Stress Modulates Cholesterol-Induced Changes in Plasma and Liver Fatty Acid Composition in Rats Fed N-6 Fatty Acid-Rich Oils (43132)

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Abstract. **The effects of dietary cholesterol (CH) and isolation stress on fatty acid compositions of plasma and liver cholesteryl ester and phospholipids were compared in growing rats fed an 18:2n-6 or an 18:3n-6 enriched semisynthetic diet for 2 weeks. Stress, CH-feeding, and dietary fats had no significant effects on plasma CH level, but CH-feeding alone elevated the liver CH concentrations. CH-feeding also modulated the liver polyunsaturated fatty acid compositions, i.e., increasing 18:2n-6 levels, and reducing 20:4n-6 levels, indicating an inhibition of the enzymes, δ-6 and δ-5-desaturases. The extent of these changes was less in rats fed 18:3n-6 than in those fed 18:2n-6. Stress, which alone had no significant effects on plasma and liver fatty acid compositions, attenuated the CH-induced changes of fatty acid levels.** [P.S.E.B.M. 1990, Vol 195]

High levels of 20:4n-6, 22:4n-6, and 22:5n-6 in tissue lipids usually reflect an active metabolism of n-6 fatty acids. In liver, the pathway of a series of elongation and desaturation steps leading to the synthesis of these long chain n-6 polyunsaturated fatty acids (PUFA) has been established (1). The activity of the rate-limiting enzyme, δ-6-desaturase (D6D) (2), is readily modulated by various nutritional and hormonal factors (3).

Since many of the PUFA-modulating factors, such as cholesterol (CH), saturated fats, stress, diabetes, obesity, aging, and gender, also constitute the major risk factors of cardiovascular disease (CVD) (4), there is much interest in the relationship between the development of CVD and PUFA (particularly essential fatty acids, EFA) metabolism (5). A high plasma CH level is one of the major factors facilitating the progress of CVD (6, 7). Lowering plasma CH may reduce the incidence of CVD (8). Since dietary n-6 EFA have been demonstrated to be effective in lowering plasma CH levels (9, 10), the interaction between CH and EFA (11, 12) may play an important role in the pathogenesis of CVD.

However, as the development of CVD is a complex process, the effects of other risk factors on the interaction of CH and EFA should also be examined. In this study, we investigated whether stress might modulate the CH-induced effect on plasma and liver fatty acid compositions in growing rats given either an 18:2n-6- or an 18:3n-6-enriched diet. The aim of dietary supplementation with 18:3n-6 was to bypass the previously known CH and stress-inhibition of D6D activity in liver microsomes (13–19). In addition, 18:3n-6 has been shown to attenuate cardiovascular responses to isolation induced stress in borderline hypertensive rats (20, 21) and to lower plasma CH levels in normal rats (16, 22).

Materials and Methods

Forty-eight weanling male Wistar Kyoto rats (WKY, 3-week-old) purchased from Taconic Farms (Germantown, NY) were used in this study. All rats were group-housed, six per cage, in a humidity (40%)-, temperature (21°C)-, and light (8:00–22:00 hr)-controlled room. They were maintained on a fat-free semisynthetic diet (Teklad Test Diets, Madison, WI) supplemented with 3% by weight safflower oil (SFO)

Received February 9, 1990. [P.S.E.B.M. 1990, Vol 195]
Accepted May 22, 1990.

0037-9727/90/1951-0136$2.00/0

for a period of 5 weeks. The rats weighed about 250 g each and were randomly divided into two dietary groups of 24 rats each and were given the rat-free diet supplemented with 3% SFO or 3% evening primrose oil (EPO). The fatty acid composition of the oil supplements is shown in Table I. In each dietary group, rats were separated into two groups (stressed and nonstressed) of 12 each. The nonstressed rats were group-housed, while the stressed rats were placed in individual cages for a 2-week period of isolation stress (20, 21). Half of the stressed and nonstressed rats ($n = 6$) received an addition of 0.3% cholesterol in their respective diets. Previous evidence (20, 21) indicates that blood pressure in the stressed rats begins to rise between 2 and 4 weeks. Thus, a 2-week stress period was chosen to examine whether changes in tissue lipid profiles predispose the elevation of blood pressure.

In this study, supplementation of oil at 3% (w/w) provides 5% calories of n-6 fatty acids which exceeds the minimal dosage required to avoid the development of EFA deficiency (23). The rationale for adding 0.3% CH to the diet was to maintain the oil to CH ratio (10:1) in the diet as in our previous studies (16, 17). In addition, we have previously shown that feeding rats with 0.25% CH significantly elevated the liver CH contents within 3 days (18). Before and after the stress and feeding study, systolic blood pressures were measured indirectly using the tail cuff technique as described previously (20).

At the end of the study, rats were anesthetized with halothane and killed by exsanguination via cardiac puncture. Plasma was separated by centrifugation. Livers were rapidly removed and frozen at −80°C until analyzed. Plasma and liver lipids were extracted using the method described by Folch *et al.* (24). Lipid fractions, e.g., cholesteryl esters (CE), triacylglycerols, and total phospholipids (PL) were separated by thin layer chromatography using the solvent system, hexane:diethyl ether:acetic acid, 80:20:1 (v/v/v). Fatty acids in the different lipid fractions were methylated and analyzed on a Hewlett-Packard gas chromatograph (model 5880A, Hewlett-Packard, Missisauga, Ontario, Canada) equipped with a flame ionization detector, and a glass column (2.5 mm i.d. × 180 cm) packed with 10% Silar 10C coated on 100/120 mesh Gas Chrom Q (Applied Science, State College, PA). Helium was used as the carrier gas at a constant flow rate of 30 ml/min. The oven was programmed to rise from an initial temperature of 160°C for 3 min to 190°C at a rate of 5°C/min, and maintained for a period of 10 min. The temperature was then increased to 220°C at a rate of 2°C/min and maintained at that temperature for 5 min. Fatty acid methyl esters were identified by comparing their retention times with those of commercial standards (Nu-Chek-Prep, Inc., Elysian, MN and Supleco, Inc., Bellefonte, PA).

Table I. Fatty Acid Composition of Oil Supplement

Fatty acid	Evening primrose	Safflower
16:0	6.2	6.8
18:0	1.8	2.1
18:1n-9	10.6	10.7
18:2n-6	71.8	80.0
18:3n-6	9.1	—
Others	0.5	0.4

Results are expressed as mean ± SD of six rats. The effects of CH, stress, and CH/stress interaction on the blood pressure and the fatty acid composition of different lipid fractions within groups fed the same fat were analyzed by two-way analysis of variance and Duncan's multiple range test (25). The significance of differences between the EPO- and SFO-fed groups was assessed using Student's t test.

Results

During the 2-week feeding period, the gains in body weight were higher in the stressed rats (60.7 ± 7.7, 71.7 ± 4.2, 67.5 ± 12.2, and 61.5 ± 6.0 g, respectively, for the SFO/non-CH-fed, SFO/CH-fed, EPO/non-CH-fed, and EPO/CH-fed group) as compared with the nonstressed rats (49.0 ± 3.3, 46.2 ± 3.3, 47.2 ± 6.0, and 48.0 ± 5.9 g, respectively). However, body weight gains were not affected by either fat or CH supplementation. Prior to feeding, the systolic blood pressure of the rats was 119.0 ± 2.1 mm Hg. At the end of the study, the blood pressure values were significantly ($P < 0.01$) elevated in the stressed rats (124.3 ± 2.2, 126.2 ± 2.7, 128.5 ± 2.2, and 129.0 ± 4.0 mm Hg, respectively) as compared with those in the nonstressed groups (119.0 ± 2.2, 119.3 ± 4.1, 118.5 ± 2.7, and 119.5 ± 1.7 mm Hg, respectively). Again, there were no significant differences between the SFO- and EPO-fed and between the CH-fed and non-CH-fed groups. In the non-CH-fed rats, neither stress nor dietary fat had a significant effect on the CH content of the plasma (1.02 ± 0.11, 0.90 ± 0.11, 0.80 ± 0.12, and 0.81 ± 0.30 mg/ml, respectively for the SFO/nonstressed, EPO/nonstressed, SFO/stressed, and EPO/stressed groups) or the liver (1.13 ± 0.19, 1.22 ± 0.23, 1.15 ± 0.11, and 1.21 ± 0.17 mg/g, respectively). CH feeding did not affect the plasma CH levels, but it significantly ($P < 0.01$) increased the liver CH content (1.91 ± 0.34, 1.78 ± 0.15, 1.83 ± 0.21, and 1.79 ± 0.26 mg/g, respectively). The changes in liver CH content were not significantly different between the SFO- and EPO-fed rats and between the stressed and nonstressed rats.

The content of PUFA in plasma and liver triacylglycerols was very small and was not significantly affected by either CH feeding or stress (data not shown). Similarly, the levels of dietary n-3 PUFA were negligible and had no significant effects on n-3 PUFA content of

Table II. Effects of Cholesterol and Stress on n-6 Polyunsaturated Fatty Acid Composition (% of Total Fatty Acids) of Plasma Phospholipids and Cholesteryl Esters in Rat Fed EPO or SFO (mean ± SD, $n = 6$)[a]

Fatty acid	No stress		With stress		Main effect
	Non-CH-fed	CH-fed	Non-CH-fed	CH-fed	
Phospholipids					
Σ (*n*-6)					
EPO	45.2 ± 3.3	43.6 ± 1.0	44.7 ± 2.0	45.4 ± 3.4	
SFO	45.0 ± 0.4	44.9 ± 5.2	46.5 ± 1.2	44.5 ± 1.4	
18:2					
EPO	13.3 ± 2.7	13.6 ± 1.8	12.3 ± 1.4	14.6 ± 2.2	
SFO	11.0 ± 0.7a	19.5 ± 3.3b	13.4 ± 0.9c	14.5 ± 1.6c	C,C/S
20:3					
EPO	0.5 ± 0.2a	1.3 ± 0.4b	0.6 ± 0.2a	1.1 ± 0.4b	C
SFO	0.3 ± 0.05a	1.3 ± 0.4b	0.3 ± 0.09a	1.0 ± 0.5b	C
20:4					
EPO	27.6 ± 2.2	26.5 ± 2.0	27.4 ± 1.9	26.2 ± 2.6	
SFO	29.9 ± 1.1a	21.7 ± 3.2b	27.6 ± 1.1a	25.9 ± 2.9a	C,C/S
22:4					
EPO	0.5 ± 0.3a	0.3 ± 0.3a	1.1 ± 0.2b	0.5 ± 0.4a	S,C/S
SFO	0.4 ± 0.2a	0.3 ± 0.2a	0.9 ± 0.2b	0.6 ± 0.03c	S,C/S
22:5					
EPO	3.0 ± 1.0a	1.4 ± 0.7b	2.8 ± 0.5a	2.5 ± 1.7a	C,C/S
SFO	3.4 ± 0.5a	1.3 ± 0.4b	4.3 ± 0.6c	2.3 ± 0.3d	C,S
Cholesteryl esters					
Σ (*n*-6)					
EPO	76.0 ± 3.6a	61.9 ± 3.5b	76.7 ± 1.6a	67.8 ± 2.4c	C,C/S
SFO	74.0 ± 3.7a	53.9 ± 3.4b	71.5 ± 7.2a	60.9 ± 3.7c	C,C/S
18:2					
EPO	10.2 ± 0.5	11.6 ± 1.3	12.2 ± 2.8	11.2 ± 1.1	
SFO	10.8 ± 1.0	11.5 ± 1.5	12.3 ± 1.3	11.9 ± 0.9	
18:3					
EPO	2.4 ± 0.4	2.2 ± 0.2	2.4 ± 0.1	3.5 ± 1.4	
SFO	0.6 ± 0.1a	1.0 ± 0.09b	0.7 ± 0.3a	2.1 ± 2.5a,b	C
20:3					
EPO	0.2 ± 0.08a	0.5 ± 0.1b	0.1 ± 0.02a	0.3 ± 0.1c	C,C/S
SFO	0.3 ± 0.2a	0.4 ± 0.07a	0.1 ± 0.05b	0.2 ± 0.08c	C,C/S
20:4					
EPO	62.0 ± 3.3a	46.8 ± 4.7b	61.1 ± 3.9a	52.0 ± 4.3b	C
SFO	61.0 ± 3.9a	40.2 ± 3.5b	57.6 ± 7.2a	46.0 ± 3.5b	C

[a] Data were analyzed by two-way analysis of variance and Duncan's multiple range test. Means within a line (same fat treatment) not sharing the same letter are significantly different at $P < 0.05$. The underlined data are significantly different from the EPO-fed group (within same stress or CH-treated groups) at $P < 0.05$. C,S,C/S: These values were significantly affected by CH feeding, stress, or CH/stress interaction at $P < 0.05$.

plasma and liver lipids (data not shown). Therefore, only the n-6 PUFA composition of plasma and liver PL and CE is presented. In this report, the levels of fatty acids were expressed in percent. Earlier, Iritani and Narita (26) demonstrated that dietary fat manipulation does not affect the tissue PL concentrations and suggested that percentage of fatty acid changes reflect well the absolute changes in PL. Moreover, CH feeding increased liver CH content, but these changes were not significantly different between the 18:2n-6 and 18:3n-6 fed rats and between the stressed and nonstressed rats. Hence, comparison by percentage of differences in fatty acids should also reflect well the actual PUFA changes in liver CE.

Table II shows the changes of n-6 PUFA composition in plasma PL and CE. In plasma PL, neither CH feeding nor stress had any significant effect on the sum of n-6 fatty acids. However, CH feeding suppressed the levels of 22:4n-6 (in the stressed rats) and 22:5n-6 (in all except the EPO/stressed rats), while it elevated those of 20:3n-6. CH feeding increased the levels of 18:2n-6, whereas it reduced the levels of 20:4n-6 in the nonstressed SFO-fed rats. These effects were attenuated in the stressed SFO-fed rats, and there were no effects in the stressed EPO-fed rats. In plasma CE, EPO feeding as compared with SFO feeding increased the levels of 18:3n-6. CH feeding decreased the sum of n-6 fatty acids (mainly 20:4n-6). However, these changes were less in the stressed than in the nonstressed rats and less in the EPO-fed than in the SFO-fed rats. CH feeding

Table III. Effects of Cholesterol and Stress on the Fatty Acid Composition (% of Total Fatty Acids) of Liver Phospholipids and Cholesteryl Esters in Rat Fed EPO or SFO (mean ± SD, $n = 6$)[a]

Fatty acid	No stress		With stress		Main effect
	Non-CH-fed	CH-fed	Non-CH-fed	CH-fed	
Phospholipids					
Σ (*n*-6)					
EPO	50.2 ± 1.9	47.4 ± 1.2	48.2 ± 1.4	50.1 ± 2.9	
SFO	48.5 ± 1.4a	44.4 ± 1.8b	49.2 ± 0.7a	48.3 ± 1.8a	C,C/S
18:2					
EPO	6.9 ± 0.6a	9.5 ± 1.6b	7.4 ± 1.3a	10.1 ± 2.8a,b	C
SFO	5.9 ± 1.0a	9.1 ± 1.9b	8.4 ± 0.7b	9.6 ± 1.9b	C,S
20:3					
EPO	0.9 ± 0.3a	1.6 ± 0.5b	0.7 ± 0.2a	1.3 ± 0.5b	C
SFO	0.8 ± 0.1a	1.7 ± 0.3b	0.3 ± 0.03c	1.1 ± 0.5a,b	C
20:4					
EPO	35.0 ± 1.1a	31.9 ± 1.6b	33.9 ± 1.5a	33.2 ± 1.6a	C,C/S
SFO	34.0 ± 0.8a	28.9 ± 1.1b	33.7 ± 0.4a	33.5 ± 2.5a	C,C/S
22:4					
EPO	1.2 ± 0.2a	0.9 ± 0.1b	1.3 ± 0.1a	1.2 ± 0.5a,b	C,C/S
SFO	1.0 ± 0.1a	0.9 ± 0.1b	1.0 ± 0.2a	0.8 ± 0.2a,b	C
22:5					
EPO	5.5 ± 1.6a	2.8 ± 1.0b	4.5 ± 0.9a	3.5 ± 0.8a,b	C
SFO	6.2 ± 0.8a	3.0 ± 0.8b	5.6 ± 1.1a	3.1 ± 0.5b	C
Cholesteryl esters					
Σ (*n*-6)					
EPO	26.6 ± 3.4a	14.3 ± 1.4b	18.0 ± 1.0c	15.8 ± 2.6b,c	C
SFO	23.7 ± 4.5a	10.6 ± 1.4b	24.6 ± 2.3a	11.8 ± 0.6c	C
18:2					
EPO	8.6 ± 0.6a	10.0 ± 1.0b	8.7 ± 1.4a,b	10.4 ± 2.0a,b	C
SFO	8.7 ± 1.5	7.8 ± 1.4	10.9 ± 1.1	7.7 ± 0.6	
18:3					
EPO	1.2 ± 0.4a	0.3 ± 0.05b	1.2 ± 0.9a	0.4 ± 0.1a,b	C
SFO	0.9 ± 1.1a	0.2 ± 0.04	0.4 ± 0.2	0.6 ± 0.3	
20:3					
EPO	0.2 ± 0.02a	0.6 ± 0.1b	0.2 ± 0.1a	0.6 ± 0.4b	C
SFO	0.3 ± 0.1	0.1 ± 0.04	0.2 ± 0.1	0.3 ± 0.1	
20:4					
EPO	15.2 ± 3.3a	2.9 ± 0.5b	7.5 ± 2.0c	3.5 ± 0.4b	C
SFO	12.3 ± 4.1a	2.2 ± 0.3b	11.9 ± 1.8a	2.7 ± 0.3b	C

[a] Data were analyzed by two-way analysis of variance and Duncan's multiple range test. Means within a line (same fat treatment) not sharing the same letter are significantly different at $P < 0.05$. The underlined data are significantly different from the EPO-fed group (within same stress or CH-treated groups) at $P < 0.05$. C,S,C/S: Values were significantly affected by CH feeding, stress, or CH/stress interaction at $P < 0.05$.

elevated the levels of 20:3n-6 in both nonstressed and stressed rats. The elevation was greater in the EPO-fed than in the SFO-fed rats and also greater in the nonstressed than in the stressed rats.

Table III shows the effects of dietary cholesterol, fats, and stress on liver PL and CE fatty acid composition. In liver PL, CH feeding elevated the levels of 18:2n-6 and 20:3n-6, whereas it reduced those of 22:4n-6 (in the nonstressed groups) and 22:5n-6 in both stressed and nonstressed rats. CH feeding also suppressed the levels of 20:4n-6 and the sum of n-6 fatty acids in the nonstressed, but not in the stressed rats. In liver CE, CH feeding suppressed the levels of 18:3n-6 (in the nonstressed rats), 20:4n-6, and the sum of n-6 PUFA (in all rats). CH feeding also elevated the levels of 20:3n-6 in the EPO-fed but not in the SFO-fed rats.

Discussion

The present study examined the interrelation of dietary CH, fats, and stress alone and/or together on plasma and liver PUFA compositions in growing rats fed a diet containing 18:2n-6 or 18:3n-6. Since CH feeding (18, 19) and stress hormones (13–15), such as catecholamines, have been shown to suppress the activities of δ-6-desaturase and δ-5-desaturase, a synergetic inhibitory effect on EFA metabolism would be expected in rats subjected to a combination of CH feeding and stress.

In this study, CH feeding raised the liver CH

content, but not plasma CH concentrations. This might be due to the relatively low fat (3%) and CH (0.3%) in comparison with our previous CH feeding study (16) (10% fat and 1% CH), and also the short experimental period (2 vs 5 weeks). Nevertheless, CH feeding reduced the levels of 20:4n-6 in plasma and liver CE and liver PL, and increased those of 18:2n-6 in liver PL. A reduced conversion of 18:2n-6 into 20:4n-6 in conjunction with an increase in 20:3n-6 levels indicates a CH-induced suppression of the activities of δ-5-desaturase (16–19). Since the CH-induced reduction of 20:4n-6 levels were generally less, whereas the levels of 20:3n-6 were consistently higher in EPO-fed than in SFO-fed rats, this indicates that 18:3n-6 provided in dietary EPO was effectively elongated to 20:3n-6 and some of 20:3n-6 was eventually desaturated to 20:4n-6.

The isolation stress in the present study only marginally raised the blood pressure. This was also due to the short isolation time (2 weeks) as it has been shown in earlier studies that blood pressure is still on the rise at 2 weeks (20, 21). Stress did not significantly affect the plasma and liver PL fatty acid compositions in rats fed no CH. However, when CH was given to rats, particularly to those fed SFO, stress attenuated the CH-induced suppression of 20:4n-6 levels. This result coincided with a reduction of 18:2n-6 and 20:3n-6 levels and an elevation of 22:4n-6 and 22:5n-6 levels in the stressed CH-fed rats, suggesting that stress might partially alleviate the CH-induced inhibition of D5D and D6D activities. This conclusion is contrary to an expected synergetic inhibitory effect.

The mechanism explaining the different actions of stress between the *in vivo* (enhancing D5D and D6D activity as shown by fatty acid pattern in the present study) and *in vitro* [inhibiting D5D and D6D activity as reported by de Goméz Dumm *et al.*, (13–15)] is difficult to explain; as is the role of dietary CH in the control of desaturase activity. It is known that CH feeding increases the CH content and the rigidity of the microsomal membranes. It also reduces the synthesis of long chain PUFA, and hence the membrane permeability. Leikin and Brenner (27) have attributed the low fatty acid desaturase activity in CH-fed animals to a decreased membrane fluidity. Thus, CH feeding modifies the membrane composition and microenvironment which may interfere with the availability and binding of substrates. Stress is known to stimulate the secretion of catecholamines (epinephrines and norepinephrines) (28, 29), which in *in vitro* suppress EFA metabolism (13–15). In this study, stress alone marginally raised the blood pressure, but had no significant effect on plasma and liver fatty acid composition in rats. The extent to which blood pressure and fatty acid profile responded to stress was also not affected by either EPO feeding or SFO feeding, indicating that stress alone has no significant effect on *in vivo* EFA metabolism. However, this study shows that stress could attenuate the CH-induced changes of fatty acid levels, suggesting that the *in vitro* inhibitory effect of stress on EFA metabolism might not prevail in *in vivo* conditions. It is possible that prolonged stress in CH-fed rats with reduced formation of long chain EFA, might enhance the activities of EFA metabolizing enzymes, raising them to normal levels (3) and beyond in order to meet the physiologic needs.

This work was supported in part by grants from the Natural Science and Engineering Research Council of Canada (Y-S. H. and D. E. M.).

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