Psychosocial Stress, Catecholamines, and Essential Fatty Acid Metabolism in Rats (43677)

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Abstract. **To examine the effects of psychosocial stress and the "stress hormone," epinephrine, on essential fatty acid metabolism in rats, two studies were conducted. In the first, the effects of four weeks of (i) social isolation and (ii) group housing (control) on liver microsomal Δ^6 and Δ^5 *n*-6 desaturase activity were studied in group-reared male normotensive (Wistar Kyoto) and spontaneously hypertensive (SHR) rats (n = 5/group). The second study examined the effects of acute ip epinephrine (0.0, 1.0, 2.0, and 4.0 mg/kg) 6 hr prior to and following an ig dose (4 g/kg) of safflower oil (rich in 18:2*n*-6, LA) on plasma and liver LA, 20:4*n*-6 (AA), and LA/AA ratios in adult essential fatty acid deficient Sprague-Dawley rats (n = 6/group). In the first experiment, isolation stress significantly inhibited the activity of Δ^6 ($P < 0.05$) and Δ^5 ($P < 0.01$) desaturase in the normotensive rats and of Δ^5 desaturase in the SHR ($P < 0.05$). In the second study, epinephrine increased plasma and liver LA at doses 1.0 and 2.0 mg/kg in most of the fractions examined, and suppressed AA levels. The response of the LA/AA ratio to epinephrine varied between tissues and among lipid fractions, but increased this ratio at the moderate doses (2.0–4.0 mg/kg) of epinephrine in most cases. These data suggest that psychosocial stressors are capable of inhibiting the rate limiting steps of essential fatty acid metabolism and that this response is more pronounced in the SHR than in the Wistar Kyoto. They also suggest that epinephrine is capable of altering the *in vivo* metabolism of essential fatty acids in the rat.**

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Several studies have demonstrated that dietary supplementation with either 18:3*n*-6 (gamma linolenic acid, GLA) or 20:5*n*-3 (eicosapentaenoic acid) is capable of attenuating the pressor and tachycardic responses to social isolation stress in normotensive and genetically hypertensive rats (1–5). Studies in humans have also observed a GLA-induced attenuation of blood pressure and heart rate response to acute psychological stress (6). In contrast, dietary supplementation with the *n*-6 and *n*-3 essential fatty acid (EFA) precursors, 18:2*n*-6 (linoleic acid, LA) and 18:3*n*-3 (alpha linolenic acid, LN) (Fig. 1) are without effect (1–6). It has been postulated that the effectiveness of the EFA metabolites, but not the EFAs themselves, reflects an inhibition of EFA metabolism at its rate limiting steps, Δ^6 and Δ^5 desaturation, by the elevated circulating epinephrine levels associated with this psychological stress (5). This is consistent with several published reports of an inhibition of the activity of Δ^6 and Δ^5 desaturase *in vitro* by the *in vitro* addition of cortisol, norepinephrine, and epinephrine (7–9). However, neither the inhibition of desaturase activity by psychological stress of social isolation nor the inhibition of the metabolism (desaturation and elongation) of EFA in the presence of elevations of circulating epinephrine has been demonstrated.

The present study examined (i) the effects of chronic social isolation stress on the desaturase activity of liver microsomes in normotensive and geneti-

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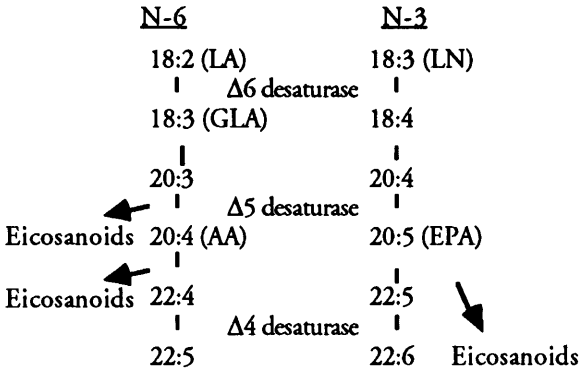

Figure 1. The metabolic pathway of *n*-6 and *n*-3 essential fatty acids in the rat.

cally hypertensive rats *in vitro* and (ii) the effects of circulating epinephrine on *in vivo* metabolism of LA in normotensive rats.

Materials and Methods

Experiment 1. Ten adult male normotensive Wistar Kyoto (WKY) and 10 spontaneously hypertensive (SHR) rats were group housed, five per cage, for a period of one week following weaning (at four weeks *post partum*) in a temperature- (21° ± 1°C) and light cycle-controlled (12L/12D) environment. Chow (#5001; Ralston-Purina, St. Louis, MO) and tap water were provided *ad libitum*. Each strain was then divided into two treatment groups, for a four-week period of (i) group housing (controls, $n = 5$ per strain) or (ii) social isolation ($n = 5$ per strain). Social isolation, i.e., transferring group housed rats to individual cages, has been shown to produce a chronic, adrenal-dependent elevation of blood pressure in normotensive rats (1–5, 10–12). At the end of the experimental period, rats were killed by exsanguination under halothane anesthesia, livers were quickly removed, rinsed in physiological saline, quick frozen in liquid nitrogen, and stored at −90°C until analysis. Four days after tissue collection, liver microsomes were isolated, as previously described (13). The activities of Δ^6 and Δ^5 desaturase were then determined by the *in vitro* conversion of [1-^{14}C]-18:2*n*-6 to [1-^{14}C]-18:3*n*-6 and [1-^{14}C]-20:3*n*-6 to [1-^{14}C]-20:4*n*-6, respectively, as described previously (14, 15).

Experiment 2. Thirty adult female Sprague-Dawley rats were used in the study. Rats were fed an EFA-deficient diet (#85238; Teklad, Madison, WI) for eight weeks post weaning (at four weeks *post partum*) in order to deplete endogenous EFA stores (16). Following this depletion period, animals were injected ip at 0900 hr with either epinephrine (Sigma Chemical Co., St. Louis, MO) at doses of 1.0, 2.0, or 4.0 mg/kg (six per group) or vehicle (control group, $n = 6$). This was followed by an ig dose of safflower oil (4 g/kg, see Table I for fatty acid composition), which is high in LA, at 1500 hr. A second dose of epinephrine, similar to the first, was administered at 2100 hr. Animals were killed by exsanguination under anesthesia at 0900 hr the following day, and liver and heparinized blood samples were collected for fatty acid analysis. Lipids were extracted from plasma and liver samples according to the method of Folch *et al.* (17). Tissue phospholipid, triglyceride, cholesterol ester, and non-esterified fatty acids were separated by thin layer chromatography (16) and their fatty acid composition analyzed on a Hewlett-Packard gas chromatograph equipped with a capillary column (Supelcowax 10; Supelco, Bellafonte, PA) and flame ionization detector, as previously described (16). Fatty acids were identified by comparing retention times of fatty acid methyl esters with those of commercial standards (Nu-Chek Prep, Elysian, MN). From these data, the levels of LA and 20:4*n*-6 (arachidonic acid, AA), and the LA/AA ratio were calculated.

Table I. Fatty Acid Composition (% Total Fatty Acids) of Safflower Oil

Fatty acid	% total fatty acids
16:0	7.7
16:1*n*-7	0.1
18:0	2.5
18:1*n*-9	11.7
18:2*n*-6	70.8
18:3*n*-3	3.5
20:0	1.4
Others	2.3
	100.0

Statistics. Desaturase activity data were analyzed using a two-way analysis of variance (strain × housing condition). Fatty acid data were analyzed using a one-way analysis of variance. Where a significance of $P < 0.05$ was achieved, further comparisons were made using the Student Newman-Keuls test.

Results

Effects of Isolation Stress on Δ6 and Δ5 Desaturation. The effects of chronic isolation stress on the conversion of 18:2*n*-6 to 18:3*n*-6 (Δ^6 desaturation) and of 20:3*n*-6 to 20:4*n*-6 (Δ^5 desaturation) by liver microsomes *in vitro* are shown in Fig. 2. Social isolation significantly reduced Δ^6 desaturation vs group housing in the normotensive WKY ($P < 0.05$). Isolation also tended to reduce Δ^6 desaturation in the SHR, but this did not attain statistical significance. In addition, Δ^6 desaturation in group housed (control) SHR was significantly greater than that in the WKY ($P < 0.05$). In contrast, social isolation significantly inhibited Δ^5 desaturation in both WKY ($P < 0.01$) and SHR ($P < 0.05$) vs control animals. In addition, control Δ^5 desaturation was similar in the WKY and SHR, and was approximately 10- and 6-fold higher than that of Δ^6 desaturation in the WKY and SHR, respectively.

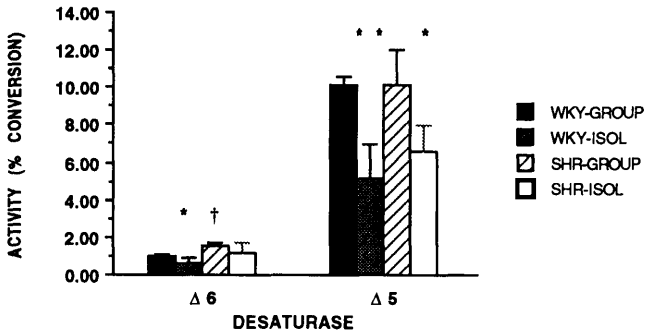

Figure 2. *In vitro* activity (% conversion) of Δ^6 and Δ^5 desaturase in adult male WKY and SHR rat livers following four weeks of either group housing (control) or social isolation (n = 5/group). Values represent $\bar{X}$ ± SEM. *P < 0.05 vs group-housed. **P < 0.01 vs group-housed. †P < 0.05 vs WKY.

Effects of Epinephrine on *in Vivo* EFA Metabolism. The effects of epinephrine on EFA content in plasma and liver phospholipids of EFA-deficient Sprague-Dawley rats is shown in Figure 3. In both tissues, the response of LA to epinephrine exhibited a biphasic pattern in which 1.0 mg/kg significantly increased phospholipid LA (P < 0.001), and 4.0 mg/kg significantly lowered LA (P < 0.001) vs vehicle. In contrast, all doses of epinephrine significantly depressed phospholipid AA levels (P < 0.001) in both tissues, with AA levels reaching a plateau at 2.0 mg/kg. In both tissues, the LA/AA increased with increasing doses of epinephrine up to 2.0 mg/kg, and then plateaued.

In the triglyceride fraction (Fig. 4), plasma LA increased linearly with increasing epinephrine doses up to 2.0 mg/kg (P < 0.001 vs vehicle). At 4.0 mg/kg epinephrine, LA levels declined but remained significantly higher than those of the vehicle group (P < 0.05). Liver triglyceride LA levels at the lowest dose of epinephrine were similar to those in the vehicle group, while higher doses significantly decreased LA (P < 0.01). In both plasma and liver, AA levels were reduced by epinephrine (P < 0.05), with no dose response being evident. The triglyceride LA/AA ratio was significantly increased by all dose of epinephrine in the plasma (P < 0.001) and by 1.0 and 2.0 mg/kg in the liver (P < 0.001).

In plasma and liver cholesterol esters (Fig. 5), a complex response pattern was also observed. In plasma, LA was increased by 1.0 mg/kg epinephrine (P < 0.01) and decreased by 4.0 mg/kg (P < 0.01). In liver, the 1.0 mg/kg epinephrine did not alter LA levels, whereas 2.0 and 4.0 mg/kg significantly lowered LA (P < 0.001). In the plasma cholesterol esters, epinephrine significantly lowered AA (P < 0.001), reaching a plateau at 2.0 mg/kg, whereas no significant changes occurred in the liver AA. The effects of epinephrine on cholesterol ester LA/AA ratios was qual-

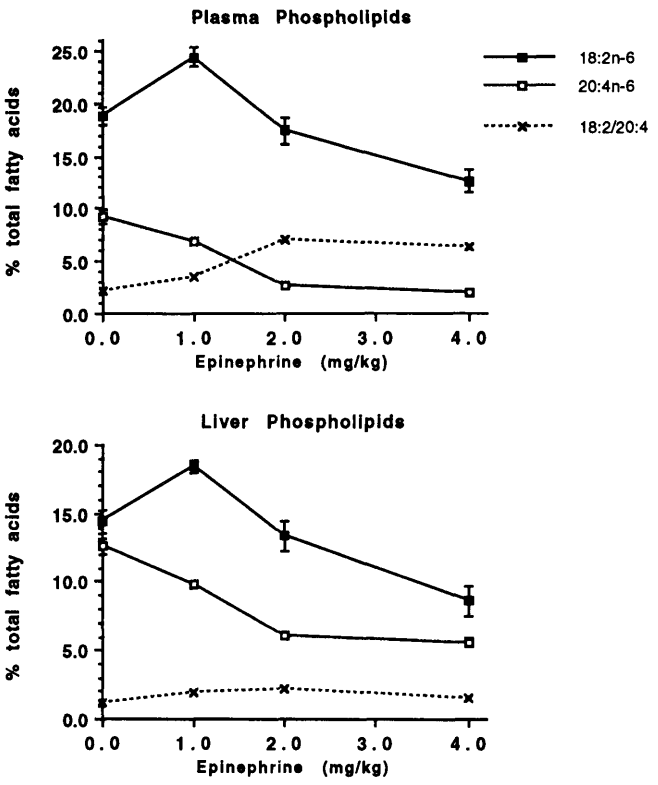

Figure 3. Linoleic (18:2n-6) and arachidonic (20:4n-6) acid levels in plasma and liver phospholipids of female EFA-deficient Sprague-Dawley rats administered ip epinephrine and an ig dose of safflower oil (n = 6/group). Values represent $\bar{X}$ ± SEM.

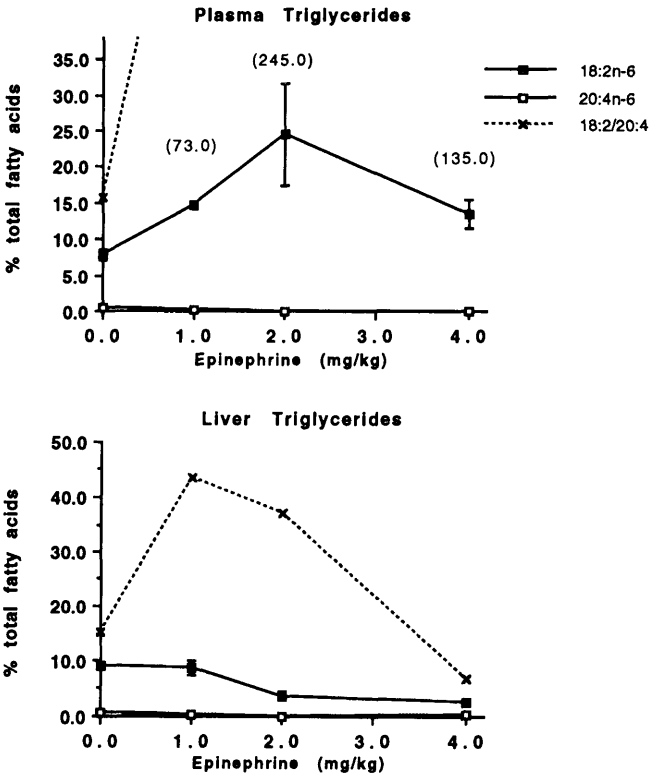

Figure 4. Linoleic (18:2n-6) and arachidonic (20:4n-6) acid levels in plasma and liver triglycerides of female EFA-deficient Sprague-Dawley rats administered ip epinephrine and an ig dose of safflower oil (n = 6/group). Values represent $\bar{X}$ ± SEM. Values in parentheses represent mean 18:2n-6/20:4n-6 ratio.

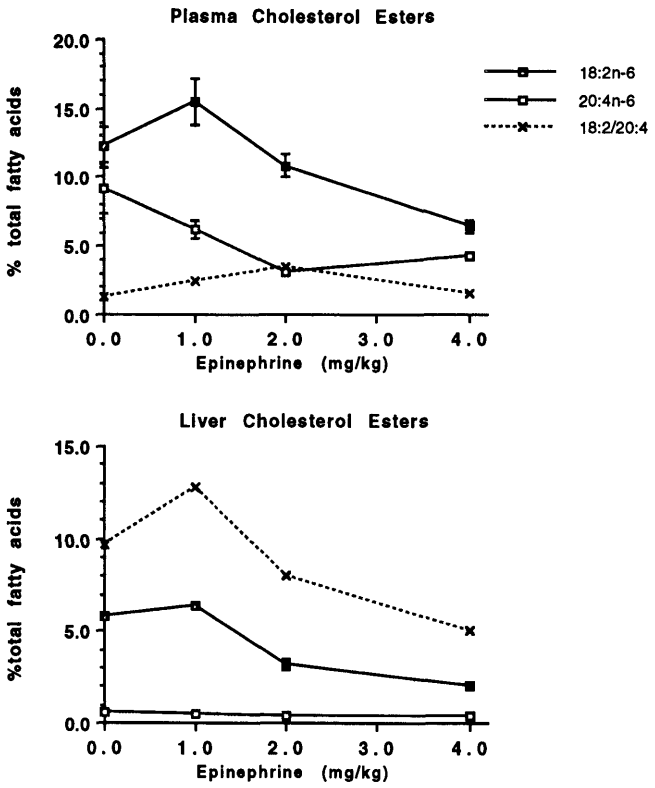

Figure 5. Linoleic (18:2*n*-6) and arachidonic (20:4*n*-6) acid levels in plasma and liver cholesterol esters of female EFA-deficient Sprague-Dawley rats administered ip epinephrine and an ig dose of safflower oil (n = 6/group). Values represent $\bar{X} \pm$ SEM.

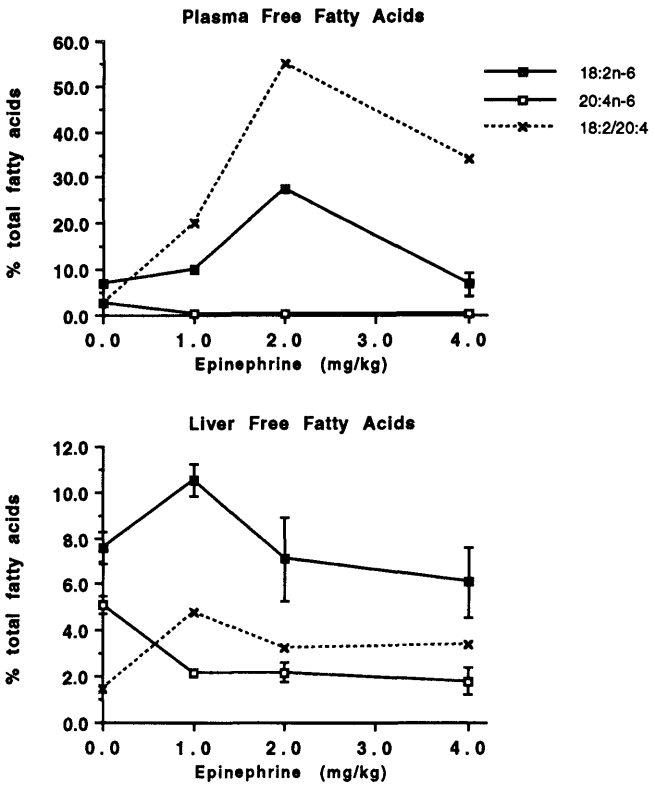

Figure 6. Linoleic (18:2*n*-6) and arachidonic (20:4*n*-6) acid levels in plasma and liver free fatty acids of female EFA-deficient Sprague-Dawley rats administered ip epinephrine and an ig dose of safflower oil (n = 6/group). Values represent $\bar{X} \pm$ SEM.

itatively similar in plasma and liver. Low doses (1.0 mg/kg in both, 2.0 mg/kg in plasma) increased this ratio. In plasma, 4.0 mg/kg epinephrine did not affect the ratio vs vehicle, and in the liver it significantly reduced the LA/AA ratio ($P < 0.05$).

In the free fatty acid fraction of plasma (Fig. 6), LA was affected only by 2.0 mg/kg epinephrine, which increased its levels vs vehicle ($P < 0.001$). In contrast, AA levels were reduced at all doses of epinephrine. In liver (Fig. 6), LA was increased vs controls only at a dose of 1.0 mg/kg epinephrine ($P < 0.001$), and AA levels were reduced in groups receiving epinephrine at doses of 1.0, 2.0, and 4.0 mg/kg ($P < 0.001$). In both tissues the LA/AA ratio in the free fatty acid fraction was increased vs vehicle at all doses of epinephrine.

Discussion

The assumption that social isolation stress will inhibit the rate limiting steps of EFA metabolism is based upon previous reports of an inhibition of liver microsomal desaturase activity *in vitro* by acutely administered stress hormones (7–9). The results of the present study confirm this hypothesis, as four weeks of social isolation stress significantly inhibited *in vitro* Δ^6 desaturase activity in the SHR, and Δ^5 desaturase activity in both WKY and SHR. The failure of isolation stress to significantly reduce Δ^6 desaturase activity in the WKY ($P < 0.10$) most likely reflects a lack of statistical power due to the small sample size used in the study (n = 5), rather than a qualitative difference in the response of the two strains of rat to the stressor.

The present observation that the activity of liver microsomal Δ^5 desaturase in chronically stressed animals was approximately 6- to 10-fold higher than that of Δ^6 desaturase activity suggests that the conversion of LA to GLA is much slower than the conversion of 20:3*n*-6 to AA during stress. We also cannot exclude the possibility of an impairment of the liver microsomal γ-linolenyl-CoA chain elongation step, as has been demonstrated in certain pathophysiological conditions (18). Based upon this observation, dietary supplementation with GLA during chronic stress might be an effective means of countering stress-related reductions in tissue levels of post-Δ^6 desaturase EFA metabolites (and/or their eicosanoid products) which might result from stress-induced reductions in desaturase activity.

Previous studies have reported a significant reduction in Δ^6 and Δ^5 desaturase activities in the hypertensive SHR as compared to the normotensive WKY (19). In contrast, the present data suggest that Δ^6 desaturase activity in the SHR is significantly greater than in the WKY, and that Δ^5 desaturase activity is similar between the two strains in unstressed animals. While there is no ready explanation for the discrepancy in

findings among the studies, it is possible that differences in the age of the animals, and/or the season in which the various studies were conducted might be factors, as both age and season have been shown to significantly alter desaturase activity (20). The deep-freezing of the livers for several days prior to analysis appears to have little influence on microsomal Δ^6 and Δ^5 desaturase activity (21).

The results of the second experiment complement those of the first study and demonstrate that catecholamines, such as those released from the adrenal gland, are capable of altering the conversion of LA to longer chain and more highly polyunsaturated fatty acids *in vivo*. As circulating epinephrine levels are known to increase during chronic isolation stress (5), this mechanism may be relevant to the observed beneficial effects of dietary supplementation with post-Δ^6 desaturase metabolites of LA on stress-induced hypertension in this paradigm. Several of the post-Δ^6 desaturase fatty acids, i.e., 20:3*n*-6 and AA, are direct precursors for eicosanoid synthesis, and orally administered LA has been shown to produce an increase in urinary prostanoid metabolites within a 24-hr period (22). Thus, a stress-related release of adrenal epinephrine might inhibit the conversion of LA to the eicosanoid substrates, 20:3*n*-6 and AA. This, in turn, could alter eicosanoid-dependent functions and play a role in the etiology of stress-related pathologies. While the present experiments utilized EFA-deficient animals, in order to deplete endogenous labile pools of LA and AA (16), these results are probably applicable to EFA-replete animals, as dietary LA is known to influence eicosanoid production in normal rats (23) and acute administration of epinephrine has been shown to alter plasma AA distribution and levels in humans (24). Finally, the biphasic effect of epinephrine on circulating plasma and liver LA levels in the present study suggests that the highest dose may influence other aspects of EFA metabolism, e.g., absorption, oxidation, and/or incorporation into tissue lipid pools via acyltransferase enzymes. However, further work is needed to verify the involvement of these mechanisms.

Together, the findings of this study support the concept that psychophysiological stress impairs the metabolism of essential fatty acids and point to the need for additional studies to ascertain whether dietary supplementation with post-Δ^6 desaturation *n*-6 fatty acids is capable of preventing the physiological effects of epinephrine.

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