Differential effects of dietary canola, soybean, and cod liver oils on arachidonic acid content of the rat adrenal glands

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We investigated the effect of canola, soybean, corn and cod liver oils, and lard fat on arachidonic acid content of the adrenal gland lipids. Adrenal gland lipids from rats fed canola oil, lard fat, and cod liver oil contained much lower levels of linoleic acid (18:2, n-6) (6.8, 3.6, and 2.9%, respectively) compared with those of the corn oil and soybean groups. The arachidonic acid (20:4, n-6) content varied slightly (13.4% to 14.5%) between different dietary groups with the exception of the cod liver oil group. Adrenals from rats receiving cod liver oil contained only 1,650 ng of arachidonic acid (20:4, n-6) compared with those from lard, corn oil, soybean oil, and canola oil groups, which contained 6,000, 9,740, 6,500, and 8,500 ng, respectively. Docosatetraenoic acid (22:4, n-6) and docosapentaenoic acid (22:5, n-6) were found only in adrenals from the corn oil group and amounted to 5.3% of the total fatty acids. On the other hand, soybean- and canola oil-fed groups contained detectable levels of α-linolenic acid (0.5% to 2%) and docosahexaenoic acid (22:6, n-3) (1.4% to 1.9%). Adrenals from the cod liver oil group contained 4.2% 20:5 (n-3), 3.3% 22:5 (n-3), and 6.7% 22:6 (n-3) fatty acids. The greater accumulation of long chain n-3 fatty acids in the cod liver oil group is likely to have resulted from the differences in the acyltransferase-mediated uptake and metabolic conversion and retroconversion of eicosapentaenoic acid (20:5, n-3). The results of this study demonstrate that soybean oil, canola oil, and cod liver oil modulate arachidonic acid as well as its precursor pools in a differential manner in adrenal glands, and that these diet-induced changes may be important for the eicosanoid-mediated adrenal functions such as steroidogenesis and the release of adrenocorticotropic hormone and catecholamines.
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Keywords: soybean oil; canola oil; cod liver oil; arachidonic acid; eicosanoids; adrenal gland

Introduction

The adrenal gland (medulla and cortex) produces four main classes of hormones: catecholamines, glucocorticoids, mineralocorticoids, and androgens. Catecholamines include dopamine (found mainly in the central nervous system), norepinephrine (found in the sympathetic nervous system), and epinephrine (found in the adrenal medulla). Glucocorticoids, mineralocorticoids, and androgens are produced from the adrenal cortex. The adrenal cortex utilizes the cholesterol synthesized from acetate and/or plasma low density lipoprotein (LDL) cholesterol as the precursor of adrenal hormones.[1] The steroid-secreting cells of the adrenal cortex are characterized by an extensive smooth endoplasmic reticulum (SER), a few parallel arrangements of rough endoplasmic reticulum (RER), abundant pleomorphic mitochondria, and a prominent Golgi complex. The lipid content of adrenal cortex averages about 15% of total wet weight. One-third of this is cholesterol ester, which is rich in long chain polyunsaturated fatty acids such as arachidonic acid (20:4, n-6) and docosatetraenoic acid (22:4, n-6).

The adrenal gland contains cholesterol, cholesterol

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ester, triglycerides, and phospholipids. Like any other tissue membranes, the adrenal membranes are largely made up of phospholipids. The bovine adrenal plasma membrane is composed of 46% phosphatidylcholine (PC), 22% phosphatidylethanolamine (PE), 9% phosphatidylserine (PS), 4% phosphatidylinositol (PI), and 1% phosphatidic acid (PA).[2] Furthermore, it has been extensively reported that adrenal membrane phospholipids relative to other tissues are more enriched in arachidonic acid (20:4, n-6), a major precursor of highly active eicosanoids.[3-8] However, a detailed molecular species distribution of the individual adrenal gland phospholipids, including ether lipids, remains to be documented, and the lipids such as ether lipids, PI, and PS may have much higher levels of arachidonic acid (20:4, n-6) when compared with the same phospholipids in other tissues.[9,10]

A change in membrane fatty acid compositions leads to altered membrane properties such as membrane fluidity, receptor affinities, ion fluxes, membrane-bound enzyme activities, and lipid turnover.[11,12] In particular, the diet-induced changes in membrane long chain n-6 polyunsaturated fatty acids (PUFA), including arachidonic acid (20:4, n-6), docosatetraenoic acid (22:4, n-6), and docosapentaenoic acid (22:5, n-6) have assumed a greater importance due to the fact that these fatty acids are further metabolized to highly biologically active eicosanoids.[2] Several in vitro studies with different cell types from the adrenal gland of several species have demonstrated that the eicosanoids formed from arachidonic acid (20:4, n-6) participate in a wide range of adrenal functions such as blood pressure regulation, the synthesis of adrenocortical hormones, and the release of catecholamines and steroidogenesis.[13-23] Furthermore, it has been shown that hormones such as adrenocorticotropic hormone (ACTH) and epinephrine modulate the enzymes involved in the desaturation and elongation of linoleic acid (18:2, n-6) to other longer chain PUFA in rat tissues.[24-27] However, the precise relationship between fatty acid metabolism, eicosanoids, and adrenal hormones still remains poorly defined.

Because the eicosanoids derived from arachidonic acid (20:4, n-6) are ubiquitous and appear to affect a variety of endocrine and metabolic systems, a great deal of interest has arisen recently in the modulation by diet of membrane levels of arachidonic acid (20:4, n-6) and its precursors [linoleic acid (18:2, n-6), dihomo-γ-linolenic acid (20:3, n-6)].[28,29] Only a limited number of studies exist in the literature on the effects of different dietary fats on adrenal gland fatty acid compositions. The present study was undertaken to specifically evaluate the in vivo effects of canola oil, soybean oil, corn oil, cod liver oil, and saturated fat (lard) on arachidonic acid and its precursor pools in adrenal glands.

Methods and materials

Animals

Fifty male weanling Wistar rats weighing 70 to 75 g (Charles River Canada, St. Constant, Quebec, Canada) were individually housed in suspended stainless-steel cages in a temperature- (22 to 23° C) and humidity-controlled room on a 12-hr light:dark cycle upon arrival.

Diets

The rats were fed one of five experimental diets containing 10% fat (wt/wt) ad libitum. The fat in the diets was composed of one of the following: lard fat (LRD), corn oil (CRN), soybean oil (SOY), canola oil (CAN), or cod liver oil (COD) *(Table 1)*. Dry ingredients were mixed separately and kept frozen at −25° C until required. Diets were prepared with appropriate fat/oils and dry ingredients were mixed three times per week and provided to animals with fresh diet daily. Diets were also kept frozen between feeding. The fatty acid composition of the dietary fat/oils employed in the present study is given in *Table 2*. Animals were fed for a period of 7 weeks during which time food consumption and body weight were recorded weekly.

Tissue collection procedure

After 7 weeks of feeding, rats were anesthetized using metofane (Pitman-Moore, Washington Crossing, NJ USA). Adrenals were swiftly excised, trimmed of perirenal fat, washed in ice-cold saline, weighed, and immediately frozen in liquid nitrogen, and stored at −80° C until required for further analysis.

Lipid extraction from the tissue

Paired adrenals were homogenized in 1 mL ice-cold saline, and lipids extracted from these homogenates by the addition of 3.75 mL of chloroform:methanol (1:2, vol/vol), 1.25 mL of chloroform, and 1.25 mL of saline.[30] Following the removal of water-soluble materials, the lower chloroform phase was dried under oxygen-free nitrogen, and lipids were resuspended in 1 mL chloroform:methanol (2:1, vol/vol) and stored at −80° C until analyzed for fatty acids.

Analyses of fatty acids

Known aliquots (0.2 mL) of lipid extracts were transferred to screw-cap culture tubes containing a known amount of internal standard, heptadecanoic acid (17:0). Chloroform was evaporated under oxygen-free nitrogen, and the dried lipid was immediately dissolved in 2.5 mL of 6 mol/L methanolic sulfuric acid (vol/vol). Lipid samples were transmethylated at 80° C for 2 hr. After transmethylation, samples were cooled at laboratory temperature, and fatty acid methyl esters were extracted by the addition of petroleum ether (40° to 60° C) and 1 mL of water. The petroleum ether phase was removed, evaporated under nitrogen, and redissolved in a small amount of hexane.[28,29] The methyl esters obtained from adrenal lipid extracts were analyzed on a fused silica capillary column (SP2330, 30 meter, Supelco Inc., Bellefonte, PA USA), using a Perkin-Elmer gas-liquid chromatograph (Norwalk, CT USA) fitted with a flame ionization detector (FID). The conditions employed were as follows: initial column temperature, 140° C; final temperature, 210° C; programmed at the rate of 4° C/min and held at 210° C for 30 min; injector temperature 250° C; detector temperature 250° C; carrier gas, helium.[28] Unknown fatty acids peaks from adrenal lipid samples were identified by comparing their retention times with corresponding standard fatty acids. Certain long chain fatty acids were further confirmed by analyzing samples in the presence of standard fatty acids. We also determined the absolute amounts of long chain fatty acids such as arachidonic acid (20:4, n-6),

Table 1 Composition of diets*

Ingredients	Groups (wt %)				
	LRD	CRN	SOY	CAN	COD
Vitamin-free casein†	20.0	20.0	20.0	20.0	20.0
Choline chloride	0.2	0.2	0.2	0.2	0.2
Corn starch	10.0	10.0	10.0	10.0	10.0
Sucrose	50.0	50.0	50.0	50.0	50.0
Vitamin mix‡	1.0	1.0	1.0	1.0	1.0
Mineral mix§	4.5	4.5	4.5	4.5	4.5
DL-methionine	0.3	0.3	0.3	0.3	0.3
Fiber¶	4.0	4.0	4.0	4.0	4.0
Lard‖	10.0	0.0	0.0	0.0	0.0
Corn oil**	0.0	10.0	0.0	0.0	0.0
Soybean oil††	0.0	0.0	10.0	0.0	0.0
Canola oil††	0.0	0.0	0.0	10.0	0.0
Cod liver oil‡‡	0.0	0.0	0.0	0.0	10.0
Santoquin antioxidant§§	0.005	0.005	0.005	0.005	0.005

*Diets were isocaloric and provided 4100 kcal/kg (17,238 kj/kg) diet. LRD, lard fat; CRN, corn oil; SOY, soybean oil; CAN, canola oil; COD, cod liver oil.
†U.S. Biochemicals, Cleveland, OH USA.
‡Supplied (mg/g diet): thiamine HCl, 6; riboflavin, 6; pyridoxine HCl, 7; nicotinic acid, 30; DL-calcium pantothenate, 32; folic acid, 2; d-biotin, 0.2; vitamin B_{12}, 0.1; inositol, 1000; vitamin A palmitate, 8 (500,000 IU/g); ergocalciferol, 2.5; DL-α-tocopherol acetate, 0.02; menadione, 0.5.
§Provided (mg/100 g diet): calcium carbonate, 720; cupric sulfate, 5.3; calcium diphosphate, 1130; ferric citrate ($5H_2O$), 15.4; potassium chloride, 730; potassium iodide, 0.3; zinc carbonate, 5.3; sodium diphosphate, 600; chromium acetate, 1.0; zinc sulfate, 8.4; magnesium chloride, 29.3; manganese chloride, 2.7; sodium selenite, 0.2 mg.
¶Alpha floc, Lee Chemical, Toronto, Ontario, Canada.
‖Tenderflake, Canada Packers, Toronto, Ontario, Canada.
**Mazola, Best Foods Canada, Etobicoke, Ontario, Canada.
††Donated by Canada Packers, Toronto, Ontario, Canada.
‡‡Life Brand, Shoppers Drug Mart, Toronto, Ontario, Canada.
§§Monsanto Chemical, Toronto, Ontario, Canada.

Table 2 Fatty acid composition dietary lipids

Fatty acids	Fatty acids (wt %)				
	LRD	CRN	SOY	CAN	COD
14:0 and 14:1	1.6	—	—	—	6.3
16:0 and 16:1	26.5	11.1	10.4	5.0	22.6
18:0	12.9	1.5	3.5	1.5	1.9
18:1 (n-9)	45.6	27.7	25.6	59.7	20.6
18:2 (n-6)	10.2	56.7	50.2	21.3	3.1
18:3 (n-3)	0.5	1.4	7.8	8.5	1.0
18:4 (n-3)	—	—	—	—	2.5
20:1	1.2	0.5	—	2.0	12.3
20:5 (n-3)	—	—	—	—	8.4
22:1	—	—	—	—	7.4
22:5 (n-3)	—	—	—	—	1.1
22:6 (n-3)	—	—	—	—	7.7
Others	1.5	1.1	2.5	1.5	2.5

Only the values for fatty acids representing >0.5% of total fatty acids are given in Table 2.
LRD, lard fat; CRN, corn oil; SOY, soybean oil; CAN, canola oil; COD, cod liver oil.

eicosapentaenoic acid (20:5, n-3), and docosahexaenoic acid (22:6, n-6) using heptadecanoic acid (17:0) as an internal standard in the adrenal glands.

Statistics

Results were analyzed by one-way analysis of variance[31] and the level of significance between groups was determined on SPSS P.C. + 4.0 (University of Western Ontario, London, Ontario) using Scheffe's test.

Results

We found no significant differences in food intake, body weights, and adrenal mass (μg/g body weight) between different dietary groups *(Table 3)*.

A detailed summary of the fatty acid composition of the total lipids obtained from the adrenal gland homogenate is summarized in *Table 4*. Saturated and certain monounsaturated fatty acids such as myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), and oleic acid (18:1, n-7) were least affected by the type of dietary fat. On the other hand, the adrenals from animals fed LRD or CAN contained higher levels of oleic acid (18:1, n-9) as compared with other experimental groups. Both of these dietary fats were also rich in oleic acid (18:1, n-9). In regard to linoleic acid (18:2, n-6), significant differences were noted between different dietary groups. The percentage of linoleic acid (18:2, n-6) detected in adrenal gland lipids from different experimental groups was found to be in the following order: CRN (16.6%), SOY (15.9%), CAN (6.8%), LRD (3.6%), COD (2.9%). The changes in linoleic acid (18:2, n-6) content of the adrenals closely reflected the linoleic acid (18:2, n-6) content of the fats fed. Arachidonic acid (20:4, n-6) levels varied from 13.4% to 14.5% between different dietary groups with

Table 3 Final body weight, food intake, and adrenal mass of rats fed diets containing 10% lard fat, corn oil, soybean oil, canola oil or cod liver oil for seven weeks

	Dietary groups				
	LRD	CRN	SOY	CAN	COD
Body weight*, g	375 ± 9 (9)	399 ± 10 (7)	303 ± 9 (9)	389 ± 15 (10)	418 ± 16 (8)
Food intake, g/d	22 ± 2	24 ± 1	22 ± 1	23 ± 1	23 ± 1
Adrenal mass† μg/g body weight	160 ± 10 (9)	160 ± 10 (7)	140 ± 10 (9)	150 ± 10 (9)	130 ± 20 (8)

LRD, lard fat; CRN, corn oil; SOY, soybean oil; CAN, canola oil; COD, cod liver oil.
*The mean initial body weight of the rats was 73 g. Values represent the mean ± SE. The number of animals used in each group is indicated in parentheses.
†Values represent the mean ± SE. The number of adrenal pairs is indicated in parentheses.

Table 4 Effect of lard fat, soybean oil, canola oil, and cod liver oil on adrenal gland fatty acid composition

	Fatty acids (wt %)*†				
Fatty acid	LRD (7)	CRN (7)	SOY (8)	CAN (8)	COD (9)
14:0	1.50 ± 0.1^{a}	1.10 ± 0.1^{a}	1.20 ± 0.1^{a}	1.20 ± 0.1^{a}	2.90 ± 0.2^{b}
16:0	18.7 ± 0.9	17.5 ± 0.7	18.0 ± 0.7	15.2 ± 0.7	18.8 ± 1.9
16:1	4.0 ± 0.4^{ab}	3.2 ± 0.3^{ab}	3.4 ± 0.4^{ab}	2.3 ± 0.4^{a}	4.9 ± 0.7^{b}
18:0	14.3 ± 0.8	12.7 ± 1.1	13.8 ± 1.1	13.0 ± 0.7	13.8 ± 1.3
18:1 (n-9)	25.7 ± 1.0^{b}	17.0 ± 1.0^{a}	17.1 ± 0.8^{a}	27.0 ± 1.4^{b}	19.4 ± 1.0^{a}
18:1 (n-7)	3.4 ± 0.2^{b}	2.1 ± 0.2^{a}	2.2 ± 0.1^{a}	2.9 ± 0.2^{ab}	3.6 ± 0.3^{b}
18:2 (n-6)	3.6 ± 0.2^{a}	16.6 ± 1.5^{c}	15.9 ± 0.8^{c}	6.8 ± 0.4^{bc}	2.9 ± 0.2^{a}
18:3 (n-3)	—	—	1.4 ± 0.1	1.4 ± 0.1	—
20:1 (n-9)	0.9 ± 0.1	trace	trace	0.9 ± 0.1	1.1 ± 0.1
Unidentified	0.9 ± 0.1	—	—	—	—
20:3 (n-6)	—	1.0 ± 0.2	0.9 ± 0.1	0.7 ± 0.2	0.5 ± 0.2
20:4 (n-6)	13.4 ± 0.7^{a}	14.5 ± 1.5^{a}	14.5 ± 1.3^{a}	13.7 ± 1.1^{a}	6.6 ± 0.8^{b}
20:5 (n-3)	trace	—	trace	0.7 ± 0.1	4.2 ± 0.2
22:4 (n-6)	3.2 ± 0.5	3.9 ± 0.5	2.7 ± 0.3	2.4 ± 0.3	trace
22:5 (n-6)	—	1.4 ± 0.2	trace	trace	trace
22:5 (n-3)	—	—	0.9 ± 0.1^{a}	1.0 ± 0.2^{a}	3.3 ± 0.3^{b}
22:6 (n-3)	0.8 ± 0.1^{a}	—	1.4 ± 0.2^{a}	1.9 ± 0.3^{a}	6.7 ± 0.7^{b}
Subtotal	90.4	91.5	93.5	90.8	88.1
Others	9.6	8.5	6.5	9.2	10.9

Only the values for fatty acids representing > 0.5% of total fatty acids are given in this table.
LRD, lard fat; CRN, corn oil; SOY, soybean oil; CAN, canola oil; COD, cod liver oil.
*The values represent the mean ± SE. Values with different superscripts in a row are different from one another ($P < 0.05$).
†Number of animals is indicated in parentheses.

the exception of COD. The percentage of arachidonic acid in the COD group (20:4, n-6) was found to be 45% of that found in the other groups (6% of total versus 14%). In addition, differences were also noted in other n-6 fatty acids such as docosatetraenoic acid (22:4, n-6) and docosapentaenoic acid (22:5, n-6), both of which are products of further metabolism of arachidonic acid (20:4, n-6). We found these two fatty acids in substantial quantities (5.3% of the total fatty acids) only in adrenals from animals given CRN oil. It should also be noted that the relative decrease observed in the absolute amounts of adrenal n-6 PUFA was similar to that found in the percentage of total fatty acids *(Table 5)*.

In regard to n-3 fatty acids, very little or no α-linolenic acid (18:3, n-3) was detected in adrenals from animals given dietary fat/oils such as LRD, CRN, and COD, which were low in this fatty acid *(Table 2)*. On the other hand, the adrenals from animals fed SOY or CAN, which contained 7.8% to 8.5% of α-linolenic acid (18:3, n-3) *(Table 2)*, had detectable levels of this fatty acid, which ranged from 0.5–2% of the total fatty acids. Adrenals from these groups were also found to contain quantifiable amounts of eicosapentaenoic acid (20:5, n-3), 22:5 (n-3), and docosahexaenoic acid (22:6, n-3). These n-3 fatty acids were not detected in adrenals from the CRN oil or LRD fat groups. However, differences in these n-3 fatty acids were more striking in the COD group compared with all other dietary groups. A high degree of enrichment of adrenal lipids with eicosapentaenoic acid (20:5, n-3), 22:5 (n-3), and docosahexaenoic acid (22:6, n-3) was apparent in animals given COD containing 8.4% eicosapentaenoic acid (20:5, n-3) and 7.7% docosahexaenoic acid (22:6, n-3). The ratio of eicosapentaenoic acid (20:5, n-3) to docosahexaenoic acid (22:6, n-3) in total lipids of the adrenals from these animals was found to be 0.63, indicating a substantial

Table 5 The absolute amounts (expressed as ng) of n-6 and n-3 long chain polyunsaturated fatty acids in rat adrenal glands

Fatty acid	Groups				
	LRD (5)	CRN (5)	SOY (5)	CAN (5)	COD (5)
n-6					
20:4	6,050 ± 900	9,740 ± 1220	6,500 ± 890	8,500 ± 920	1,650 ± 150
22:4	1,170 ± 55	2,420 ± 120	885 ± 70	1,685 ± 95	trace
22:5	44 ± 12	895 ± 75	170 ± 25	145 ± 25	trace
n-3					
20:5	trace	—	—	290 ± 30	1,165 ± 85
22:5	—	—	330 ± 35	730 ± 55	760 ± 65
22:6	trace	—	540 ± 35	1,030 ± 85	1,530 ± 135

Values represent amounts of individual long chain n-6 and n-3 polyunsaturated fatty acids per pair of adrenal glands (ng/pair of adrenal glands) as quantitated by gas-liquid chromatography using 17:0 as an internal standard.
Values are expressed as the mean ± SE. The number of pairs used in each group is indicated in parentheses.
LRD, lard fat; CRN, corn oil; SOY, soybean oil; CAN, canola oil; COD, cod liver oil.

incorporation of docosahexaenoic acid (22:6, n-3). Similar differences were apparent even in the absolute amounts of these fatty acids as summarized in *Table 5*. In general, adrenals from the corn and cod liver oil groups contained the highest amounts of n-6 and n-3 fatty acids, respectively. In addition, small but significant amounts of long chain n-3 fatty acids were detected in adrenals from rats fed soybean and canola oils. Compared with percentage differences as summarized in *Table 4*, the absolute tissue levels of long chain fatty acids reported in this study *(Table 5)* clearly demonstrate marked differences between different dietary groups.

Discussion

Although the role of dietary fats (saturated as well as unsaturated) in human health has been extensively investigated, the biochemical mechanisms by which these dietary fats may lead to chronic diseases still remain poorly understood. However, the discovery of eicosanoids has led to renewed interest in defining a biochemical basis of the relationship between dietary fatty acids, eicosanoids, and pathophysiological functions. The present study addresses, for the first time, the effect of specific dietary fats (LRD, CRN, SOY, CAN, and COD) containing different levels of saturated, monounsaturated, and polyunsaturated fatty acids (PUFA) on the fatty acid composition of the adrenal gland lipids. We undertook this study for two reasons: (a) the adrenal gland is capable of making fatty acid products in situ via the desaturation and elongation pathways and (b) the adrenal membrane phospholipids and cholesterol ester both appear to be relatively rich in arachidonic acid (20:4, n-6) and, thus, diet could have significant effects on PUFA levels in these lipid fractions and subsequently, in the eicosanoids derived from these PUFA. Several studies have demonstrated that cells of the adrenal gland can synthesize a variety of eicosanoids, including prostaglandins (E_2, D_2), prostacyclin I_2 (PGI_2), leukotrienes, and hydroxy fatty acids.[2,32] The metabolites derived from arachidonic acid (20:4, n-6) appear to have specific effects on adrenal metabolism and functions.[13–19] There is, however, little information on the potential effects of the metabolites formed from PUFA other than arachidonic acid. Because adrenal lipids are very rich in PUFA and actively produce eicosanoids, we hypothesized that formation of arachidonic acid (20:4, n-6) and its subsequent steady state relative to other tissues can be readily modulated by dietary fats.

Our results demonstrate that oleic acid (18:1, n-9) is one of the major fatty acids in the adrenal gland lipids. Furthermore, it appears that membrane oleic acid (18:1, n-9) levels were moderately influenced by diets rich in this fatty acid (LRD and CAN). We have reported similar findings recently in rat platelets.[28]

In regard to linoleic acid (18:2, n-6), the tissue differences between various dietary groups support that this fatty acid in the adrenal gland is really affected by dietary fats. For example, this fatty acid was decreased by 78%, 4%, 59%, and 82.5% in the experimental group given lard fat (18:2, n-6; 3.1%), soybean oil, canola oil, and cod liver oil, respectively, compared with that found in the corn oil group (18:2, n-6; 56.7%). The differences observed in linoleic acid (18:2, n-6) content of the rat adrenal glands in LRD, CAN, and COD groups appear to have resulted from the lower levels of this fatty acid in the dietary fats. In the present study, diets containing the large quantities of linoleic acid (18:2, n-6) resulted in the highest levels of dihomo-γ-linolenic acid (20:3, n-6). Similar values have been reported by others for the rat adrenal cholesterol ester and phospholipids when fed either 10% (by wt) corn or other vegetable oil-based diet[7,8] or commercial nonpurified diet.[4,6,33]

On the other hand, the results obtained for the arachidonic acid (20:4, n-6) content of the adrenals were not as striking as those for linoleic acid (18:2, n-6). These findings appear to reflect the ability of the adrenal gland to readily metabolize dietary linoleic acid (18:2, n-6) to arachidonic acid (20:4, n-6) despite marked differences in the diet.[25] Our results also demonstrate that the total arachidonic acid (20:4, n-6) content in rat adrenal gland homogenates when expressed as a percentage

of total fatty acids was similar among LRD, CRN, SOY, and CAN groups (varied from 13.4% to 14.5% of the total fatty acids). It appears that it is the turnover of linoleic acid (18:2, n-6) that constitutes an important biochemical pathway in the synthesis and maintenance of arachidonic acid (20:4, n-6) in biological membranes. The adrenal gland, which maintained similar levels of arachidonic acid (20:4, n-6) between different dietary groups despite marked differences in the amount of linoleic acid (18:2, n-6) in the diet, is not an exception. Although we found no significant differences among these dietary groups, the arachidonic acid (20:4, n-6) content was reduced by 55% in the COD group. This reduction appears to have resulted from the substitution of arachidonic acid (20:4, n-6) with long chain n-3 fatty acids such as eicosapentaenoic acid (20:5, n-3) and docosahexaenoic acid (22:6, n-3) and is in general agreement with the uptake of these long chain n-3 PUFA by specific acyltransferases. It has been suggested that long chain PUFA are preferentially taken up via the pathways that involve specific acyltransferases in other tissues.[34] In addition, the greater accumulation of docosahexaenoic acid (22:6, n-3) found in the adrenal tissue as compared with other tissues[28] may have resulted from the reduced retroconversion of this fatty acid to eicosapentaenoic acid (20:5, n-3). Retroconversion of docosahexaenoic acid (22:6, n-3) to eicosapentaenoic acid (20:5, n-3) has been known to occur in vivo in humans,[35] in cultures of human retinoblastoma cells,[36] and in rat liver.[37] It is of further interest that the percentage of total arachidonic acid (20:4, n-6) reported in this study is similar to that reported by others for cholesterol esters[4,7,38] but somewhat lower than that of adrenal phospholipids that contain about 40% arachidonic acid.[6,8,33,38,39] The lower percentage values observed in this study appear to have resulted from the fact that both phospholipids and triglycerides were analyzed as a single fraction. It has been reported that triglycerides, which make up as much as 53% of the total lipids in adrenals, contain only 1.5% to 3.5% arachidonic acid (20:4, n-6).[6,8,39] Our results also indicate that docosatetraenoic acid (22:4, n-6), most of which appears to be present in cholesterol esters,[4-6] was reduced by 30% and 35% in the SOY and CAN groups. The adrenals from the COD group contained very little of this fatty acid, which suggests that cod liver oil may cause a greater reduction of docosatetraenoic acid (22:4, n-6) in the cholesterol ester fraction. Cholesterol esters are more enriched with docosatetraenoic acid (22:4, n-6) when compared with other lipid fractions such as phospholipids and triglycerides. However, the mechanism(s) by which cod liver oil facilitates either a reduction of n-6 fatty acids, including docosatetraenoic acid (22:4, n-6), in cholesterol esters or a selective/preferential incorporation of eicosapentaenoic acid (20:5, n-3) and/or docosahexaenoic acid (22:6, n-3) into cholesterol esters, remains to be investigated. Furthermore, the implications of such fatty acid changes on adrenal steroid metabolism warrant further investigation.

The present study also demonstrates that SOY and CAN, which contained 8% to 8.5% α-linolenic acid, exerted little effect on the deposition of this fatty acid in adrenal lipids. These results are in general agreement with previous reports on the effect of dietary fats rich in α-linolenic acid.[40,41] It has been recently suggested that low tissue deposition of α-linolenic acid following administration of diets rich in this fatty acid may be due to its rapid oxidation.[41] However, the accumulation of small amounts of eicosapentaenoic acid (20:5, n-3) and docosahexaenoic acid (22:6, n-3) in these groups support the idea that α-linolenic acid is metabolized to eicosapentaenoic acid (20:5, n-3) and docosahexaenoic acid (22:6, n-3) via the desaturation and elongation pathways. It is known that the adrenal gland is capable of metabolizing essential fatty acids via these pathways.[24-27]

In conclusion, the results of this study indicate that the adrenal lipid fatty acid composition undergoes rapid changes in response to dietary fatty acids. The differences in n-6 and n-3 fatty acids among treatment groups were more striking when expressed as absolute amounts per pair of adrenal glands. The reduction in n-6 fatty acids was somewhat more striking in the COD group, and these animals also incorporated relatively high levels of eicosapentaenoic acid (20:5, n-3) and docosahexaenoic acid (22:6, n-3) into their adrenal lipids as compared with other tissues such as platelets and neutrophils.[28,29] Although the present study did not address fatty acid changes in specific lipid fractions, we suggest that the fatty acids incorporated into different adrenal lipid fractions vary and probably involve different mechanisms(s), which may have direct implications on eicosanoid metabolism and thus, eicosanoid-mediated adrenal functions. The fatty acid changes observed in response to COD and other dietary fats could translate into parallel changes in the formation of respective eicosanoids. The demonstration of changes in eicosanoid formation under different dietary conditions is crucial because of the importance of eicosanoids in steroidogenesis, in the release of ACTH and catecholamines, and in related adrenal functions.

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