 ± 0.0^{b}
C14:0	8.5 ± 0.1^{a}	1.5 ± 0.0^{b}	1.4 ± 0.0^{b}
C16:0	23.9 ± 0.3^{a}	22.5 ± 0.3^{b}	21.3 ± 0.4^{c}
C16:1	6.1 ± 0.3^{a}	4.2 ± 0.2^{c}	4.9 ± 0.2^{b}
C18:0	2.9 ± 0.1^{b}	3.7 ± 0.3^{b}	4.7 ± 0.3^{a}
cis C18:1 (n-9)	24.6 ± 0.2^{c}	41.5 ± 0.4^{a}	30.2 ± 0.2^{b}
trans C18:1 (n-9)	0.2 ± 0.0^{b}	n.d.	12.4 ± 0.5^{a}
C18:2 (n-6)	20.6 ± 0.5^{b}	25.7 ± 0.2^{a}	24.1 ± 0.8^{a}
C18:3 (n-3)	0.5 ± 0.0^{b}	0.7 ± 0.0^{b}	2.2 ± 0.1^{a}
C20:4 (n-6)	0.3 ± 0.1^{ab}	0.4 ± 0.0^{a}	0.2 ± 0.0^{b}
Σ SFA	47.5 ± 0.8^{a}	28.1 ± 0.5^{b}	27.9 ± 0.6^{b}
Σ MUFA	30.8 ± 0.4^{c}	45.7 ± 0.4^{b}	47.5 ± 0.5^{a}
Σ PUFA	21.5 ± 0.6^{b}	26.8 ± 0.3^{a}	26.6 ± 0.8^{a}
18:2 (n-6)/20:4 (n-6)	79.7 ± 9.8^{b}	74.3 ± 8.3^{b}	143.6 ± 7.6^{a}

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

TABLE 6-2

Fatty acid composition of perirenal adipose tissue (mole%) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Fatty acid	Dietary group		
	SFA	CFA	TFA
C10:0	1.0 ± 0.1[a]	0.4 ± 0.0[b]	0.5 ± 0.1[b]
C12:0	13.4 ± 0.5[a]	0.2 ± 0.0[b]	0.2 ± 0.0[b]
C14:0	9.3 ± 0.1[a]	1.7 ± 0.1[b]	1.8 ± 0.0[b]
C16:0	26.4 ± 0.5[a]	25.4 ± 0.7[a]	25.2 ± 0.7[a]
C16:1	5.8 ± 0.3[a]	4.3 ± 0.2[b]	5.6 ± 0.3[a]
C18:0	3.5 ± 0.4[a]	3.6 ± 0.2[a]	3.5 ± 0.1[a]
cis C18:1 (n-9)	23.5 ± 0.4[c]	40.7 ± 0.6[a]	29.3 ± 0.2[b]
trans C18:1 (n-9)	0.2 ± 0.0[b]	0.2 ± 0.1[b]	11.8 ± 0.3[a]
C18:2 (n-6)	16.3 ± 0.5[c]	22.7 ± 0.6[a]	20.2 ± 0.7[b]
C18:3 (n-3)	0.2 ± 0.0[c]	0.4 ± 0.0[b]	2.0 ± 0.0[a]
C20:4 (n-6)	0.4 ± 0.3[a]	0.3 ± 0.1[b]	0.2 ± 0.0[b]
Σ SFA	53.6 ± 0.5[a]	31.4 ± 0.9[b]	31.2 ± 0.7[b]
Σ MUFA	29.4 ± 0.3[c]	45.2 ± 0.4[b]	46.6 ± 0.4[a]
Σ PUFA	17.0 ± 0.4[b]	23.4 ± 0.6[a]	22.2 ± 0.7[a]
18:2 (n-6)/20:4 (n-6)	94.9 ± 17.7[a]	23.4 ± 0.6[a]	22.2 ± 0.7[a]

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids.

TABLE 6-3

Fatty acid composition of inguinal adipose tissue (mole%) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Fatty acid	Dietary group		
	SFA	CFA	TFA
C10:0	1.0 ± 0.1^{a}	0.5 ± 0.1^{b}	0.4 ± 0.1^{b}
C12:0	14.9 ± 0.3^{a}	0.5 ± 0.1^{b}	0.4 ± 0.0^{b}
C14:0	9.1 ± 0.2^{a}	1.6 ± 0.1^{b}	1.7 ± 0.0^{b}
C16:0	22.9 ± 0.5^{a}	22.4 ± 0.3^{ab}	21.4 ± 0.3^{b}
C16:1	4.0 ± 0.3^{a}	2.7 ± 0.1^{b}	3.7 ± 0.3^{a}
C18:0	3.5 ± 0.2^{b}	3.7 ± 0.1^{ab}	4.1 ± 0.2^{a}
cis C18:1 (n-9)	22.8 ± 0.3^{c}	41.1 ± 0.4^{a}	30.0 ± 0.3^{b}
trans C18:1 (n-9)	0.2 ± 0.0^{b}	0.1 ± 0.0^{b}	11.8 ± 0.4^{a}
C18:2 (n-6)	20.8 ± 0.7^{b}	26.1 ± 0.5^{a}	24.4 ± 0.6^{a}
C18:3 (n-3)	0.4 ± 0.0^{c}	0.5 ± 0.0^{b}	2.0 ± 0.0^{a}
C20:4 (n-6)	0.3 ± 0.1^{b}	0.5 ± 0.1^{a}	0.2 ± 0.0^{b}
Σ SFA	51.4 ± 0.8^{a}	28.8 ± 0.4^{b}	27.9 ± 0.3^{b}
Σ MUFA	27.0 ± 0.2^{c}	44.0 ± 0.4^{b}	45.5 ± 0.7^{a}
Σ PUFA	21.6 ± 0.7^{b}	27.2 ± 0.4^{a}	26.7 ± 0.6^{a}
18:2 (n-6)/20:4 (n-6)	86.8 ± 12.2^{b}	71.9 ± 15.1^{b}	130.4 ± 7.1^{a}

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids.

TABLE 6-4

Fatty acid composition of liver phospholipids (mole%) in rats fed saturated, cis or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Fatty acid	Dietary group		
	SFA	CFA	TFA
C10:0	0.8 ± 0.2^{a}	1.0 ± 0.7^{a}	0.1 ± 0.0^{a}
C12:0	0.7 ± 0.1^{a}	0.7 ± 0.1^{a}	0.6 ± 0.0^{a}
C14:0	0.9 ± 0.1^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}
C16:0	21.6 ± 0.3^{a}	20.9 ± 0.4^{a}	19.2 ± 0.6^{b}
C16:1	1.7 ± 0.2^{a}	1.0 ± 0.1^{b}	1.6 ± 0.2^{a}
C18:0	22.7 ± 0.4^{a}	21.9 ± 0.4^{a}	16.1 ± 0.4^{b}
cis C18:1 (n-9)	4.6 ± 0.1^{c}	6.8 ± 0.1^{b}	7.8 ± 0.2^{a}
trans C18:1 (n-9)	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}	8.1 ± 0.2^{a}
C18:2 (n-6)	16.7 ± 0.4^{b}	16.1 ± 0.2^{b}	20.2 ± 0.3^{a}
C18:3 (n-3)	n.d.	n.d.	0.10 ± 0.0
C20:4 (n-6)	29.9 ± 0.4^{a}	30.8 ± 0.4^{a}	25.6 ± 0.4^{b}
Σ SFA	46.7 ± 0.3^{a}	44.8 ± 0.6^{b}	36.4 ± 0.4^{c}
Σ MUFA	6.5 ± 0.4^{c}	8.1 ± 0.2^{b}	17.5 ± 0.2^{a}
Σ PUFA	46.8 ± 0.4^{a}	47.1 ± 0.5^{a}	46.0 ± 0.4^{a}
18:2 (n-6)/20:4 (n-6)	0.6 ± 0.0^{b}	0.5 ± 0.0^{b}	0.8 ± 0.0^{a}

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

TABLE 6-5

Fatty acid composition of liver neutral lipids (mole%) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Fatty acid	Dietary group		
	SFA	CFA	TFA
C10:0	0.7 ± 0.2[a]	1.8 ± 0.7[a]	2.6 ± 1.0[a]
C12:0	2.5 ± 0.1[a]	2.1 ± 0.2[b]	2.0 ± 0.1[b]
C14:0	4.5 ± 0.2[a]	1.5 ± 0.0[b]	1.5 ± 0.0[b]
C16:0	32.5 ± 0.6[a]	26.7 ± 0.4[b]	26.9 ± 0.6[b]
C16:1	6.1 ± 0.9[a]	3.0 ± 0.2[b]	5.0 ± 0.5[a]
C18:0	3.9 ± 0.2[a]	3.3 ± 0.2[b]	3.3 ± 0.1[b]
cis C18:1 (n-9)	25.2 ± 0.3[b]	35.3 ± 0.7[a]	26.5 ± 0.4[b]
trans C18:1 (n-9)	0.3 ± 0.2[b]	n.d.	8.0 ± 0.5[a]
C18:2 (n-6)	20.5 ± 1.4[a]	22.4 ± 0.5[a]	20.5 ± 1.2[a]
C18:3 (n-3)	n.d.	n.d.	1.4 ± 0.1
C20:4 (n-6)	3.5 ± 0.3[a]	3.4 ± 0.3[a]	2.1 ± 0.1[b]
Σ SFA	44.1 ± 0.8[a]	35.4 ± 1.1[b]	36.2 ± 1.4[b]
Σ MUFA	31.6 ± 1.2[b]	38.4 ± 0.8[a]	39.5 ± 0.5[a]
Σ PUFA	24.4 ± 1.8[a]	26.3 ± 0.6[a]	24.3 ± 1.3[a]
18:2 (n-6)/20:4 (n-6)	6.0 ± 0.2[b]	6.9 ± 0.6[b]	9.8 ± 0.6[a]

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

Skeletal muscle. The fatty acid composition of the skeletal muscle PL and NL were summarized in **Tables 6-6 and 6-7**. In the PL fraction, the deposition of elaidic acid significantly decreased the percentages of palmitic, stearic, oleic, and arachidonic acids and significantly increased the level of linoleic acid **(Table 6-6)**. In contrast, oleic acid was significantly reduced and palmitoleic and linolenic acids were significantly increased as the elaidic acid incorporated into the NL fraction (**Table 6-7**). In the PL fraction, a lower percentage of total SFA and higher percentage of total MUFA were found in rats fed the TFA diet than in those fed the CFA diet (**Table 6-6**). In the NL fraction, feeding the TFA diet increased the proportion of total MUFA compared to feeding of the CFA diet (**Table 6-7**).

Small intestine. Tables 6-8 and 6-9 present the fatty acid PL and NL content of the small intestine. In the PL fraction, the incorporation of elaidic acid decreased the proportions of palmitic, stearic, and oleic acids and increased the proportion of linoleic acid (**Table 6-8**). In the NL fraction, the TFA group had significantly higher percentages of elaidic and linolenic acids and significantly lower percentages of oleic and linoleic acids than the CFA group (**Table 6-9**). Higher percentages of total MUFA and PUFA as well as lower percentage of total SFA in the TFA group than in the CFA group was observed in the PL fraction (**Table 6-8**), whereas there was no dietary fat effect on the proportions of total SFA, MUFA, and PUFA in the NL fraction (**Table 6-9**).

Plasma FA composition in Experiment 2. The fatty acid compositions of plasma in rats after 2 weeks of dietary treatment is shown in **Table 6-10**. In the total lipids of plasma, rats received the TFA diet had significantly lower percentages of palmitic, stearic, oleic, and docosahexaenoic acids than those fed the CFA diet ($P < 0.05$) (**Table**

TABLE 6-6

Fatty acid composition of skeletal muscle phospholipids (mole%) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Fatty acid	Dietary group		
	SFA	CFA	TFA
C10:0	n.d.	n.d.	n.d.
C12:0	n.d.	n.d.	n.d.
C14:0	0.7 ± 0.2^{a}	0.3 ± 0.2^{ab}	0.1 ± 0.1^{b}
C16:0	22.7 ± 0.8^{a}	22.9 ± 0.5^{a}	20.8 ± 0.4^{b}
C16:1	1.6 ± 0.9^{a}	0.8 ± 0.2^{a}	0.6 ± 0.2^{a}
C18:0	17.8 ± 0.7^{a}	16.8 ± 0.3^{a}	14.2 ± 0.1^{b}
cis C18:1 (n-9)	5.2 ± 0.4^{b}	7.5 ± 0.7^{a}	5.5 ± 0.3^{b}
trans C18:1 (n-9)	n.d.	n.d.	5.0 ± 0.2
C18:2 (n-6)	22.6 ± 0.7^{a}	19.8 ± 0.5^{b}	23.3 ± 0.5^{a}
C18:3 (n-3)	n.d.	n.d.	0.1 ± 0.1
C20:4 (n-6)	17.8 ± 1.0^{ab}	18.7 ± 0.8^{a}	15.8 ± 0.6^{b}
Σ SFA	41.2 ± 1.2^{a}	40.1 ± 0.5^{a}	35.1 ± 0.3^{b}
Σ MUFA	6.9 ± 0.8^{b}	8.3 ± 0.8^{b}	11.1 ± 0.6^{a}
Σ PUFA	51.7 ± 0.8^{a}	51.5 ± 1.1^{a}	53.7 ± 0.4^{a}
18:2 (n-6)/20:4 (n-6)	1.3 ± 0.1^{b}	1.1 ± 0.0^{c}	1.5 ± 0.1^{a}

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

TABLE 6-7

Fatty acid composition of skeletal muscle neutral lipids (mole%) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Fatty acid	Dietary group		
	SFA	CFA	TFA
C10:0	n.d.	n.d.	n.d.
C12:0	3.7 ± 0.7	n.d.	n.d.
C14:0	8.0 ± 0.5[a]	2.1 ± 0.2[b]	1.9 ± 0.1[b]
C16:0	28.3 ± 0.7[a]	24.9 ± 0.8[b]	23.0 ± 0.4[b]
C16:1	4.7 ± 0.4[a]	3.1 ± 0.1[b]	4.3 ± 0.2[a]
C18:0	5.5 ± 0.4[a]	5.2 ± 0.3[a]	5.1 ± 0.2[a]
cis C18:1 (n-9)	25.2 ± 0.5[c]	36.9 ± 0.8[a]	27.7 ± 0.4[b]
trans C18:1 (n-9)	n.d.	n.d.	10.5 ± 0.4
C18:2 (n-6)	20.6 ± 0.6[b]	23.1 ± 0.6[a]	21.4 ± 0.6[ab]
C18:3 (n-3)	0.4 ± 0.1[b]	0.3 ± 0.1[b]	1.8 ± 0.1[a]
C20:4 (n-6)	1.4 ± 0.2[a]	2.0 ± 0.3[a]	1.4 ± 0.2[a]
Σ SFA	45.7 ± 0.5[a]	33.7 ± 1.4[b]	31.3 ± 1.1[b]
Σ MUFA	30.0 ± 0.3[c]	40.0 ± 0.8[b]	42.4 ± 0.9[a]
Σ PUFA	24.3 ± 0.5[a]	26.2 ± 0.9[a]	26.2 ± 0.6[a]
18:2 (n-6)/20:4 (n-6)	15.7 ± 2.7[a]	12.8 ± 2.1[a]	16.7 ± 2.1[a]

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

TABLE 6-8

Fatty acid composition of small intestinal phospholipids (mole%) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Fatty acid	Dietary group		
	SFA	CFA	TFA
C10:0	4.0 ± 1.5[a]	6.1 ± 2.1[a]	3.1 ± 1.1[a]
C12:0	0.7 ± 0.1[a]	0.8 ± 0.1[a]	0.6 ± 0.1[a]
C14:0	1.6 ± 0.1[a]	0.8 ± 0.1[b]	0.7 ± 0.1[b]
C16:0	17.1 ± 0.3[a]	17.6 ± 0.8[a]	15.3 ± 0.4[b]
C16:1	0.4 ± 0.1[a]	0.5 ± 0.0[a]	0.5 ± 0.0[a]
C18:0	24.4 ± 0.7[a]	21.5 ± 0.5[b]	18.7 ± 0.4[c]
cis C18:1 (n-9)	7.3 ± 0.4[c]	11.6 ± 0.4[a]	10.3 ± 0.3[b]
trans C18:1 (n-9)	0.1 ± 0.0[b]	0.2 ± 0.0[b]	5.6 ± 0.3[a]
C18:2 (n-6)	23.0 ± 1.4[ab]	19.2 ± 1.0[b]	25.2 ± 1.4[a]
C18:3 (n-3)	n.d.	n.d.	0.3 ± 0.0
C20:4 (n-6)	17.0 ± 0.5[a]	17.2 ± 1.3[a]	14.8 ± 0.8[a]
Σ SFA	48.8 ± 1.0[a]	47.8 ± 1.4[a]	39.1 ± 1.1[b]
Σ MUFA	8.3 ± 0.5[c]	13.0 ± 0.5[b]	16.8 ± 0.3[a]
Σ PUFA	43.0 ± 1.0[a]	39.2 ± 1.2[b]	44.2 ± 1.1[a]
18:2 (n-6)/20:4 (n-6)	1.4 ± 0.1[ab]	1.2 ± 0.1[b]	1.7 ± 0.2[a]

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

TABLE 6-9

Fatty acid composition of small intestinal neutral lipids (mole%) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Fatty acid	Dietary group		
	SFA	CFA	TFA
C10:0	1.2 ± 0.9[a]	0.9 ± 0.4[a]	1.4 ± 0.6[a]
C12:0	8.7 ± 1.0[a]	0.5 ± 0.1[b]	0.5 ± 0.1[b]
C14:0	9.1 ± 0.5[a]	1.6 ± 0.1[b]	1.7 ± 0.1[b]
C16:0	24.8 ± 0.8[a]	22.4 ± 0.5[a]	22.1 ± 1.3[a]
C16:1	3.9 ± 0.4[a]	3.0 ± 0.1[a]	4.0 ± 0.6[a]
C18:0	6.1 ± 0.9[a]	4.4 ± 0.1[a]	5.0 ± 0.6[a]
cis C18:1 (n-9)	22.5 ± 1.5[c]	40.4 ± 0.5[a]	27.7 ± 1.2[b]
trans C18:1 (n-9)	0.2 ± 0.0[b]	0.1 ± 0.0[b]	12.0 ± 0.6[a]
C18:2 (n-6)	19.2 ± 0.3[c]	23.6 ± 0.4[a]	21.2 ± 0.8[b]
C18:3 (n-3)	0.3 ± 0.0[b]	0.4 ± 0.0[b]	1.8 ± 0.1[a]
C20:4 (n-6)	2.8 ± 1.1[a]	1.6 ± 0.2[a]	1.6 ± 0.6[a]
Σ SFA	50.4 ± 1.0[a]	30.3 ± 0.5[b]	31.1 ± 0.5[b]
Σ MUFA	27.0 ± 1.8[b]	43.9 ± 0.5[a]	43.9 ± 1.2[a]
Σ PUFA	22.7 ± 1.4[a]	25.9 ± 0.3[a]	25.0 ± 1.5[a]
18:2 (n-6)/20:4 (n-6)	10.9 ± 2.5[a]	16.6 ± 2.4[a]	19.0 ± 4.2[a]

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids.

6-10). Feeding the TFA diet resulted in a significant increase in the percentage of linoleic acid ($P < 0.05$) and no decrease in the percentage of arachidonic acid, compared with feeding of the CFA diet (**Table 6-10**). A significant amount of elaidic acid was present in the TFA group and a trace amount of elaidic acid was also found in the CFA group (**Table 6-10**). The different dietary fats led to variations in the total content of the different fatty acid classes. Compared with the CFA group, the TFA group had significantly higher percentages of total MUFA and PUFA, accompanied by a significantly lower percentage of total SFA (**Table 6-10**).

After an acute dose of ethanol, within the CFA group, the levels of myristic and palmitoleic acids increased and those of oleic and linoleic acids decreased in the CFA group (**Table 6-11**). Within the TFA group, a reduced percentage of linoleic and elevated percentage of arachidonic acid were found after the administration of ethanol (**Table 6-11**).

Liver microsomal fatty acid composition in Experiment 2. The fatty acid composition of microsomal PL and NL isolated from the livers of rats fed CFA or TFA diets for 2 weeks are summarized in **Tables 6-12 to 6-15**. The characteristic changes of the fatty acid profile in plasma were also observed in the microsomes. In both the PL (**Tables 6-12**) and NL (**Tables 6-14**) fractions, the percentages of palmitic, stearic and oleic acids were significantly lower in the TFA group that those in the CFA group ($P < 0.05$). The significantly higher percentage of linoleic acid was accompanied by an invariable level of arachidonic acid in the TFA group compared to the CFA group. Elaidic acid was only found in rats fed the TFA diet. Overall, feeding of TFA led to a decreased percentage of total SFA, an increased percentage of total MUFA, and an unaffected percentage of total

TABLE 6-10

Fatty acid composition of plasma total lipids (mole%) in rats fed cis or trans fatty acids diet for 2 weeks from Experiment 2 [1,2]

Fatty acid	Dietary group	
	CFA	TFA
C10:0	n.d.	n.d.
C12:0	n.d.	n.d.
C14:0	0.6 ± 0.0^a	0.6 ± 0.0^a
C16:0	24.8 ± 0.3^a	21.8 ± 0.3^b
C16:1	1.3 ± 0.1^a	1.5 ± 0.1^a
C18:0	17.3 ± 0.3^a	12.7 ± 0.2^b
cis C18:1 (n-9)	9.8 ± 0.1^a	8.6 ± 0.2^b
trans C18:1 (n-9)	0.2 ± 0.0^b	7.7 ± 0.1^a
C18:2 (n-6)	21.0 ± 0.6^b	23.8 ± 0.6^a
C18:3 (n-3)	n.d.	n.d.
C20:4 (n-6)	20.6 ± 0.5^a	19.4 ± 0.6^a
C22:6 (n-3)	2.6 ± 0.1^a	2.4 ± 0.1^b
Σ SFA	43.2 ± 0.4^a	35.5 ± 0.4^b
Σ MUFA	11.4 ± 0.2^b	17.8 ± 0.2^a
Σ PUFA	45.4 ± 0.3^b	46.7 ± 0.4^a
18:2 (n-6)/20:4 (n-6)	1.0 ± 0.1^b	1.3 ± 0.1^a

[1] Values are group means ± SEM (n = 12) and those bearing the same superscript letters in a row are not significant different at $P < 0.05$.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

TABLE 6-11

Fatty acid composition of plasma total lipids (mole%) in rats fed cis or trans fatty acids diet with or without ethanol administration before being killed from Experiment 2 [1,2]

Fatty acid	Dietary group			
	CFA		TFA	
	no EtOH[a]	with EtOH[a]	no EtOH	with EtOH
C10:0	n.d.	n.d.	n.d.	n.d.
C12:0	n.d.	n.d.	n.d.	n.d.
C14:0	0.6 ± 0.0^{b}	0.7 ± 0.0^{a}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}
C16:0	24.2 ± 0.5^{a}	25.3 ± 0.1^{a}	22.0 ± 0.5^{b}	21.6 ± 0.4^{b}
C16:1	1.0 ± 0.1^{b}	1.5 ± 0.1^{a}	1.3 ± 0.1^{ab}	1.7 ± 0.2^{a}
C18:0	17.1 ± 0.4^{a}	17.5 ± 0.4^{a}	12.5 ± 0.2^{b}	13.0 ± 0.2^{b}
cis C18:1 (n-9)	10.1 ± 0.1^{a}	9.4 ± 0.2^{b}	8.6 ± 0.2^{c}	8.6 ± 0.2^{c}
trans C18:1 (n-9)	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	7.8 ± 0.2^{a}	7.5 ± 0.2^{a}
C18:2 (n-6)	22.7 ± 0.3^{b}	19.2 ± 0.5^{c}	25.2 ± 0.6^{a}	22.4 ± 0.9^{b}
C18:3 (n-3)	0.1 ± 0.0	0.2 ± 0.1	n.d.	n.d.
C20:4 (n-6)	19.5 ± 0.7^{a}	21.8 ± 0.5^{a}	18.3 ± 0.8^{b}	20.6 ± 0.8^{a}
C22:6 (n-3)	2.7 ± 0.1^{a}	2.6 ± 0.1^{ab}	2.4 ± 0.1^{b}	2.3 ± 0.1^{ab}
Σ SFA	42.4 ± 0.5^{a}	43.9 ± 0.3^{a}	35.4 ± 0.6^{b}	35.5 ± 0.6^{b}
Σ MUFA	11.6 ± 0.2^{b}	11.3 ± 0.3^{b}	17.6 ± 0.3^{a}	18.0 ± 0.4^{a}
Σ PUFA	46.2 ± 0.5^{ab}	45.1 ± 0.2^{b}	47.1 ± 0.5^{a}	46.7 ± 0.5^{a}
18:2 (n-6)/20:4 (n-6)	1.2 ± 0.0^{b}	0.9 ± 0.0^{c}	1.4 ± 0.1^{a}	1.1 ± 0.1^{b}

[1] Values are group means ± SEM (n = 6) and those bearing the same superscript letters in a row are not significant different at $P < 0.05$.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid; EtOH, ethanol; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

[a] With ethanol, animals received an oral dose (3 g/kg body weight) of ethanol (13%, v/v) 2 hours before being killed; no ethanol, animals received no ethanol before being killed.

TABLE 6-12

Fatty acid composition in hepatic microsomal phospholipids (mole%) in rats fed cis or trans fatty acids diet for 2 weeks from Experiment 2 [1,2]

Fatty acid	Dietary group	
	CFA	TFA
C10:0	n.d.	n.d.
C12:0	n.d.	n.d.
C14:0	0.6 ± 0.1[a]	0.5 ± 0.1[a]
C16:0	22.3 ± 0.5[a]	19.3 ± 0.4[b]
C16:1	1.7 ± 0.2[a]	2.0 ± 0.2[a]
C18:0	18.3 ± 0.4[a]	13.3 ± 0.2[b]
cis C18:1 (n-9)	7.0 ± 0.1[a]	6.6 ± 0.1[b]
trans C18:1 (n-9)	n.d.	7.9 ± 0.3
C18:2 (n-6)	12.3 ± 0.3[b]	14.7 ± 0.5[a]
C18:3 (n-3)	n.d.	n.d.
C20:4 (n-6)	30.1 ± 0.4[a]	29.1 ± 0.5[a]
C22:6 (n-3)	4.9 ± 0.4[a]	4.0 ± 0.5[a]
Σ SFA	41.7 ± 0.6[a]	33.3 ± 0.5[b]
Σ MUFA	8.8 ± 0.3[b]	16.5 ± 0.3[a]
Σ PUFA	49.5 ± 0.5[a]	50.2 ± 0.6[a]
18:2 (n-6)/20:4 (n-6)	0.4 ± 0.0[b]	0.5 ± 0.0[a]

[1] Values are means ± SEM (n = 12) and those bearing the same superscript letters in a row are not significant different at $P < 0.05$.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

TABLE 6-13

Fatty acid composition of hepatic microsomal phospholipids (mole%) in rats fed cis or trans fatty acids diet with or without ethanol administration before being killed from Experiment 2 [1,2]

Fatty acid	Dietary group			
	CFA		TFA	
	no EtOH[a]	with EtOH[a]	no EtOH	with EtOH
C10:0	n.d.	n.d.	n.d.	n.d.
C12:0	n.d.	n.d.	n.d.	n.d.
C14:0	0.6 ± 0.0^{a}	0.7 ± 0.1^{a}	0.4 ± 0.1^{a}	0.6 ± 0.1^{a}
C16:0	21.5 ± 0.7^{ab}	23.2 ± 0.7^{a}	19.1 ± 0.6^{c}	19.6 ± 0.6^{bc}
C16:1	1.4 ± 0.2^{a}	1.9 ± 0.4^{a}	1.7 ± 0.2^{a}	2.4 ± 0.3^{a}
C18:0	18.5 ± 0.5^{a}	18.1 ± 0.7^{a}	13.3 ± 0.2^{b}	13.2 ± 0.4^{b}
cis C18:1 (n-9)	7.1 ± 0.3^{a}	7.0 ± 0.1^{ab}	6.5 ± 0.1^{b}	6.7 ± 0.2^{ab}
trans C18:1 (n-9)	n.d.	n.d.	7.8 ± 0.4^{a}	8.0 ± 0.4^{a}
C18:2 (n-6)	12.8 ± 0.5^{bc}	11.8 ± 0.4^{c}	15.1 ± 0.5^{a}	14.4 ± 1.0^{ab}
C18:3 (n-3)	n.d.	n.d.	n.d.	n.d.
C20:4 (n-6)	29.4 ± 0.5^{a}	30.7 ± 0.6^{a}	28.7 ± 0.7^{a}	29.4 ± 0.7^{a}
C22:6 (n-3)	5.3 ± 0.1^{a}	4.5 ± 0.9^{a}	4.2 ± 0.6^{a}	3.8 ± 0.8^{a}
Σ SFA	41.1 ± 0.5^{a}	42.3 ± 1.1^{a}	33.1 ± 0.6^{b}	33.6 ± 0.8^{b}
Σ MUFA	8.6 ± 0.4^{c}	8.9 ± 0.4^{c}	16.0 ± 0.4^{b}	17.1 ± 0.3^{a}
Σ PUFA	50.8 ± 0.5^{a}	49.1 ± 0.9^{a}	51.4 ± 0.8^{a}	49.7 ± 0.8^{a}
18:2 (n-6)/20:4 (n-6)	0.4 ± 0.0^{bc}	0.4 ± 0.0^{c}	0.5 ± 0.0^{a}	0.5 ± 0.0^{ab}

[1] Values are means ± SEM (n = 6) and those bearing the same superscript letters in a row are not significant different at $P < 0.05$.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid; EtOH, ethanol; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

[a] With ethanol, animals received an oral dose (3 g/kg body weight) of ethanol (13%, v/v) 2 hours before being killed; no ethanol, animals received no ethanol before being killed.

TABLE 6-14

Fatty acid composition in hepatic microsomal neutral lipids (mole%) in rats fed cis or trans fatty acids diet for 2 weeks from Experiment 2 [1,2]

Fatty acid	Dietary group	
	CFA	TFA
C10:0	n.d.	n.d.
C12:0	1.4 ± 0.2[a]	1.2 ± 0.1[a]
C14:0	2.8 ± 0.2[a]	2.3 ± 0.2[a]
C16:0	27.5 ± 0.8[a]	25.0 ± 0.6[b]
C16:1	5.3 ± 0.6[a]	5.4 ± 0.5[a]
C18:0	7.5 ± 0.3[a]	6.2 ± 0.2[b]
cis C18:1 (n-9)	25.7 ± 0.5[a]	19.7 ± 0.8[b]
trans C18:1 (n-9)	n.d.	8.1 ± 0.3
C18:2 (n-6)	16.6 ± 0.7[a]	18.6 ± 0.8[a]
C18:3 (n-3)	n.d.	n.d.
C20:4 (n-6)	9.8 ± 0.8[a]	9.7 ± 0.7[a]
C22:6 (n-3)	1.8 ± 0.2[a]	1.4 ± 0.3[a]
Σ SFA	39.4 ± 0.8[a]	34.8 ± 0.7[b]
Σ MUFA	31.1 ± 0.8[b]	33.3 ± 0.6[a]
Σ PUFA	29.5 ± 1.4[a]	31.9 ± 1.1[a]
18:2 (n-6)/20:4 (n-6)	1.8 ± 0.2[a]	2.0 ± 0.1[a]

[1] Values are means ± SEM (n = 12) and those bearing the same superscript letters in a row are not significant different at $P < 0.05$.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

TABLE 6-15

Fatty acid composition of hepatic microsomal neutral lipids (mole%) in rats fed cis or trans fatty acids diet with or without ethanol administration before being killed from Experiment 2 [1,2]

Fatty acid	Dietary group			
	CFA		TFA	
	no EtOH[a]	with EtOH[a]	no EtOH	with EtOH
C10:0	n.d.	n.d.	n.d.	n.d.
C12:0	1.0 ± 0.2^{b}	1.8 ± 0.2^{a}	1.1 ± 0.1^{b}	1.4 ± 0.1^{ab}
C14:0	2.2 ± 0.1^{c}	3.4 ± 0.2^{a}	1.9 ± 0.1^{c}	2.8 ± 0.2^{b}
C16:0	26.9 ± 1.2^{ab}	28.2 ± 1.1^{a}	24.1 ± 0.8^{b}	25.9 ± 0.8^{ab}
C16:1	4.1 ± 0.6^{a}	6.4 ± 0.9^{a}	4.6 ± 0.5^{a}	6.3 ± 0.9^{a}
C18:0	8.0 ± 0.5^{a}	6.9 ± 0.3^{b}	6.4 ± 0.2^{b}	6.0 ± 0.3^{b}
cis C18:1 (n-9)	25.5 ± 0.9^{a}	25.9 ± 0.6^{a}	19.7 ± 0.6^{b}	19.7 ± 0.4^{b}
trans C18:1 (n-9)	n.d.	n.d.	8.8 ± 0.2^{a}	7.4 ± 0.5^{b}
C18:2 (n-6)	17.7 ± 1.0^{ab}	15.5 ± 0.8^{b}	20.2 ± 0.6^{a}	17.0 ± 1.0^{b}
C18:3 (n-3)	n.d.	n.d.	n.d.	n.d.
C20:4 (n-6)	10.9 ± 1.6^{a}	8.7 ± 0.4^{a}	10.4 ± 1.2^{a}	9.4 ± 0.7^{a}
C22:6 (n-3)	1.6 ± 0.3^{a}	2.0 ± 0.4^{a}	1.0 ± 0.4^{a}	1.8 ± 0.2^{a}
Σ SFA	38.2 ± 0.9^{ab}	40.5 ± 1.2^{a}	33.5 ± 0.8^{c}	36.0 ± 0.9^{bc}
Σ MUFA	29.7 ± 1.2^{b}	32.4 ± 0.5^{a}	33.1 ± 0.9^{a}	33.4 ± 0.7^{a}
Σ PUFA	32.7 ± 1.8^{a}	27.5 ± 1.5^{b}	33.5 ± 1.5^{a}	30.6 ± 1.4^{ab}
18:2 (n-6)/20:4 (n-6)	1.8 ± 0.3^{a}	1.8 ± 0.1^{a}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}

[1] Values are mean ± SEM (n = 6) and those bearing the same superscript letters in a row are not significant different at $P < 0.05$.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid; EtOH, ethanol; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; Σ SFA, total saturated fatty acids; Σ MUFA, total mono-unsaturated fatty acids; Σ PUFA, total poly-unsaturated fatty acids; n.d., not detectable.

[a] With ethanol, animals received an oral dose (3 g/kg body weight) of ethanol (13%, v/v) 2 hours before being killed; no ethanol, animals received no ethanol before being killed.

PUFA compared to CFA feeding.

Administration of ethanol had only minor effects on the fatty acid profile of liver microsomal PL and NL. In the PL fraction, the only change was an increase of the total MUFA content in the TFA group after giving the acute ethanol dose (**Table 6-13**). In the microsomal NL fraction of the CFA group, the ethanol administration resulted in a significant increase in the levels of lauric and myristic acids and a significant decrease in the levels of stearic acid ($P < 0.05$) (**Table 6-15**). The overall percentage of MUFA was increased and that of PUFA was decreased in the microsomal NL of rats fed CFA and acute dose of ethanol. In the microsomal NL fraction of the TFA group, the percentage of myristic acid was significantly increased ($P < 0.05$) and the percentages of elaidic and linoleic acids were significantly decreased ($P < 0.05$) after the acute ethanol consumption (**Table 6-15**). The overall fatty acid pattern of rats fed TFA was not influenced by the acute ethanol dose.

DISCUSSION

In order to evaluate the possible relevance of fatty acid changes to ethanol metabolism, the fatty acid composition of the liver, small intestine (Experiment 1), plasma, and liver microsome (Experiment 2) were determined. In Experiment 1, the fatty acid profile was also analyzed in the adipose tissues and skeletal muscle for the determination of TFA incorporation into these tissues. It is known that adipose tissues and skeletal muscle have little relevance to ethanol metabolism. Therefore, the discussion is focused on the fatty acid analysis of liver, small intestine, plasma, and liver microsomes.

In general, the extent of incorporation of TFA varied for different tissues and lipid classes as expected. The results support a number of other studies showing that the fatty acid composition of diverse classes of tissues mirrored the lipid content of the diet (Decker and Mertz 1966, Hill et al. 1982, Moore et al. 1980, Ostlund-Lindqvist et al. 1985, Sgoutas et al. 1973, Sugano et al. 1984, Wood 1979, Wood et al. 1977). Due to the low content of elaidic acid in the diet (19 mole%) of the present study, the measured elaidic acid content in the studied tissues and lipid classes was much lower than that reported in the previous studies, where elaidic acid made up 25 to 50% of the TFA diets. It should be noted that elaidic acid was readily incorporated into the plasma and microsome over a 2-week feeding period (Experiment 2) as compared to other studies that fed TFA for 4 to 16 weeks (Blomstrand et al. 1985, Kirstein et al. 1983, Mahfouz et al. 1984, Moore et al. 1980, Morgado et al. 1998, Sgoutas et al. 1973, Sugano et al. 1984).

Elaidic acid variously influenced the distribution of other fatty acids depending on the tissue and lipid fraction studied. It has been shown that the pattern of incorporation of elaidic acid resembled that of SFA in the PL and that of the of CFA isomer (oleic acid) in the NL (Atal et al. 1994). This incorporation pattern was observed in the PL of liver and hepatic microsome, and the NL of liver and small intestine. However, in the PL of small intestine and the NL of liver microsome, feeding of TFA to rats resulted in decreased palmitic, stearic, and oleic acids as compared to feeding of CFA, suggesting that elaidic acid replaced both SFA and MUFA in these lipid fractions. Differences in the deposition and incorporation of TFA into tissue lipids in animals fed the same dietary fats may involve selective mechanisms. PUFA are incorporated exclusively into the 2-position of

membrane PL, while SFA are incorporated into the 1-position. More than 90% of elaidic acid and linoelaidic acid are incorporated into the 1-position of rat liver PL in place of SFA (Wolff and Entressangles 1994). However, it was found that elaidic acid was also preferentially incorporated into the 2-position of PL (Wood 1979). Therefore, acyl transferases that esterify fatty acids to the 1- and 2-position are sensitive to the configuration of fatty acids and may contribute to the differences in TFA deposition.

The dietary lipids related effect on linoleic and arachidonic acids were also observed in the present study. In Experiment 1, the PL of the liver (**Table 6-4**) in rats fed the TFA diet had a higher content of linoleic acid and a lower content of arachidonic acid than those found in rats fed the CFA diet. In Experiment 2, both the plasma and liver microsomes of the TFA group had significantly more linoleic acid than the CFA group, while arachidonic acid levels were not statistically different between the 2 dietary groups. This change could not be attributed to different levels of these fatty acids in the diets because the TFA diet contained less or the same content of linoleic acid than the CFA diet and neither diet contained arachidonic acid. This observation indicates the potential for inhibition by TFA in the conversion of linoleic acid to arachidonic acid. It has been shown in the previous studies that trans isomers of oleic acid exert a competitive inhibition on the activity of Δ6-desaturase (De Schrijver and Privett 1982, Kurata and Privett 1980, Lawson et al. 1983, Mahfouz et al. 1980;1984). However, α-linolenic acid, a more preferable substrate for Δ6-desaturase than linoleic acid (Sugano and Ikeda 1996), was higher in the TFA diet than in the CFA diet and thus might result in decreased conversion of linoleic acid to arachidonic acid.

Exposure to ethanol also has been shown to increase the ratio of linoleate to

arachidonate in the rat liver (French et al. 1970, Mendenhall et al. 1969, Nanji et al. 1993, Salem et al. 1996). Moreover, the liver microsomal linoleic acid concentration was lower in rats fed saturated fats and ethanol than that of animal fed corn oil and ethanol (Nanji et al. 1994a). The definite mechanism for the ethanol-related alteration in the concentrations of linoleic and arachidonic acids is not clear. However, this phenomenon may be in part explained by ethanol inhibition of Δ6- and Δ5-desaturases, which are responsible for the conversion of linoleic acid to arachidonic acid (Wang and Reitz 1983). In addition, lipid peroxidation has been demonstrated to be initiated in the hepatic endoplasmic reticulum in rats during the metabolism of ethanol (Reinke et al. 1987). Arachidonic acid is a component of cell membranes and is susceptible to lipid peroxidation (Szebeni and Eskelson 1989). In rats with continuous ethanol infusion, reduced arachidonic acid in hepatic microsomal membrane was positively correlated with raised CYP2E1 content ($r = 0.69$, $P < 0.01$) and increased conjugated dienes in the liver microsome ($r = 0.70$, $P < 0.05$) (Nanji et al. 1993). The content of liver microsomal arachidonic acid was decreased in rats fed corn oil and ethanol compared to those fed only corn oil (Nanji et al 1994a). Therefore, it was suggested that ethanol-induced lipid peroxidation in microsomes could play a role in the decrease of arachidonic acid (Nanji et al. 1993). In Experiment 1, rats fed the TFA diet for 6 weeks had an increased linolenate to arachidonate ratio in the liver PL compared to those fed the SFA and CFA diets (**Table 6-4**). The elevated ratios of linolenate to arachidonate was also observed in the plasma and liver microsomes of rats fed the TFA diet for 2 weeks (**Tables 6-10, 6-12, and 6-14**). The exact relevance of these changes in fatty acid composition with regard to altered ethanol clearance is unknown. Because the microsomal ethanol oxidizing system

(cytochrome P450) is a membrane bound enzyme system, the incorporation of TFA into the microsomal PL of the P450 system may alter the activity of CYP2E1 and thereby change the ethanol metabolism. The lowered BEC in rats fed the TFA diet as shown in Chapter 3 might be due to enhanced CYP2E1 activity. While the relationship between the dietary and microsomal fatty acid patterns is evident from the data of fatty acid analyses, the mechanism by which these changes are affecting blood ethanol clearance in not understood.

In summary, the fatty acid composition of tissues were highly reflective of the dietary fatty acid composition. The increased ratio of linoleate to arachidonate observed in the plasma and liver microsomes of TFA-fed rats supports the inhibition of desaturation and elongation of linoleic acid to arachidonic acid. This shift in the ratio of linoleic acid to arachidonic acid has been shown to increase ethanol-induced liver disease which may be accompanied by relatively faster rates of ethanol metabolism similar to that seen in the TFA group.

CHAPTER VII

CONCLUSIONS

The effects of dietary saturated fatty acids (SFA), cis fatty acids (CFA), and trans fatty acid (TFA) on blood ethanol clearance were determined in 5-week-old male Sprague-Dawley rats. Various fats, oils, and one fatty acids were blended to generate diets of comparable fatty acid content.

Two experiments were conducted to establish the effects of the types of dietary fatty acids on the blood ethanol concentrations (BEC). In Experiment 1, after 2 weeks of feeding, the BEC of SFA and CFA groups were higher than those of TFA group. However, these differences in BEC were not significant after 4 and 6 weeks of feeding the diets. Experiment 2 confirmed the decrease in BEC caused by the TFA diets in rats after 2 weeks of dietary treatment. Therefore, it is concluded that the TFA diet lowered BEC compared to the SFA or CFA diets. In addition, liver triglycerides were significantly decreased in the TFA group, which suggests that TFA may offer protection against fatty liver.

Possible mechanisms of dietary fatty acids related changes on BEC were examined in these animals. It was found that TFA enhanced blood ethanol clearance in comparison to SFA or CFA without affecting the hepatic alcohol dehydrogenase (ADH) activity after either 2 or 6 weeks of dietary treatment. The activity of carnitine palmitoyl transferase-I (CPT-I) was not affected by the types of dietary fatty acids. The urinary ethanol excretion (mg ethanol/mL urine) was higher in the TFA group than in the CFA

group during the first 4-hour post-ethanol administration, which may have contributed to the lower BEC in the TFA group. The concentrations of carnitines changed parallel with BEC among the dietary groups which are consistent with the fact that carnitine attenuates blood ethanol clearance.

The lower BEC maintained in the TFA group compared to the SFA and CFA groups may have been due to differences in the absorption of ethanol from the gastrointestinal tract. Therefore, the differences in ethanol uptake and metabolism in the small intestines was determined in everted intestinal sacs. Ethanol transport expressed as the ratio of serosal to mucosal ethanol concentrations and the *in vitro* absorption rate of ethanol was not significantly affected by dietary fatty acids. However, when the amount of ethanol metabolized to CO_2 during the transport process was taken into account, uptake and metabolism of ethanol by the proximal segments of the small intestine were significantly higher in the rats fed the TFA and CFA diet than in those fed the SFA diet. Because ethanol oxidation was not significantly different between CFA and TFA group, ethanol transport and metabolism in the small intestine can not be a mechanism and some other mechanism must be responsible for the differences in BEC between the CFA and TFA groups. Nonetheless, it is recognized that ethanol metabolism in extrahepatic tissues is important and must be reexamined in this context.

Analyses of fatty acid composition revealed that the fatty acid composition of various tissues was highly reflective of the fatty acid content of the diets. Elaidic acid was well incorporated into the adipose tissues, liver, skeletal muscle, small intestine, plasma, and hepatic microsomes of the TFA group after 2 or 6 weeks of dietary treatment. Compared with rats fed the CFA diet, the fatty acid composition in the

plasma, liver NL, and liver microsomes of rats fed the TFA diet was characterized by a significant decrease in palmitic, stearic, and oleic acids and an increase in linoleic acid. The higher linoleate to arachidonate ratio in the TFA group compared to the CFA group supports the suggestion that TFA, and elaidic acid in particular, may interfere with the conversion of linoleic acid to arachidonic acid which has been shown to protect liver from alcohol-induced injury.

LITERATURE CITED

Abbey, M. & Nestel, P. J. (1994) Plasma cholesteryl ester transfer protein activity is increased when trans-elaidic acid is substituted for cis-oleic acid in the diet. Atherosclerosis 106: 99-107.

Adamo, S., Siliprandi, N., Di Lisa, F., Carrara, M., Azzurro, M., Sartori, G., Vita, G. & Ghidini, O. (1988) Effect of L-carnitine on ethanol and acetate plasma levels after oral administration of ethanol in humans. Alcohol Clin. Exp. Res. 12: 653-654.

Adlof, R. O. & Emken, E. A. (1986) Distribution of hexadecenoic, octadecenoic and octadecadienoic acid isomers in human tissue lipids. Lipids 21: 543-547.

Agarwal, D. P. & Goedde, H. W. (1992) Pharmacogenetics of alcohol metabolism and alcoholism. Pharmacogenetics 2: 48-62.

Alam, S. Q., Ren, Y.-F. & Alam, B. S. (1989) Effect of dietary trans fatty acids on some membrane-associated enzymes and receptors in rat heart. Lipids 24: 39-44.

Ali, L., Angyal, G., Weaver, C. & Rader, J. (1996) Determination of saturated and cis and trans unsaturated fatty acids in foods using capillary column-gas chromatography. FASEB J. 10: A794.

Almendingen, K., Jordal, O., Kierulf, P., Sandstad, B. & Pedersen, J. I. (1995) Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on serum lipoproteins and Lp[a] in men. J. Lipid Res. 36: 1370-1384.

Arnon, R., Esposti, S. D. & Zern, M. A. (1995) Molecular biological aspects of alcohol-induced liver disease. Alcohol. Clin. Exp. Res. 19: 247-256.

Aro, A., Jauhiainen, M., Partanen, R., Salminen, I. & Mutanen, M. (1997) Stearic acid, trans fatty acids, and dairy fat: effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein(a), and lipid transfer proteins in healthy subjects. Am. J. Clin. Nutr. 65: 1419-1426.

Aro, A., Kardinaal, A. F. M., Salminen, I., Kark, J. D., Riemersma, R. A., Delgado-Rodriguez, M., Gomez-Aracena, J., Huttunen, J. K., Kohlmeier, L., Martin, B. C., Martin-Moreno, J. M., Mazaev, V. P., Ringstad, J., Thamm, M., van't Veer, P. & Kok, F. J. (1995) Adipose tissue isomeric trans fatty acids and risk of myocardial infarction in nine countries: the EURAMIC study. Lancet 345: 273-278.

Ascherio, A., Hennekens, C. H., Buring, J. E., Master, C., Stampfer, M. J. & Willett, W. C. (1994) Trans-fatty acids intake and risk of myocardial infarction. Circulation 89: 94-101.

ASCN/AIN Task force on trans fatty acids. (1996) Position paper on trans fatty acids. Am. J. Clin. Nutr. 63: 663-670.

Atal, S., Zamowski, M. J., Cushman, S. W. & Sampugna, J. (1994) Comparison of body weight and adipose tissue in male c57b1/6j mice fed diets with and without trans fatty acids. Lipids 29: 319-325.

Bakker, N., van't Veer, P., Zock, P. L. & the EURAMIC study group. (1997) Adipose fatty acids and cancers of the breast, prostate and colon: an ecological study. Int. J. Cancer 72: 587-591.

Baraona, E., Julkunen, R., Tannenbaum, L. & Lieber, C. S. (1986) Role of intestinal bacterial overgrowth in ethanol production and metabolism in rats. Gastroenterology 90: 103-110.

Barbour, A. D. (1933) The deposition and utilization of hydrogenation isooleic acid in the animal body. J. Bio. Chem. 101: 63-72.

Batt, R. D. (1989) Absorption, distribution, and elimination of alcohol. In: Human metabolism of alcohol (Crow, K. E. & Batt, R. D., eds.), vol. 1, Pharmacokinetics, medicolegal aspects, and general interest, pp. 3-8. CRC Press, Boca Raton, FL.

Beare-Rogers, J. L. (1983) Trans- and positional isomers f common fatty acids. Adv. Nutr. Res. 5: 171-200.

Beisswenger, T.B., Holmquist, B. & Vallee, B. L. (1985) χ-ADH is the sole alcohol dehydrogenase isozyme of mammalian brains: implications and inferences. Proc. Natl. Acad. Sci. USA 82: 8369-8373.

Berger, R. & Sachan, D. S. (1986) Elevation of blood-ethanol concentrations in carnitine-supplemented rats. Nutr. Rep. Int. 34: 153-157.

Berger, R. & Sachan, D. S. (1991) Effects of supplementary levels of L-carnitine on blood and urinary carnitines and on the portal-systemic blood-ethanol concentrations in the rat. J. Nutr. Biochem. 2: 382-386.

Bergmeyer, H. U., Gawehn, K. & Grassal, M. (1974) Enzymes as biological reagents. In: Methods of enzymatic analysis (Bergmeyer, H. U., ed.), pp. 428-430. Verlag Chemic Int., Deerfield Beach, FL.

Bernard, A., Echinard, B. & Carlier, H. (1987) Differential intestinal absorption of two fatty acid isomers: elaidic and oleic acids. Am. J. Physiol. 253: G751-G759.

Bernt, E. & Gutmann, I. (1974) Ethanol determination with alcohol dehydrogenase and NAD. In: Methods of enzymatic analysis (Bergmeyer, H. U., ed.), pp. 1499-1502. Verlag Chemic Int., Deerfield Beach, FL.

Bieber, L. L. (1988) Carnitine. Ann. Rev. Biochem. 57: 261-283.

Black, S. D. & Coon, M. J. (1987) P-450 cytochromes: structure and function. Adv. Enzymol. Relat. Areas Mol. Biol. 60:35-87.

Blackmore, D. J. & Mason, J. K. (1968) Renal clearance of urea, creatinine and alcohol. Med. Sci. Law 8: 50-53.

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

Blomstrand, R., Diczfalusy, U., Sisfontes, L. & Svensson, L. (1985) Influence of dietary partially hydrogenated vegetable and marine oils on membrane composition and function of liver microsomes and platelets in the rat. Lipids 20: 283-295.

Bøhmer, T. & Mølstad, P. (1980) Carnitine transport across the plasma membrane. In: Carnitine biosynthesis, metabolism, and functions (Frenkel, R. A. & McGarry, J. D., eds.), pp. 73-89. Academic Press, New York, NY.

Boleda, M. D., Julià, P., Moreno, A. & Parés, X. (1989) Role of extrahepatic alcohol dehydrogenase in rat ethanol metabolism. Arch. Biochem. Biophys. 274: 74-81.

Borlak, J. T. & Welch, V. A. (1994) Health implications of fatty acids. Arzneim. Forsch. Drug Res. 44: 976-981.

Bosron, W. F., Li, T.-K., Dofeldecker, W. P. & Vallee, B. L. (1979) Human liver π-alcohol dehydrogenase: kinetic and molecular properties. Biochemistry 18: 1101-1105.

Bosron, W. F., Lumeng, L. & Li, T.-K. (1988) Genetic polymorphism of enzymes of alcohol metabolism and susceptibility to alcoholic liver disease. Mol. Aspects Med. 10: 147-158.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Brändén, C.-I., Jörnvall, H., Eklund, H. & Furugren, B. (1975) Alcohol dehydrogenases. In: The enzymes, (Boyer, P. D., ed.), vol. 11, Oxidation-reduction, part A: dehydrogenase (I), electron transfer (I), pp. 103-190. Academic Press, New York, NY.

Bremer, J. (1981) The effect of fasting n the activity f liver carnitine palmitoyltransferase and its inhibition by malonyl-CoA. Biochim. Biophys. Acta 665: 628-631.

Bunout, D., Petermann, M., Ugarte, G., Barrera, G. & Iturriaga, H. (1987) Nitrogen economy in alcoholic patients without liver disease. Metabolism 36: 651-653.

Burnell, J. C. & Bosron, W. F. (1989) Genetic polymorphism of human liver alcohol dehydrogenase and kinetic properties of the isoenzymes. In: Human metabolism of alcohol (Crow, K. E. & Batt, R. D., eds.), vol. 2, Regulation, enzymology, and metabolites of ethanol, pp. 65-75. CRC Press, Boca Raton, FL.

Carter, A. L., Abney, T. O. & Lapp D. F. (1995) Biosynthesis and metabolism of carnitine. J. Child Neurol. 10: 23S-27S.

Carter, E. A. & Isselbacher, K. J. (1971) The metabolism of ethanol to carbon dioxide by stomach and small intestinal slices. Proc. Soc. Exp. Biol. Med. 138: 817-819.

Castro, G. A. (1981) Principles of digestion and absorption. In: Gastrointestinal physiology (Johnson, L. R., ed.), pp. 95-106. Second edition. The C. V. Mosby Co., St. Louis, MI.

Castro, J., Cortés, J. P. & Guzmán (1991) Properties of the mitochondrial membrane and carnitine palmitoyltransferase I in the periportal and the perivenous zone of the liver. Biochem. Pharmacol. 41: 1987-1995.

Cederbladt, G. & Lindstedt, S. (1972) A method for determination of carnitine in picomole range. Clin. Clim. Acta 37: 335-343.

Cha, Y. S. & Sachan, D. S. (1994) Opposite effects of dietary saturated and unsaturated fatty acids on ethanol-pharmacokinetics, triglycerides, and carnitine. J. Am. Coll. Nutr. 13: 338-343.

Cha, Y. S. & Sachan, D. S. (1995) Acetylcarnitine-mediated inhibition of ethanol oxidation in hepatocytes. Alcohol 12:289-294.

Chern, J. C. & Kinsella, J. E. (1983) The effects of unsaturated fatty acids on the synthesis of arachidonic acid in rat kidney cells. Biochim. Biophys. Acta 750: 465-471.

Chiang, M. T. & Lu, Y. S. (1996) Variation of plasma cholesterol levels in rats fed trans fatty acids or cis fatty acids. Int. J. Vit. Nutr. Res. 66: 263-269.

Choi, Y. R. & Bieber, L. L. (1977) A method for the isolation, identification, and quantitation of water-soluble aliphatic acylcarnitines. Anal. Biochem. 79: 413-418.

Christiansen, E. N., Gary, T. J. B. & Lake B. G. (1985) Effect of vary long chain fatty acids on peroxisomal β-oxidation in primary rat hepatocyte cultures. Lipids; 20: 929-932.

Clandinin, M. T., Cheema, S., Field, C. J., Garg, M. L., Venkatraman, J. & Clandinin, T. R. (1991) Dietary fat: exogenous determination of membrane structure and cell function. FASEB J. 5: 2761-2769.

Clement, J., Lavoue, G. & Clement G. (1965) Structure of the intestinal mucosa and lymph glycerides of rats after absorption of fats containing elaidic acid. J. Am. Oil Chem. Soc. 42: 1035-1037.

Clevidence, B. A., Judd, J. T., Schaefer, E. J., Jenner, J. L., Lichtenstein, A. H., Muesing, R. A., Wittes, J. & Sunkin, M. E. (1997) Plasma lipoprotein (a) levels in men and women consuming diets enriched in saturated, cis-, or trans-monounsaturated fatty acids. Arterioscler. Thromb. Vasc. Biol. 17: 1657-1661.

Cook, H. W. (1979) Incorporation and metabolism of the dietary trans-unsaturated fatty acid, elaidic acid, by developing rat brain. J. Neurochem. 32: 515-519.

Cooke, A. R. & Birchall, A. (1969) Absorption of ethanol from the stomach. Gastroenterology 57: 269-272.

Coots, R. H. (1964) A comparison of the metabolism of elaidic, oleic, palmitic, and stearic acids in the rats. J. Lipid Res. 5: 468-472.

Cromer, K. D., Jenkins, T. C. & Thies, E. J. (1995) Replacing cis octadecenoic acid with trans isomers in media containing rat adipocytes stimulates lipolysis and inhibits glucose utilization. J. Nutr. 125: 2394-2399.

Danielsson, O., Atrian, S., Luque, T., Hjelmqvist, L., Gonzàlez-Duarte, R. & Jörnvall, H. (1994) Fundamental molecular differences between alcohol dehydrogenases classes. Proc. Natl. Acad. Sci. USA 91: 4980-4984.

Decker, W. J. & Mertz, W. (1966) Incorporation of dietary elaidic acid in tissues and effects on fatty acid distribution. J. Nutr. 89: 165-170.

Decsi, T. & Koletzko, B. (1994) Polyunsaturated fatty acids in infant nutrition. Acta Paediatr. 395: S31-S37.

Decsi, T. & Koletzko, B. (1995) Do trans fatty acids impair linoleic acid metabolism in children? Ann. Nutr. Metab. 39: 36-41.

Degtyarenko, K. N. & Archakov, A. I. (1993) Molecular evolution of P450 superfamily and P450-containing monooxygenase systems. FEBS Lett. 332: 1-8.

De Schrijver, R. & Privett, O. S. (1982) Interrelationship between dietary trans fatty acids and the 6- and 9-desaturases in the rat. Lipids 17: 27-34.

De Schrijver, R. & Privett, O. S. (1984) Energetic efficiency and mitochondrial function in rats fed trans fatty acids. J. Nutr. 114: 1183-1191.

Di Padova, C., Worner, T. M., Julkunen, R. J. K. & Lieber, C. S. (1987) Effects of fasting and chronic alcohol consumption on the first-pass metabolism of ethanol. Gastroenterology 92: 1169-1173.

Dupont, J., White, P. J. & Feldman, E. B. (1991) Saturated and hydrogenated fats in food in relation to health. J. Am. Coll. Nutr. 10: 577-592.

Dutton, H. J. (1979) Hydrogenation of fats and its significance. In: Geometrical and positional fatty acid isomers (Emken, E. A. & Dutton, H. J., ed.), pp. 1-16. American Oil Chemists' Society, Champaign, IL.

Egwim, P. O. & Sgoutas, D. S. (1972) Effect of hydrogenated soybean fat on rat adipose tissue and on liver acetyl-CoA carboxylase and fatty acid synthetase activities. Am. J. Clin. Nutr. 25: 16-22.

Emken, E. A. (1984) Nutrition and biochemistry of trans and positional fatty acid isomers in hydrogenated oils. Ann. Rev. Nutr. 4: 339-376.

Emken, E. A. (1991) Do trans acids have adverse health consequences? In: Health effects of dietary fatty acids (Nelson, G. J., ed.), pp. 245-263. AOCS, Champaign. IL.

Emken, E. A. (1995) Trans fatty acids and coronary heart disease risk: physicochemical properties, intake, and metabolism. Am. J. Clin. Nutr. 62: 659S-669S.

Enig, M. G. (1996) Trans fatty acids in diets and databases. Cereal Food World 41: 58-63.

Enig, M. G., Atal, S., Keeney, M. & Sampugna, J. (1990) Isomeric trans fatty acids in the U.S. diet. J. Am. Coll. Nutr. 9: 471-486.

Enig, M. G., Pallansch, L. A., Sampugna. J. & Keeney, M. (1983) Fatty acid composition of the fat in selected food items with emphasis on trans components. J. Am. Oil Chem. Soc. 60: 1788-1795.

Fan, F. & Plapp, B. V. (1995) Substitutions of isoleucine residues at the adenine binding site activate horse liver alcohol dehydrogenase. Biochemistry 34: 4709-4713.

Farrés, J., Wang, T. T. Y., Cunningham, S. J. & Weiner, H. (1995) Investigation of the active site cysteine residue of rat liver mitochondrial aldehyde dehydrogenase by site-directed mutagenesis. Biochemistry 34: 2592-2598.

Feierman, D. E., Winston, G. W. & Cederbaum, A. I. (1985) Ethanol oxidation by hydroxyl radicals: role of iron chelates, superoxide, and hydrogen peroxide. Alcohol. Clin. Exp. Res. 9: 95-102.

Feinman, L. & Lieber, C. S. (1992) Nutrition: medical problems of alcoholism. In: Medical and nutritional complications of alcoholism: mechanisms and management (Lieber, C. S. ed.), pp. 515-530. Plenum medical book company, New York, NY.

Feinman, L., Korsten, M. A. & Lieber, C. S. (1992) Alcohol and digestive tract. In: Medical and nutritional complications of alcoholism: mechanisms and management (Lieber, C. S. ed.), pp. 307-340. Plenum medical book company, New York, NY.

Feller, A. G. & Rudman, D. (1988) Role of carnitine in human nutrition. J. Nutr. 118: 541-547.

Fita, I. & Rossmann, M. G. (1985) The NADPH binding site on beef liver catalase. Proc. Natl. Acad. Sci. USA 82: 1604-1608.

Food and Agriculture Organization (FAO). (1994) Global trends in the availability of edible fats and oils. In: Fats and oils in human nutrition (FAO/WHO ed.), pp. 25-32. FAO, Rome, Italy.

Foulkes, E. C. (1996) Slices and sacs: limitations on metabolic and functional studies in kidney cortex and intestine. Proc. Soc. Exp. Biol. Med. 211: 155-162.

French, S. W. (1993) Nutrition in the pathogenesis of alcoholic liver disease. Alcohol Alcohol. 28: 97-109.

French, S. W., Ihrig, T. J. & Morin R. J. (1970) Lipid composition of RBC ghosts, liver mitochondria and microsomes of ethanol-fed rats. Q. J. Stud. Alcohol. 31: 801-809.

French, S. W., Miyamoto, K. & Tsukamoto, H. (1986) Ethanol-induced hepatic fibrosis in the rat: role of the amount of dietary fat. Alcohol. Clin. Exp. Res. 10: 13S-19S.

Garland, M., Sacks, F. M., Colditz, G. A., Rimm, E. B., Sampson, L. A., Willett, W. C. & Hunter, D. J. (1998) The relation between dietary intake and adipose tissue composition of selected fatty acids in US women. Am. J. Clin. Nutr. 67: 25-30.

Giegel, J. L., Ham, A. B. & Clema, W. (1975) Manual and semiautomated procedure for measurement of triglycerides in serum. Clin. Chem. 21: 1575-1581.

Gillman, M. W., Cupples, A., Gagnon, D., Millen, B. E., Ellison, R. C. & Castelli, W. P. (1997) Margarine intake and subsequent coronary heart disease in men. Epidemiology 8: 144-149.

Goldstein, D. B. (1983) Absorption, distribution, and elimination of ethanol. In: Pharmacology of alcohol (Goldstein, D. B., ed.), pp. 3-17. Oxford University Press, New York, NY.

Gonzalez, F. J. (1992) Human cytochromes P450: problems and prospects. Trends Pharmacol. Sci. 13: 346-352.

Gruchow, H. W., Sobocinski, K. A., Barboriak, J. J. & Scheller, J. G. (1985) Alcohol consumption, nutrient intake and relative body weight among US adults. Am. J. Clin. Nutr. 42: 289-295.

Guzmán, M. & Geelen, M. J. H. (1988) Effects of ethanol feeding on the activity and regulation of hepatic carnitine palmitoyltransferase I. Arch. Biochem. Biophys. 267: 580-588.

Guzmán, M., Castro, J. & Maquedano, A. (1987) Ethanol feeding to rats reversibly decreases hepatic carnitine palmitoyltransferase activity and increases enzyme sensitivity to malonyl-CoA. Biochem. Biophys. Res. Commun. 149: 443-448.

Halliwell, B. & Gutteridge, J. M. (1986) Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch. Biochem. Biophys. 246: 501-514.

Hawkins, R. D. & Kalant, H. (1972) The metabolism of ethanol and its metabolic effects. Pharmacol. Rev. 24:67-157.

Hayashi, K., Hirata, Y., Kurushima, H., Saeki, M., Amioka, H., Nomura, S., Kuga, Y., Ohkura, Y., Ohtani, H. & Kajiyama, G. (1993) Effect of dietary hydrogenated corn oil (trans-octadecenoate rich oil) on plasma and hepatic cholesterol metabolism in the hamster. Atherosclerosis 99: 97-106.

Heckers, H., Korner, M., Tuschen, T. W. L. & Melcher, F. W. (1977) Occurrence of individual trans-isomeric fatty acids in human myocardium, jejunum and aorta in relation to different degrees of atherosclerosis. Atherosclerosis 28: 389-398.

Henry, R. J. (1967) Determination of creatine and creatinine. In: Clinical chemistry: principles and techniques (Henry, R. J., ed.), pp. 292-296. Harper and Row, New York, NY.

Hietanen, E., Hänninen, M., Laitinen, M. & Lang, M. (1978) Regulation of hepatic drug metabolism by elaidic and linoleic acids in rats. Enzyme 23:127-134.

Hill, E. G., Johnson, S. B., Lawson, L.D., Mahfouz, M. M. & Holman, R. T. (1982) Perturbation of the metabolism of essential fatty acids by dietary partially hydrogenated vegetable oil. Proc. Natl. Acad. Sci. USA 79: 953-957.

Hillers, V. N. & Massey, L. K. (1985) Interrelationships of moderate and high alcohol consumption with diet and health status. Am. J. Clin. Nutr. 41: 356-362.

Höög, J.-O. & Brandt, M. (1995) Mammalian class VI alcohol dehydrogenase: novel types of the rodent enzymes. Adv. Exp. Med. Biol. 372: 355-364.

Horowitz, M., Maddox, A., Bochner, M., Wishart, J., Bratasiuk , R., Collins, P. & Shearman, D. (1989) Relationships between gastric emptying of solid and caloric liquid meals and alcohol absorption. Am. J. Physiol. 257: G291-G298.

Hosein, E. A. & Bexton, B. (1975) Protective action of carnitine on liver lipid metabolism after ethanol administration to rats. Biochem. Pharmacol. 24: 1859-1863.

Hsu, C. M. L. & Kummerow, F. A. (1977) Influence of elaidate and erucate on heart mitochondria. Lipids 12: 486-494.

Hu, F. B., Stampfer, M. J., Manson, J. E., Rimm, E., Colditz, G. A., Rosner, B. A., Hennekens, C. H. & Willett, W. C. (1997) Dietary fat intake and the risk of coronary heart disease in women. N. Engl. J. Med. 337: 1491-1499.

Hudgins, L. C., Hirsch, J. & Emken, E. A. (1991) Correlation of isomeric fatty acids in human adipose tissue with clinical risk factors for cardiovascular disease. Am. J. Clin. Nutr. 53: 474-482.

Hunter, E. & Applewhite, T. H. (1991) Reassessment of trans fatty acid availability in the US diet. Am. J. Clin. Nutr. 54: 363-369.

Ide, T., Watanabe M., Sugano, M. & Yamamoto, I. (1987) Activities of liver mitochondrial and peroxisomal fatty acid oxidation enzymes in rats fed trans fat. Lipids 22: 6-10.

Ip, C. (1997) Review of the effects of trans fatty acids, oleic acid, n-3 polyunsaturated fatty acids, and conjugated linoleic acid on mammary carcinogenesis in animals. Am. J. Clin. Nutr. 66: 1523S-1529S.

Ip, C. & Marshall, J. R. (1996) Trans fatty acids and cancer. Nutr. Rev. 54: 138-145.

Jeloková, J., Karlsson, C., Estonius, M., Jörnvall, H. & Höög, J.-O. (1994) Features of structural zinc in mammalian alcohol dehydrogenase. Site-directed mutagenesis of the zinc ligands. Eur. J. Biochem. 225: 1015-1019.

Jenkins, T. C. (1994) Regulation of lipid metabolism in the rumen. J. Nutr. 124: 1372S-1376S.

Johnston, P. V., Johnson, O. C. & Kummerow, F. A. (1957) Occurrence of trans fatty acids in human tissue. Science 126: 698-699.

Johnston, P. V., Kummerow, F. A. & Walton, C. H. (1958) Origin of the trans fatty acids in human tissue. Proc. Soc. Exp. Biol. Med. 99: 735-736.

Jones, D. P. & Greene, E. A. (1966) Influences of dietary fat on alcoholic fatty liver. Am. J. Clin. Nutr. 18: 350-357.

Judd, J. T., Baer, D. J., Clevidence, B. A., Muesing, R. A., Chen, S. C., Weststrate, J. A., Meijer, G. W., Wittes, J., Lichtenstein, A. H., Vilella-Bach, M. & Schaefer, E. J. (1998) Effects of margarine compared with those of butter on blood lipid profiles related to cardiovascular disease risk factors in normolipemic adults fed controlled diets. Am. J. Clin. Nutr. 68: 768-777.

Judd, J. T., Clevidence, B. A., Muesing, R. A., Wittes, J., Sunkin, M. E. & Podczasy, J. J. (1994) Dietary trans fatty acids: effects on plasma lipids and lipoproteins of healthy men and women. Am. J. Clin. Nutr. 59: 861-868.

Julkunen, R. J. K., Di Padova, C. & Lieber, C. S. (1985a) First pass metabolism of ethanol - a gastrointestinal barrier against the systemic toxicity of ethanol. Life Sci 37: 567-573.

Julkunen, R. J. K., Tannenbaum, L., Baraona, E. & Lieber, C. S. (1985b) First pass metabolism of ethanol: an important determinant of blood levels after alcohol consumption. Alcohol 2: 437-441.

Kamlay, M. T. & Shore, J. D. (1983) Transient kinetic studies of substrate inhibition in the horse liver alcohol dehydrogenase reaction. Arch. Biochem. Biophys. 222: 59-66.

Kanayama, R., Takase, S., Matsuda, Y. & Takada, A. (1984) Effect of dietary fat upon ethanol metabolism in rats. Biochem. Pharmacol. 33: 3283-3287.

Katan, M. B. (1995) Exit trans fatty acids. Lancet 346: 1245-1246.

Katan, M. B. (1998) Health effects of trans fatty acids. Eur. J. Clin. Invest. 28: 257-258.

Katan, M. B. & Mensink R. P. (1992) Isomeric fatty acids and serum lipoproteins. Nutr. Rev. 50: 46-48.

Katan, M. B., Zock, P. L. & Mensink, R. P. (1995) Trans fatty acids and their effects on lipoproteins in humans. Ann. Rev. Nutr. 473-493.

Kedishvili, N. Y., Bosron, W. F., Stone, C. L., Hurley, T. D., Peggs, C. F., Thomasson, H. R., Popov, K. M., Carr, L. G., Edenberg, H. J. & Li, T.-K. (1995) Expression and kinetic characterization of recombinant human stomach alcohol dehydrogenase. Active-site amino acid sequence explains substrate specificity compared with liver isozymes. J. Biol. Chem. 270: 3625-3630.

Khosla, P. & Hayes, K. C. (1996) Dietary trans-monounsaturated fatty acids negatively impact plasma lipids in humans: critical review of the evidence. J. Am. Coll. Nutr. 15: 325-339.

Khosla, P., Hajri, T., Pronczuk, A. & Hayes, K. C. (1997) Replacing dietary palmitic acid with elaidic acid (t-C18:1Δ9) depresses HDL and increases CETP activity in cebus monkeys. J. Nutr. 127: 531S-536S.

Kirstein, D., Høy, C.-E. & Hølmer, G. (1983) Effect of dietary fats on the Δ6- and Δ5-desaturation of fatty acids in rat liver microsomes. Br. J. Nutr. 50: 749-756.

Kohlmeier, L., Simonsen, N., van't Veer, P., Strain, J. J., Martin-Moreno, J. M., Margolin, B., Huttunen, J. K., Navajas, J. F.-C., Martin, B. C., Thamm, M., Kardinaal, A. F. M. & Kok, F. J. (1997) Adipose tissue trans fatty acids and breast cancer in the European community multicenter study on antioxidants, myocardial infarction, and breast cancer. Cancer Epidemiol. 6: 705-710.

Koletzko, B. (1992) Trans fatty acids may impair biosynthesis of long-chain polyunsaturates and growth in man. Acta Paediatr. Scand. 81: 302-306.

Koop, D. R. (1989) Minor pathways of ethanol metabolism. In: Human metabolism of alcohol (Crow, K. E. & Batt, R. D., eds.), vol. 2, Regulation, enzymology, metabolites of ethanol, pp. 133-145. CRC Press, Boca Raton, FL.

Koop, D. R. (1992) Oxidative and reductive metabolism by cytochrome P450 2E1. FASEB J. 6: 724-730.

Koop, D. R. & Tierney, D. J. (1990) Multiple mechanisms in the regulation of ethanol-inducible cytochrome P450IIE1. Bioessays 12: 429-435.

Korsten, M. A., Pirola, R. C. & Lieber, C. S. (1992) Ethanol and the pancreas. In: Medical and nutritional complications of alcoholism: mechanisms and management (Lieber, C. S. ed.), pp. 341-358. Plenum Medical Book Company, New York, NY.

Krebs, H. A. & Perkins, J. R. (1970) The physiological role of liver alcohol dehydrogenase. Biochem. J. 118: 635-644.

Kricka, L. J. & Clark, P. M. S. (1979) Absorption, excretion and metabolism of ethanol. In: Biochemistry of alcohol and alcoholism (Kricka, L. J. & Clark, P. M. S., eds.), pp. 30-53. Ellis Horwood Limited, Chichester, England.

Kris-Etherton, P. M. & Nicolosi, R. J. (1995) Trans fatty acids and coronary heart disease risk. Washington, D.C.: International life science institute.

Kromhout, D., Menotti, A., Bloemberg, B., Aravanis, C., Blackburn, H., Buzina, R., Dontas, A. S., Fidanza, F., Giampaoli, S., Jansen, A., Karvonen, M., Katan, M., Nissinen, A., Nedeljkovic, S., Pekkanen, J., Pekkarinen, M., Punsar, S., Räsänen, L., Simic, B. & Toshima, H. (1995) Dietary saturated and trans fatty acids and cholesterol and 25-year mortality from coronary heart disease: the seven countries study. Preventive Med. 24: 308-315.

Kummerow, F. A. (1974) Current studies on relation of fat to health. J. Am. Oil. Chem. Soc. 51: 255-259.

Kunitoh, S., Asai, H., Imaoka, S., Funae, Y. & Monna, T. (1996) Metabolism of acetaldehyde to acetate by rat hepatic P-450s: presence of different metabolic pathway from acetaldehyde dehydrogenase system. Alcohol. Clin. Exp. Res. 20: 22A-24A.

Kurata, N. & Privett, O. S. (1980) Effects of dietary trans fatty acids on the biosynthesis of arachidonic acid in rat liver microsomes. Lipids 15: 1029-1036.

Lagrost, L. (1992) Differential effects of cis and trans fatty acid isomers, oleic and elaidic acids, on the cholesteryl ester transfer protein activity. Biochim. Biophys. Acta 1124: 159-162.

Lange, L. G., Bergman, S. R. & Sobel, B. E. (1981) Identification of fatty acid ethyl esters as products of rabbit myocardial ethanol metabolism. J. Biol. Chem. 256: 12968-12973.

Lanser, A. C., Emken, E. A. & Ohlrogge, J. B. (1986) Oxidation of oleic and elaidic acids in rat and human heart homogenates. Biochim. Biophys. Acta 875: 510-515.

Laposata, E. A. & Lange, L. G. (1986) Presence of nonoxidative ethanol metabolism in human organs commonly damaged by ethanol abuse. Science 231: 497-499.

Lawson, L. D. & Holman, R. T. (1981) β-oxidation of the geometric and positional isomers of octadecenoic acid by rat heart and liver mitochondria. Biochim. Biophys. Acta 665: 60-65.

Lawson, L. D. & Kummerow, F. A. (1979) β-oxidation of the coenzyme A esters of elaidic, oleic, and stearic acids and their full-cycle intermediates by rat heart mitochondria. Biochim. Biophys. Acta 573: 245-254.

Lawson, L. D., Hill, E. G. & Holman, R. T. (1983) Suppression of arachidonic acid in lipids of rat tissues by dietary mixed isomeric cis and trans octadecenoates. J. Nutr. 113: 1827-1835.

Layne, E. (1957) Spectrophotometric and turbidimetric methods for measuring proteins. III. Biuret method. Methods Enzymol. 3: 450-451.

Li, T-K. (1977) Enzymology of human alcohol metabolism. Adv. Enzymol. 45: 427-483.

Lichtenstein, A. H. (1995) Trans fatty acids and hydrogenated fat - what do we know? Nutr. Today 30: 102-107.

Lichtenstein, A. H. (1997) Trans fatty acids, plasma lipid levels, and risk of developing cardiovascular disease. A statement for healthcare professionals from the American heart Association. Circulation 95: 2588-2590.

Lichtenstein, A. H., Ausman, L. M., Carrasco, W., Jenner, J. L., Ordovas, J. M. & Schaefer, E. J. (1993) Hydrogenation impairs the hypolipidemic effect of corn oil in humans. Atherioscler. Thromb. 13: 154-161.

Lieber, C. S. (1991) Alcohol, liver, and nutrition. J. Am. Coll. Nutr. 10:602-632.

Lieber, C. S. (1994) Alcohol and the liver: 1994 update. Gastroenterology 106: 1085-1105.

Lieber, C. S. (1997) Ethanol metabolism, cirrhosis and alcoholism. Clin. Chim. Acta. 257: 59-84.

Lieber, C. S. & DeCarli, L. M. (1968) Ethanol oxidation by hepatic microsomes: adaptative increase after ethanol feeding. Science 162: 917-918.

Lieber, C. S. & DeCarli, L. M. (1970) Quantitative relationship between amount of dietary fat and severity of alcoholic fatty live. Am. J. Clin. Nutr. 23: 474-478.

Lieber, C. S., Lefevre, A., Spritz, N., Feinman, L. & DeCarli, L. M. (1967) Difference in hepatic metabolism of long- and medium-chain fatty acids: the role of fatty acid chain length in the production of the alcoholic fatty liver. J. Clin. Invest. 46: 1451-1460.

London, S. J., Sacks, F. M., Caesar, J., Stampfer, M. J., Siguel, E. & Willett, W. C. (1991) Fatty acid composition of subcutaneous adipose tissue and diet in

postmenopausal US women. Am. J. Clin. Nutr. 54: 340-345.

Lumeng, L., Bosron, W. F. & Li, T.-K. (1979) Quantitative correlation of ethanol elimination rates in vivo with liver alcohol dehydrogenase activities in fed, fasted and food-restricted rats. Biochem. Pharmacol. 28: 1547-1551.

Mahfouz, M. M., Johnson, S. & Holman, R. T. (1980) The effect of isomeric trans-18:1 acids on the desaturation of palmitic, linoleic and eicosa-8,11,14-trienoic acids by rat liver microsomes. Lipids 15: 101-107.

Mahfouz, M. M., Johnson, S. & Holman, R. T. (1981) Inhibition of desaturation of palmitic, linoleic and eicosa-8,11,14 trienoic acids in vitro by isomeric cis octadecenoic acids. Biochim. Biophys. Acta 663: 58-68.

Mahfouz, M. M., Smith, T. L. & Kummerow, F. A. (1984) Effect of dietary fats on desaturase activities and the biosynthesis of fatty acids in rat-liver microsomes. Lipids 19: 214-222.

Manteca, X., Fernandez, E. & Gonalons, E. (1994) Effects of oleic and elaidic acids on *in vitro* intestinal uptake of cholesterol in the rat. Arch. Int. Physiol. Biochim. Biophys. 102: 231-232.

Mardh, G., Dingley, A. L., Auld, D. S. & Vallee, B. L. (1986) Human class II (π) alcohol dehydrogenase has a redox-specific function in norepinephrine metabolism. Proc. Natl. Acad. Sci. USA 83: 8908-8912.

Mattson, F. H., Hollenbach, E. J. & Kligman, A. M. (1975) Effect of hydrogenated fat on the plasma cholesterol and triglyceride levels of man. Am. J. Clin. Nutr. 28: 726-731.

McGarry, J. D. & Brown, N. F. (1997) The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur. J. Biochem. 244: 1-14.

McManus, I. R., Contag, A. O. & Olson, R. E. (1960) Characterization of endogenous ethanol in the mammal. Science 131; 102-103.

Mendenhall, C., Bradford, R. H. & Furman, R. H. (1969) Effect of ethanol on fatty acid composition of hepatic phosphatidylcholine and phosphatidylethanolamine and on microsomal fatty acyl-CoA: Lysophophatide transferase activities in rats fed corn oil or coconut oil. Biochim. Biophys. Acta 187: 510-519.

Mensink, R. P. & Katan, M. B. (1990) Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. N. Engl. J. Med. 323 :439-445.

Mensink, R. P., Zock, P. L., Katan, M. B., Hornstra, G. (1992) Effect of dietary cis- and trans-fatty acids on serum lipoprotein (a) levels in humans. J. Lipid Res. 33: 1493-1501.

Mertz, W., Tsui, J. C., Judd, J. T., Reiser, S., Hallfrisch, J., Morris, E. R., Steele, P. D. & Lashley, E. (1991) What are people really eating? The relation between energy intake derived from estimated diet records and intake determined to maintain body weight. Am. J. Clin. Nutr. 54: 291-295.

Meydani, S. N., Lichtenstein, A. H., White, P. J., Goodnight, S. H., Elson, C. E., Woods, M., Gorbach, S. L. & Schaefer, E. J. (1991) Food use and health effects of soybean and sunflower oils. J. Am. Coll. Nutr. 10: 406-428.

Mistilis, S. P. & Garske, A. (1969) Induction of alcohol dehydrogenase in liver and gastro-intestinal tract. Australasian Ann. Med. 18: 227-231.

Moore, C. E., Alfin-Slater, R. B. & Aftergood, L. (1980) Incorporation and disappearance of trans fatty acids in rat tissues. Am. J. Clin. Nutr. 33: 2318-2323.

Morehouse, L. A., Thomas, C. E. & Aust, S. D. (1984) Superoxide generation of NADPH-cytochrome P-450 reductase: the effect of iron chelators and the role of superoxide in microsomal lipid peroxidation. Arch. Biochem. Biophys. 232: 366-377.

Morimoto, M., Zern, M. A., Hagbjörk, A.-L., Ingelman-Sundberg, M. & French, S. W. (1994) Fish oil, alcohol, and liver pathology: role of cytochrome P450 2E1. Proc. Soc. Exp. Biol. Med. 207: 197-205.

Morgan, E. T., Koop, D. R. & Coon, M. J. (1982) Catalytic activity of cytochrome P-450 isozyme 3a isolated from liver microsomes of ethanol-treated rabbits. J. Biol. Chem. 257: 13951-13957.

Morgado, N., Galleguillos, A., Sanhueza, J., Garrido, A., Nieto, S. & Valenzuela. A. (1998) Effect of the degree of hydrogenation of dietary fish oil on the trans fatty acid content and enzymatic activity of rat hepatic microsomes. Lipids 33: 669-673.

Munsch, N. & Pascaud, M. (1969a) Comparative metabolism of C-14 elaidic and oleic acids in rats. II. Distribution, turnover and desaturation. Bull. Soc. Chim. Biol. 51: 1575-1590.

Munsch, N., Strouve, C. & Pascaud, M. (1969b) Comparative metabolism of C-14 elaidic and oleic acid in rats. I. Oxidation and retention. Bull. Soc. Chim. Biol. 51: 1565-1574.

Mynatt, R. L. & Sachan, D. S. (1992) Altered redox state as a basis for carnitine-mediated attenuation of ethanol oxidation in the rat. Biochem. Arch. 8: 345-353.

Nanji, A. A. & French, S. W. (1985) Relationship between pork consumption and cirrhosis. Lancet 1 :681-683.

Nanji, A. A. & French, S. W. (1986) Dietary factors and alcoholic cirrhosis. Alcohol. Clin. Exp. Res. 10: 271-273.

Nanji, A. A. & French, S. W. (1988) Effect of different dietary fats on ethanol metabolism: implications for pathogenesis of experimental alcoholic liver disease. Int. J. Vit. Nutr. Res. 58: 475-476.

Nanji, A. A. & French, S. W. (1989) Dietary linoleic acid is required for development of experimental induced alcoholic liver disease. Life Sci. 44: 223-227.

Nanji, A. A., Mendenhall, C. L. & French, S. W. (1989) Beef fat prevents alcoholic liver disease in the rat. Alcohol. Clin. Exp. Res. 13: 15-19.

Nanji, A. A., Sadrzadeh, S. M. H. & Dannenberg, A. J. (1994a) Liver microsomal fatty acid composition in ethanol-fed rats: effect of different dietary fats and relationship to liver injury. Alcohol. Clin. Exp. Res. 18: 1024-1028.

Nanji, A. A., Zhao, S., Lamb, R. G., Dannenberg, A. J., Sadrzadeh, S. M. H. & Waxman, D. J. (1994b) Changes in cytochromes P-450, 2E1, 2B1, and 4A, and phospholipases A and C in the intragastric feeding rat model for alcoholic liver disease: relationship to dietary fats and pathologic liver injury. Alcohol. Clin. Exp. Res. 18: 902-908.

Nanji, A. A., Zhao, S., Lamb, R. G., Sadrzadeh, S. M. H., Dannenberg, A. J. & Waxman, D. J. (1993) Changes in microsomal phospholipases and arachidonic acid in experimental alcoholic liver injury: relationship to cytochrome P-450 2E1 induction and conjugated diene formation. Alcohol. Clin. Exp. Res. 17: 598-603.

Nanji, A. A., Zhao, S., Sadrzadeh, S. M. H., Dannenberg, A. J., Tahan, S. R. & Waxman, D. J. (1994c) Markedly enhanced cytochrome P450 2E1 induction and lipid peroxidation is associated with severe liver injury in fish oil-ethanol-fed rats. Alcohol. Clin. Exp. Res. 18: 1280-1285.

Nanji, A. A., Sadrzadeh, S. M. H., Yang, E. K., Fogt, F., Meydani, M. & Dannenberg, A. J. (1995) Dietary saturated fatty acids: a novel treatment for alcoholic liver disease. Gastroenterology 109:547-554.

Nebert, D. W., Nelson, D. R., Adesnik, M., Coon, M. J., Estabrook, R. W., Gonzalez, F. J., Guengerich, F. P., Gunsalus, I. C., Johnson, E. F., Kemper, B., Levin, W.,

Phillips, I. R., Sato, R. & Waterman, M. R. (1989) The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. DNA 8: 1-13.

Nelson, D. R., Kamataki, T., Waxman, D. J., Guengerich, F. P., Estabrook, R. W., Feyereisen, R., Gonzalez, F. J., Coon, M. J., Gunsalus, I. C., Gotoh, O., Okuda, K. & Nebert, D. W. (1993) The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. DNA Cell Biol. 12: 1-51.

Nestel, P., Noakes, M., Belling, B., McArthur, R., Clifton, P., Janus, E. & Abbey, M. (1992) Plasma lipoprotein lipid and Lp[a] changes with substitution of elaidic acid for oleic acid in the diet. J. Lipid Res. 33: 1029-1036.

Ohlrogge, J. B. (1983) Distribution in human tissues of fatty acid isomers from hydrogenated oils. In: Dietary fats and health (Perkins, E. G. & Visek, W. J., eds.), pp. 359-374. Champaign, IL: AOCS.

Ohlrogge, J. B., Emken, E. A. & Gulley, R. M. (1981) Human tissue lipids: occurrence of fatty acid isomers from dietary hydrogenated oils. J. Lipid Res. 22: 955-960.

Ohlrogge, J. B., Gulley, R. M. & Emken, E. A. (1982) Occurrence of octadecenoic fatty acid isomers from hydrogenated fats in human tissue lipid classes. Lipids 17: 551-557.

Ono, K. & Fredrickson, D. S. (1964) The metabolism of ^{14}C-labeled cis and trans isomers of octadecenoic and octadecadienoic acids. J. Bio. Chem. 239: 2482-2488.

Orme-Johnson, W. H. & Ziegler, D. M. (1965) Alcohol mixed function. Oxidase activity of mammalian liver microsomes. Biochem. Biophys. Res. Commun. 21: 78-82.

Östlund-Lindqvist, A.-M., Albanus, L. & Croon, L-B. (1985) Effect of dietary trans fatty acids on microsomal enzymes and membranes. Lipids 20: 620-624.

Panigrahi, K. & Sampugna, J. (1993) Effects of trans fatty acids on lipid accumulation in 3T3-L1 cells. Lipids 28: 1069-1074.

Park, Y., Albright, K. J., Liu, W., Storkson, J. M., Cook, M. E. & Pariza, M. W. (1997) Effect of conjugated linoleic acid on body composition in mice. Lipids 32: 853-858.

Pedrosa, M. C., Russell, R. M., Saltzman, J. R., Golner, B. B., Dallal, G. E., Seppe, T. E., Oates, E., Egerer, G. & Seitz, H. K. (1996) Gastric emptying, a major modulator of first pass metabolism of ethanol in elderly subjects. Scand. J. Gastroenterol. 31: 671-677.

Perkins, E. G., McCarthy, T. P., O'brien, M. A. & Kummerow, F. A. (1977) The application of packed column gas chromatographic analysis to the determination of trans unsaturation. J. Am. Oil Chem. Soc. 54: 279-281.

Persson, B., Krook, M. & Jörnvall, H. (1991) Characteristics of short-chain alcohol dehydrogenases and related enzymes. Eur. J. Biochem. 200: 537-543.

Petrek, J. A., Hudgins, L. C., Ho, M., Bajorunas, D. R. & Hirsch, J. (1997) Fatty acid composition of adipose tissue, an indication of dietary fatty acids, and breast cancer prognosis. J. Clin. Oncol. 15: 1377-1384.

Pettersen, J. & Opstvedt, J. (1989) Trans fatty acids. 3. Fatty acid composition of lipids of the brain and other organs in the newborn piglets. Lipids 24: 616-624.

Pettersen, J. & Opstvedt, J. (1992) Trans fatty acids. 5. Fatty acid composition of lipids of the brain and other organs in suckling piglets. Lipids 27: 761-769.

Pietinen, P., Ascherio, A., Korhonen, P., Harman, A. M., Willett, W. C., Albanes, D. & Virtamo, J. (1997) Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The alpha-Tocopherol, beta-carotene cancer prevention study. Am. J. Epidemiol. 145: 876-887.

Power, G. W. & Newsholm, E. A. (1997) Dietary fatty acids influence the activity and metabolic control of mitochondrial carnitine palmitoyltransferase I in rat heart and skeletal muscle. J. Nutr. 127: 2142-2150.

Power, G. W., Yaqoob, P., Harvey, D. J., Newsholm, E. A. & Calder, P. C. (1994) The effect of dietary lipid manipulation on hepatic mitochondrial phospholipid fatty acid composition and carnitine palmitoyltransferase I activity. Biochem. Mol. Bio. Int. 34: 671-684.

Rajagopalan, K. V., Fridovich, O. & Handler, P. (1964) Hepatic aldehyde oxidase: III. The substrate binding site. J. Biol. Chem 239: 2027-2035.

Reinke, L. A., Lai, E. K., DuBose, C. M. & McCay, P. B. (1987) Reactive free radical generation *in vivo* in heart and liver of ethanol-fed rats: correlation with radical formation *in vitro*. Proc. Natl. Acad. Sci. USA 84: 9223-9227.

Rhew, T. H. & Sachan, D. S. (1986) Dose-dependent lipotropic effect of carnitine in chronic alcoholic rats. J. Nutr. 116: 2263-2269.

Roberts, D. C. K. (1995) Dietary trans fatty acids and cancer. A review of the evidence. Food Australia 47: 263-265.

Roberts, T. L., Wood, D. A., Riemersma, R. A., Gallagher, P. J. & Lampe, F. C. (1995) Trans isomers of oleic and linoleic acids in adipose tissue and sudden cardiac death. Lancet 345: 278-282.

Roine, R. P., Gentry, T., Lim, R. T., Baraona, E. & Lieber, C. S. (1991) Effect of concentration of ingested ethanol on blood alcohol levels. Alcohol. Clin. Exp. Res. 15: 734-738.

Rosenthal, M. D. & Doloresco, M. A. (1984) The effect of trans fatty acids on fatty acyl Δ5 desaturation by human skin fibroblasts. Lipids 19: 869-874.

Rosenthal, M. D. & Whitehurst, M. C. (1983) Selective effects of isomeric cis and trans fatty acids on fatty acyl Δ9 and Δ6 desaturation by human skin fibroblasts. Biochim. Biophys. Acta 753: 450-459.

Rubin, E., Hutterer, F. & Lieber, C. S. (1968) Ethanol increases hepatic smooth endoplasmic reticulum and drug metabolizing enzymes. Science 159: 1469-1470.

Ruttenberg, H., Davidson, L. M., Little, N. A., Klurfeld, D. M. & Kritchevsky, D. (1983) Influence of trans unsaturated fats on experimental atherosclerosis in rabbits. J. Nutr. 113: 835-844.

Sachan, D. S. (1992) Carnitine-mediated attenuation of ethanol metabolism. In: Current concepts in carnitine research (Carter, A. L., ed.), pp. 79-91. CRC Press, Boca Raton, FL.

Sachan, D. S. & Berger, R. (1987) Attenuation of ethanol metabolism by supplementary carnitine in rats. Alcohol 4:31-35.

Sachan, D. S. & Berger, R. (1993) Specificity of carnitine in attenuation of ethanol metabolism in the rat. Biochem. Arch. 9: 141-146.

Sachan, D. S. & Cha, Y. S. (1994) Acetylcarnitine inhibits alcohol dehydrogenase. Biochem. Biophys. Res. Commun. 203: 1496-1501.

Sachan, D. S. & Davis, C. L. (1969) Hydrogenation of linoleic acid by a rumen spirochete. J. Bacteriol. 98: 300-301.

Sachan, D. S & Mynatt, R. L. (1993) Wheat gluten-based diet retarded ethanol metabolism by altering alcohol dehydrogenase and not carnitine status in adult rats. J. Am. Coll. Nutr. 12: 170-175.

Sachan, D. S. & Rhew, T. H. (1983) Lipotropic effect of carnitine on alcohol-induced hepatic steatosis. Nutr. Rep. Int. 27: 1221-1226.

Sachan, D. S., Rhew, T. H. & Ruark, R. A. (1984) Ameliorating effects of carnitine and its precursors on alcohol-induced fatty liver. Am. J. Clin. Nutr. 39: 738-744.

Saggerson, D. (1986) Carnitine palmitoyltransferase in extrahepatic tissues. Biochem. Soc. Trans. 14: 679-681.

Salem, N., Reyzer, M. & Karanian, J. (1996) Losses of arachidonic acid in rat liver after alcohol inhalation. Lipids 31: S153-S156.

Schonbaum, G. R. & Chance, B. (1976) Catalase. In: The enzymes (Boyer, P. D., ed.), vol. 13, Oxidation-Reduction, part C: Dehydrogenases (II), Oxidases (II), hydrogen peroxide cleavage, pp 363-408. Academic Press, New York, NY.

Schrock, C. G. & Connor, W. E. (1975) Incorporation of the dietary trans fatty acid (C18:1) into the serum lipids, the serum lipoproteins and adipose tissue. Am. J. Clin. Nutr. 28: 1020-1027.

Schulz, H. (1991) Beta oxidation of fatty acids. Biochim. Biophys. Acta 1081: 109-120.

Scopes, R. K. (1983) An iron-activated alcohol dehydrogenase. FEBS Lett. 156: 303-306.

Seitz, H. K. & Oneta, C. M. (1998) Gastrointestinal alcohol dehydrogenases. Nutr. Rev. 56: 52-60.

Senti, F. R. ed. (1985) Health aspects of dietary trans fatty acids. Life sciences research office, Fed. Am. Soc. Exp. Biol. Bethesda, MD.

Sgoutas, D. & Kummerow, F. A. (1970) Incorporation of trans-fatty acids into tissue lipids. Am. J. Clin. Nutr. 23: 1111-1119.

Sgoutas, D. S., Jones, R. & Leight, M. (1973) Distribution of dietary elaidic acid in subcellular particles of rat liver. Int. J. Biochem. 4: 437-445.

Sherlock, S. (1995) Alcoholic liver disease. Lancet 345: 227-229.

Shimp, J. L., Bruckner, G. & Kinsella, J. E. (1982) The effects of dietary trilinoelaidin on fatty acid and acyl desaturases in rat liver. J. Nutr. 112: 722-735.

Sinclair, R. G. (1935) The metabolism of the phospholipids. VIII. The passage of elaidic acid into tissue phospholipids. Evidence of the intermediary role of liver phospholipid in fat metabolism. J. Bio. Chem. 111: 515-526.

Slover, H. T., Thompson, R. H., Davis, C. S. & Merola, G. V. (1985) Lipids in margarines and margarine-like foods. J. Am. Oil Chem. Soc. 62: 775-786.

Smith, L. M., Clifford, A. J., Creveling, R. K. & Hamblin, C. L. (1985) Lipid content and fatty acid profiles of various deep-fat fried foods. J. Am. Oil Chem. Soc. 62: 996-999.

Smith, M. O., Cha, Y.-S. & Sachan, D. S. (1994) Carnitine prolongs the half-life of ethanol in broilers. Comp. Biochem. Physiol. 109A: 177-180.

Smith, T., DeMaster, E. G., Furne, J. K., Springfield, J. & Levitt, M. D. (1992) First-pass gastric mucosal metabolism of ethanol is negligible in the rat. J. Clin. Inv. 89: 1801-1806.

Sommerfeld, M. (1983) Trans unsaturated fatty acids in natural products and processed foods. Prog. Lipid Res. 22: 221-233.

Stowell, L. I. (1989) Nutritional aspects of alcohol. In: Human metabolism of alcohol, (Crow, K. E. & Batt, R. D., eds.), vol. 1, Pharmacokinetics, medicolegal aspects, and general interest, pp. 187-201. CRC Press, Boca Raton, FL.

Subbaiah, P. V., Subramanian, V. S. & Liu, M. (1998) Trans unsaturated fatty acids inhibit lecithin:cholesterol acyltransferase and alter its positional specificity. J. Lipid Res. 39: 1438-1447.

Sugano, M. & Ikeda, I. (1996) Metabolic interactions between essential and trans-fatty acids. Curr. Opin. Lipidol. 7: 38-42.

Sugano, M., Ryu, K. & Ide, T. (1984) Cholesterol dynamics in rats fed cis- and trans-octadecenoate in the form of triglyceride. J. Lipid Res. 25: 474-485.

Sundram, K., Ismail, A., Hayes, K. C., Jeyamalar, R. & Pathmanathan, R. (1997) Trans (elaidic) fatty acids adversely affect the lipoprotein profile relative to specific saturated fatty acids in humans. J. Nutr. 127: 514S-520S.

Svensson, L. (1983) The effect of dietary partially hydrogenated marine oils on desaturation of fatty acids in rat liver microsomes. Lipids 18: 171-178.

Szebeni, J. & Eskelson, C. D. (1989) Diagnosis of alcohol use by lipid changes. In: Diagnosis of alcohol abuse, (Watson, R. R., ed.), pp. 107-124. CRC Press, Boca Raton, FL.

Takada, A., Matsuda, Y. & Takase, S. (1986) Effects of dietary fat on alcohol-pyrazole hepatitis in rats: the pathogenetic role of the nonalcohol dehydrogenase pathway in alcohol-induced hepatic cell injury. Alcohol. Clin. Exp. Res. 10: 403-411.

Takahashi, H., Johansson, I., French, S. W. & Ingelman-Sundberg, M. (1992) Effects of dietary fat composition on activities of the microsomal ethanol oxidizing system

and ethanol-inducible cytochrome P450 (CYP2E1) in the liver of rats chronically fed ethanol. Pharmacol. Toxicol. 70: 347-352.

Taussky, H. H. (1954) A microcolorimetric determination of creatinine in the urine by the Jaffee reation. J. Biol. Chem. 208: 853-861.

Teschke, R. & Gellert, J. (1986) Hepatic microsomal ethanol-oxidizing system (MEOS): metabolic aspects and clinical implications. Alcohol. Clin. Exp. Res. 10:20S-32S.

Teter, B. B., Sampugna, J. & Keeney, M. (1990) Milk fat depression in C57B1/6J mice consuming partially hydrogenated fat. J. Nutr. 120: 818-824.

Thomas, L. H., Winter, J. A. & Scott, R. G. (1983a) Concentration of 18:1 and 16:1 transunsaturated fatty acids in the adipose body tissue of decedents dying of ischaemic heart disease compared with control analysis by gas liquid chromatography. J. Epidemiol. Community Health 37: 16-21.

Thomas, L. H., Winter, J. A. & Scott, R. G. (1983b) Concentration of transunsaturated fatty acids in the adipose body tissue of decedents dying of ischaemic heart disease compared with controls. J. Epidemiol. Community Health 37: 22-24.

Thomas, L. H., Jones, P. R., Winter, J. A. & Smith, H. (1981) Hydrogenated oils and fats: the presence of chemically-modified fatty acids in human adipose tissue. Am. J. Clin. Nutr. 34: 877-886.

Thomson, A. B. R. & Rajotte, R. (1983) Effect of dietary modification on the uptake of glucose, fatty acids, and alcohols in diabetic rats. Am. J. Clin. Nutr. 38: 394-403.

Thomson, A. B. R., Keelan, M., Clandinin, M. T. (1991) Feeding rats a diet enriched with saturated fatty acids prevents the inhibitory effects of acute and chronic ethanol exposure on the in vitro uptake of hexoses and lipids. Biochim. Biophys. Acta. 1084: 122-128.

Thomson, A. B. R., Garg, M., Keelan, M. & Doring, K. (1994) Feeding trans fatty acids to rats has no effect on the intestinal uptake of glucose, fatty acids or cholesterol. Digestion 55: 405-409.

Thomson, A. B. R., Keelan, M., Clandinin, M. T. & Walker, K. (1986) Dietary fat selectively alters transport properties of rat jejunum. J. Clin. Invest. 77: 279-288.

Thomson, A. B. R., Keelan, M., Garg, M. L. & Clandinin, M. T. (1989) Influence of dietary fat composition on intestinal absorption in the rat. Lipids 24: 494-501.

Thomson, A. D. & Majumdar, S. K. (1981) The influence of ethanol o intestinal absorption and utilization of nutrients. Clin. Gastroenterol. 10: 263-293.

Thompson, G. R. (1989) Lipid related consequences of intestinal malabsorption. Gut Festschrift. 30: 29-34.

Troisi, R., Willett, W. C. & Weiss, S. T. (1992) Trans-fatty acid intake in relation to serum lipid concentrations in adult men. Am. J. Clin. Nutr. 56: 1019-1024.

Tsao, Y. K. & Lands, W. E. M. (1980) Cell growth with trans fatty acids is affected by adenosine 3,5-monophosphate and membrane fluidity. Science 207: 777-779.

Tsukamoto, H. & French, S. W. (1985) Alcohol-induced liver fibrosis in rats. Hepatology 5:983.

Tsukamoto, H., French, S. W., Benson, N., Delgado, G., Rao, G. A., Larkin, E. C. & Largman, C. (1985) Severe and progressive steatosis and focal necrosis in rat liver induced by continuous intragastric infusion of ethanol and low fat diet. Hepatology 5: 224-232.

Tsukamoto, H., Towner, S. J., Ciofalo, L. M. & French, S. W. (1986) Ethanol-induced liver fibrosis in rats fed high fat diet. Hepatology 6: 814-822.

Tygstrup, N., Ranek, L., Ramscoe, K. & Keiding, S. (1974) The effect of submaximal ethanol elimination on hepatic redox levels in man. In: Alcohol and aldehyde metabolizing systems, (Thurman, R. G., Yonetani, T., Williamson, J. R. & Chance, B., eds), pp. 469-481, Academic Press, New York, NY.

van Tol, A. Zock, P. L., van Gent, T., Scheek, L. M. & Katan, M. B. (1995) Dietary trans fatty acids increase serum cholesterylester transfer protein activity in man. Atherosclerosis 115: 129-134.

Wang, D. L. & Reitz, R. C. (1983) Ethanol ingestion and polyunsaturated fatty acids: effects on the acyl-CoA desaturases. Alcohol. Clin. Exp. Res. 7: 220-226.

Watkins, B. A. (1988) Influences of biotin deficiency and dietary trans-fatty acids on tissue lipids in chickens. Br. J. Nutr. 61: 99-111.

Watson, P. E. (1989) Total body water and blood alcohol levels: updating the fundamentals. In: Human metabolism of alcohol, (Crow, K. E. & Batt, R. D., eds.), vol. 1, Pharmacokinetics, medicolegal aspects, and general interest, pp. 41-56. CRC Press, Boca Raton, FL.

White, R. E. & Coon, M. J. (1980) Oxygen activation by cytochrome P-450. Ann. Rev. Biochem. 49: 315-356.

Willett, W. C. & Ascherio, A. (1994) Trans fatty acids: are the effects only marginal? Am. J. Public Health 84: 722-724.

Willett, W. C., Stampfer, M. J. & Manson, J. E. (1993) Intake of trans fatty acids and risk of coronary heart disease among women. Lancet 341: 581-585.

Wilson, T. H. & Wiseman, G. (1954) The use of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. J. Physiol. 123: 116-125.

Winkler, K., Lundquist, F. & Tygstrup, N. (1969) The hepatic metabolism of ethanol in patients with cirrhosis of the liver. Scand. J. Clin. Lab. Invest. 23: 59-69.

Wolff, R. L. & Entressangles, B. (1994) Steady-state fluorescence polarization study of structurally defined phospholipids from liver mitochondria of rats fed elaidic acid. Bochim. Biophys. Acta 1211: 198-206.

Wood, R. (1979) Incorporation of dietary cis and trans octadecenoate isomers in the lipid classes of various rat tissues. Lipids 14: 975-982.

Wood, R., Chumbler, F. & Wiegand, R. (1977) Incorporation of dietary cis and trans isomers of octadecenoate in lipid classes of liver and hepatoma. J. Biol. Chem. 252: 1965-1970.

Wood, R., Kubena, K., O'Brien, B., Tseng, S. & Martin, G. (1993) Effects of butter, mono- and polyunsaturated fatty acid-enriched butter, trans fatty acid margarine, and zero trans fatty acid margarine on serum lipids and lipoproteins in healthy men. J. Lipid Res. 34: 1-11.

Wootan, M., Liebman, B. & Rosofsky, W. (1996) Trans: the phantom fat. Nutr. Action 23: 1, 10-13.

Yoo, J.-S. H., Hong, J.-Y., Ning, S. M. & Yang, C. S. (1990) Roles of dietary corn oil in the regulation of cytochromes P450 and glutathione S-transferases in rat liver. J. Nutr. 120: 1718-1726.

Yoo, J.-S. H., Ning, S. M, Pantuck, C. B., Pantuck, E. J. & Yang, C. S. (1991) Regulation of hepatic microsomal cytochrome P450IIE1 level by dietary lipids and carbohydrates in rats. J. Nutr. 121: 959-965.

Yoo, J.-S. H., Smith, T. J., Ning, S. M., Lee, M-J., Thomas, P. E. & Yang, C. S. (1992) Modulation of the levels of cytochromes P450 in rat liver and lung by dietary lipid. Biochem. Pharmacol. 43: 2535-2542.

Zevenbergen, J. L., Houtsmuller, U. M. T. & Gottenbos, J. J. (1988) Linoleic acid requirement of rats fed trans fatty acids. Lipids 23: 178-186.

Zheng, Y. W., Bey, M., Liu, H. & Felder, M. R. (1993) Molecular basis of the alcohol dehydrogenase-negative deer mouse: evidence for deletion of the gene for class I enzyme and identification of a possible new enzyme class. J. Biol. Chem. 268: 24933-24939.

Zock, P. L. & Katan, M. B. (1992) Hydrogenation alternatives: effects of trans fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. J. Lipid Res. 33: 399-410.

Zock, P. L. & Mensink, R. P. (1996) Dietary trans-fatty acids and serum lipoproteins in humans. Curr. Opin. Lipidol. 7: 34-37.

APPENDIXES

APPENDIX I

ETHANOL CONCENTRATIONS

TABLE I-1

Blood ethanol concentrations (BEC, mmol/L) in rats fed saturated, cis, or trans fatty acids diet for 2 weeks from Experiment 1 [1,2]

Time (minutes)	Dietary group		
	SFA	CFA	TFA
15	8.97 ± 1.49[a]	8.12 ± 1.54[a]	2.83 ± 1.54[b]
30	15.27 ± 1.04[a]	14.97 ± 1.98[a]	5.92 ± 2.34[b]
60	19.25 ± 1.80[a]	19.83 ± 2.34[a]	12.30 ± 3.07[b]
90	21.88 ± 1.55[a]	21.91 ± 2.45[a]	13.26 ± 1.19[b]
120	23.91 ± 1.76[a]	23.07 ± 2.26[a]	13.05 ± 1.70[b]
180	24.91 ± 2.12[a]	23.68 ± 2.16[a]	16.41 ± 4.03[a]
240	23.39 ± 1.91[a]	21.13 ± 1.11[a]	11.46 ± 3.91[b]
300	16.45 ± 1.58[a]	14.33 ± 1.81[ab]	7.55 ± 3.08[b]
360	13.55 ± 0.62[a]	12.70 ± 2.05[a]	7.70 ± 4.53[a]

[1] Values are group means ± SEM (n = 5) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid.

TABLE I-2

Blood ethanol concentrations (BEC, mmol/L) in rats fed saturated, cis, or trans fatty acids diet for 4 weeks from Experiment 1 [1,2]

Time (minutes)	Dietary group		
	SFA	CFA	TFA
15	9.03 ± 1.30[a]	10.54 ± 1.82[a]	11.19 ± 2.00[a]
30	16.35 ± 1.25[a]	17.91 ± 1.47[a]	14.83 ± 1.80[a]
60	21.51 ± 1.25[a]	23.63 ± 1.92[a]	18.25 ± 2.15[a]
90	25.27 ± 1.18[a]	27.09 ± 2.78[a]	21.13 ± 1.72[a]
120	31.49 ± 0.56[a]	29.94 ± 3.17[a]	25.19 ± 2.39[a]
180	27.43 ± 0.95[a]	26.29 ± 3.09[a]	23.71 ± 2.68[a]
240	26.21 ± 1.72[a]	24.12 ± 3.72[a]	24.23 ± 1.99[a]
300	20.95 ± 2.63[a]	18.14 ± 3.60[a]	21.06 ± 2.21[a]
360	16.71 ± 2.34[a]	14.82 ± 3.21[a]	16.59 ± 1.69[a]

[1] Values are group means ± SEM (n = 6) and those with the same superscripts in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid.

TABLE I-3

Blood ethanol concentrations (BEC, mmol/L) in rats fed saturated, cis, or trans fatty acids diet for 6 weeks from Experiment 1 [1,2]

Time (minutes)	Dietary group		
	SFA	CFA	TFA
15	14.63 ± 2.37[a]	14.75 ± 3.12[a]	16.92 ± 3.18[a]
30	21.14 ± 4.13[a]	20.71 ± 2.84[a]	25.17 ± 3.89[a]
60	26.18 ± 1.60[a]	31.75 ± 5.48[a]	38.54 ± 7.42[a]
90	39.66 ± 7.53[a]	35.82 ± 7.58[a]	45.25 ± 9.09[a]
120	46.62 ± 8.55[a]	39.06 ± 7.86[a]	36.15 ± 3.91[a]
180	34.84 ± 2.63[a]	28.08 ± 1.40[a]	31.50 ± 2.49[a]
240	30.16 ± 3.14[a]	27.74 ± 2.69[a]	28.46 ± 1.92[a]
300	26.63 ± 2.64[a]	22.49 ± 2.58[a]	27.49 ± 2.55[a]
360	20.56 ± 2.92[a]	12.68 ± 2.34[a]	17.37 ± 1.69[a]

[1] Values are group means ± SEM (n = 5) and those with the same superscripts in a row are not significantly different $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid.

TABLE I-4

Blood ethanol concentrations (BEC, mmol/L) in rats fed cis or trans fatty acids diet for 2 weeks from Experiment 2 [1,2]

Time (minutes)	Dietary group	
	CFA	TFA
30	19.86 ± 1.69[a]	17.61 ± 1.31[a]
60	22.77 ± 2.13[a]	17.19 ± 1.40[b]
90	22.32 ± 2.34[a]	15.63 ± 1.59[b]
120	22.49 ± 2.40[a]	15.53 ± 1.69[b]
150	19.82 ± 2.56[a]	13.79 ± 1.73[a]
180	18.18 ± 2.69[a]	13.11 ± 2.04[a]
210	14.22 ± 2.55[a]	10.84 ± 1.99[a]
240	12.51 ± 2.41[a]	8.12 ± 2.39[b]
270	9.92 ± 2.30[a]	7.93 ± 2.64[a]
300	6.76 ± 2.01[a]	4.96 ± 1.73[a]
360	1.82 ± 0.80[a]	1.21 ± 0.53[a]

[1] Values are means ± SEM (n = 12) and those with the same superscript letters in a row are not significant different at $P < 0.05$.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid.

TABLE I-5

Urinary ethanol concentrations (UEC, mg/mg creatinine) in rats fed cis or trans fatty acids diet for 2 weeks from Experiment 2 [1,2]

Collection time (hour)	Dietary group	
	CFA	TFA
2	6.0 ± 1.5[a]	4.7 ± 0.7[a]
4	2.0 ± 0.6[a]	1.3 ± 0.5[a]
6	0.3 ± 0.1[a]	0.2 ± 0.0[a]
8	0.1 ± 0.0[a]	0.1 ± 0.0[a]
24	0.0 ± 0.0[a]	0.0 ± 0.0[a]

[1] Values are means ± SEM (n = 12) and those with the same superscript letters in a row are not significant different at $P < 0.05$. Creatinine was determined in the urine of the given collection period and related to the mg of ethanol per mg of creatinine.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid.

TABLE I-6

Urinary ethanol concentrations (UEC, mg/mL urine) in rats fed cis or trans fatty acids diet for 2 weeks from Experiment 2 [1,2]

Collection time (hour)	Dietary group	
	CFA	TFA
2	1.3 ± 0.2^{a} (3.0 ± 0.4)	1.1 ± 0.1^{a} (3.0 ± 0.3)
4	0.8 ± 0.2^{a} (1.1 ± 0.2)	0.5 ± 0.1^{a} (1.3 ± 0.3)
6	0.2 ± 0.1^{a} (0.6 ± 0.2)	0.1 ± 0.0^{a} (0.7 ± 0.1)
8	0.1 ± 0.0^{a} (0.4 ± 0.2)	0.0 ± 0.0^{a} (0.5 ± 0.2)
24	0.0 ± 0.0^{a} (8.2 ± 0.8)	0.0 ± 0.0^{a} (9.8 ± 0.9)

[1] Values are means ± SEM (n = 12) and those with the same superscript letters in a row are not significant different at $P < 0.05$. Number in parenthesis refers to the mL of urine in a particular collection period.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid.

TABLE I-7

Urinary ethanol concentrations (UEC, mg/collection period) in rats fed cis or trans fatty acids diet for 2 weeks from Experiment 2 [1,2]

Collection time (hour)	Dietary group	
	CFA	TFA
2	4.2 ± 0.9^a	3.8 ± 0.8^a
4	1.0 ± 0.3^a	0.8 ± 0.4^a
6	0.2 ± 0.1^a	0.1 ± 0.0^a
8	0.1 ± 0.0^a	0.0 ± 0.0^a
24	0.4 ± 0.0^a	0.3 ± 0.0^a

[1] Values are means ± SEM (n = 12) and those with the same superscript letters in a row are not significant different at $P < 0.05$.

[2] Abbreviations used: CFA, cis fatty acids; TFA, trans fatty acid.

APPENDIX II

CARNITINE CONCENTRATIONS

TABLE II-1

Plasma carnitine concentrations (μmol/L) in rats fed saturated, cis, or trans fatty acids diet for 0, 2, 4, and 6 weeks from Experiment 2 [1,2]

	Dietary group		
	SFA	CFA	TFA
0 week:			
NEC	19.4 ± 1.1[a]	19.6 ± 2.1[a]	17.3 ± 0.8[a]
ASAC	6.8 ± 0.7[a]	8.0 ± 0.9[a]	7.7 ± 0.6[a]
AIAC	1.3 ± 0.5[a]	0.7 ± 0.2[a]	1.0 ± 0.4[a]
TC	27.5 ± 1.1[a]	28.3 ± 3.1[a]	26.0 ± 1.0[a]
2 weeks:			
NEC	24.5 ± 2.1[a]	24.5 ± 2.3[a]	21.2 ± 1.5[a]
ASAC	9.2 ± 1.4[a]	9.0 ± 0.8[a]	5.7 ± 0.7[b]
AIAC	2.5 ± 0.2[a]	2.3 ± 0.2[a]	1.4 ± 0.1[b]
TC	36.2 ± 3.2[a]	35.7 ± 3.1[a]	28.3 ± 1.8[a]
4 weeks:			
NEC	29.6 ± 4.0[a]	29.7 ± 3.1[a]	26.0 ± 2.0[a]
ASAC	8.6 ± 0.6[a]	8.1 ± 1.4[a]	6.5 ± 0.4[a]
AIAC	2.4 ± 0.2[a]	1.8 ± 0.3[ab]	1.6 ± 0.2[b]
TC	40.5 ± 4.4[a]	39.6 ± 3.9[a]	34.0 ± 2.1[a]

Continued

TABLE II-1 (Continued)

Plasma carnitine concentrations (μmol/L) in rats fed saturated, cis, or trans fatty acids diet for 0, 2, 4, and 6 weeks from Experiment 2 [1,2]

	Dietary group		
	SFA	CFA	TFA
6 weeks:			
NEC	36.8 ± 4.4[a]	40.4 ± 3.6[a]	31.2 ± 1.4[a]
ASAC	14.4 ± 1.3[a]	11.3 ± 1.1[ab]	8.7 ± 1.0[b]
AIAC	4.3 ± 0.4[a]	4.0 ± 0.3[a]	3.3 ± 0.2[a]
TC	55.5 ± 5.3[a]	55.7 ± 4.7[a]	43.1 ± 1.1[a]

[1] Values are means ± SEM (n = 6) and those with the same superscript letters in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; NEC, nonesterified carnitine; ASAC, acid-soluble acylcarnitine; AIAC, acid-insoluble acylcarnitine; TC, total carnitine.

TABLE II-2

Urinary excretion carnitine concentrations (μmol/day) in rats fed saturated, cis or trans fatty acids diet for 0, 2, 4, and 6 weeks from Experiment 2 [1,2]

	Dietary group		
	SFA	CFA	TFA
0 week:			
NEC	0.17 ± 0.03[a]	0.14 ± 0.01[a]	0.14 ± 0.01[a]
ASAC	0.19 ± 0.02[a]	0.19 ± 0.01[a]	0.21 ± 0.02[a]
AIAC	0.03 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]
TC	0.39 ± 0.05[a]	0.34 ± 0.01[a]	0.36 ± 0.02[a]
2 weeks:			
NEC	0.16 ± 0.02[a]	0.17 ± 0.02[a]	0.16 ± 0.02[a]
ASAC	0.07 ± 0.01[a]	0.09 ± 0.00[a]	0.09 ± 0.01[a]
AIAC	0.02 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
TC	0.25 ± 0.02[a]	0.27 ± 0.02[a]	0.27 ± 0.02[a]
4 weeks:			
NEC	0.23 ± 0.03[a]	0.23 ± 0.04[a]	0.20 ± 0.03[a]
ASAC	0.13 ± 0.01[a]	0.16 ± 0.02[a]	0.15 ± 0.02[a]
AIAC	0.02 ± 0.00[a]	0.01 ± 0.00[b]	0.01 ± 0.00[b]
TC	0.38 ± 0. 04[a]	0.39 ± 0.06[a]	0.36 ± 0.04[a]
6 weeks:			
NEC	0.33 ± 0.06[a]	0.43 ± 0.07[a]	0.28 ± 0.04[a]
ASAC	0.14 ± 0.02[a]	0.14 ± 0.02[a]	0.15 ± 0.02[a]
AIAC	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.04 ± 0.00[a]
TC	0.49 ± 0.07[a]	0.59 ± 0.08[a]	0.46 ± 0.06[a]

[1] Values are means ± SEM (n = 6) and those with the same superscript letters in a row are not significantly different at $P < 0.05$.

[2] Abbreviations used: SFA, saturated fatty acids; CFA, cis fatty acids; TFA, trans fatty acid; NEC, nonesterified carnitine; ASAC, acid-soluble acylcarnitine; AIAC, acid-insoluble acylcarnitine; TC, total carnitine.

APPENDIX III

FATTY ACID ANALYSIS

Part A. Extraction of fatty acids

1. Homogenize the tissue sample of adipose tissue, liver, muscle, or small intestine in cold saline to yield a concentration at 1 mg/mL using a glass homogenizer. For plasma and microsome, 250 μL is used for fatty acid assay.

2. Transfer 800 μL of homogenate into teflon coated 16 x 125 screw top tubes and add 3 mL of chloroform:methanol (1:2, v/v).

3. Vortex and incubate the mixture for 30 minutes on ice in dark condition.

4. Add 200 μL of saturated NaCl and 1 mL chloroform to each sample tube.

5. Vortex and incubate the mixture for 15 minutes on ice in dark condition.

6. Centrifuge at 900 x g for 3 minutes to separate the lipid and non-lipid phases.

7. Remove chloroform layer (bottom layer) and transfer to 12 x 75 mm test tube.

8. Extract the lipids one more time by adding 1 mL of chloroform and follow the steps 5 to 7.

9. Evaporate chloroform under nitrogen to dryness.

10. Resuspend the lipid extraction in 50 μL of chloroform.

11. Separate polar fraction (phospholipids) and non-polar fraction (triglyceride, cholesterol, and free fatty acids) with thin layer chromatography (TLC) by applying 25 μL of lipid extract to TLC silica gel 60 HP-TLC plate.

12. Place TLC plate into a glass chamber containing chloroform:methanol (8:1, v/v) and run TLC for about 15 minutes or until solvent reach the top line. Dry TLC plate under hood.

Part B. Saponification, acidification, and methylation of fatty acids

13. Phospholipid fraction is scraped from point of origin of plate using razor blade and placed in a 16 x 125 mm screw top tube.

14. Add 100 μL of C15:0 (1 mg/mL in iso-octane) as internal standard to the screw

top tube.

15. Add 250 μL of toluene and 500 μL of 0.5 N KOH/MeOH for fatty acid saponification. Make sure the caps are tightly fitted. Incubate the tube at 86°C water bath for 8 minutes.

16. Cool the tube to room temperature.

17. Add 1 mL of 0.7N HCl/MeOH to the tube for fatty acid acidification.

18. Add 2 mL of hexane to extract the fatty acids. Vortex well and centrifuge at 900 x g for 3 minutes.

19. Transfer the top layer to a 12 x 75 mm tube.

20. Repeat steps 17 to 19 and evaporate hexane to dryness under nitrogen gas.

21. Resuspend lipids in 200 μL of hexane and transfer to a 2 mL vial. Evaporate hexane to dryness under nitrogen gas.

22. Repeat step 21.

23. Under the hood, add 150 to 200 μL of ethereal diazomethane to the vial for fatty acid methylation. Place the cap on the vial loosely immediately after adding diazomethane and incubate for 10 minutes.

24. Evaporate the vial to dryness under the hood.

25. Add hexane to the vial to resuspend the methylated fatty acids. Cap the vial and vortex well.

    * Amount of hexane used for resuspension of methylated fatty acids:
    For adipose tissue, 120 μL; liver, 100 μL; microsome, 250 μL; muscle, 500 μL; plasma, 100 μL; small intestine, 15 μL.

26. Analyze fatty acids via gas chromatograph.

Vita

Mei-Shin Mong was born in Taichung City, Taiwan, Republic of China (R. O. C.), on January 8, 1964. She attended the elementary and junior high school in that city and graduated from Feng Yuan high school in Taichung Hsien in 1982. After attending the Providence University in Taichung City, Taiwan, R. O. C. from September of 1982 to June of 1986, she graduated with a Bachelor of Science degree majoring in Food and Nutrition. She attended the University of North Carolina at Greensboro beginning in August of 1988 and received a Master of Science degree majoring in Nutrition in August of 1991. She entered the Ph.D. program at the University of Tennessee at Knoxville in August 1992. She married Chun-Hung Chiu in 1993 and their son was born in 1998. In May 1999, she earned a Ph.D. degree in Human Ecology with a concentration in nutrition.