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Perturbation of the metabolism of essential fatty acids by dietary partially hydrogenated vegetable oil

(isomeric octadecenoic acids/desaturation/chain elongation/polyunsaturated acids)

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ABSTRACT **Rats were fed purified diets containing (*i*) partially hydrogenated soybean oil as source of isomeric octadecenoic acids, (*ii*) hydrogenated coconut oil as source of saturated fatty acids, and (*iii*) a low level of corn oil as low-fat control. All diets contained 18% of the linoleate requirement. Rat liver and heart phospholipids were analyzed by gas/liquid chromatography for fatty acids, and liver microsomes were assayed for desaturase (acyl-CoA, hydrogen-donor: oxidoreductase, EC 1.14.99.5) activities. Products of desaturation reactions measured analytically provided more information with greater statistical significance than did the enzymatic assays. Rats fed isomeric octadecenoic acids showed more severe essential fatty acid deficiency than did saturated-fat and control groups. The suppression of linoleate metabolites was largely due to decreased Δ5 and Δ6 desaturase activities. At several levels of linoleate, the deficiency was more severe at the higher level of isomeric octadecenoic acids. Increasing the intake of linoleate to 7.5% of calories did not suppress deposition of isomeric unsaturated acids in tissue lipids.**

High levels of polyunsaturated fatty acids (Pufa) in many dietary vegetable oils make them extremely vulnerable to autoxidation, decreasing their Pufa content and producing objectionable and toxic products. This characteristic limits the useful lifetime in exposure to air and causes problems in distribution and storage. Partial hydrogenation of vegetable oils produces fats of desired physical property, lessens rancidity and off-flavors, increases shelf life, and makes available inexpensive food fats on a large scale, but it diminishes Pufa content, increases saturated acids, and increases *cis* and *trans* positional isomers of monoenoic acids, many of which are rare in nature and whose metabolism is not known. *trans* isomeric acids are better known because they are more abundant in hydrogenated oils and are more easily measured than the *cis* isomers that accompany them. *trans* acids are known to be incorporated into tissue lipids at appreciable levels (1–6), and isomers of common unsaturated fatty acids affect Pufa metabolism (6–10). Dietary fat containing appreciable isomeric 18:1† acids intensifies essential fatty acid (Efa) deficiency and affects desaturase (acyl-CoA, hydrogen-donor: oxidoreductase, EC 1.14.99.5) activities assayed *in vitro* (6). Effects of isomeric unsaturated acids on Efa metabolism are studied here in greater detail, using an analytical approach to measure metabolic activities at each step in the conversion of dietary Efa to longer and more highly unsaturated acids. This study compares such analyses with enzymatic assays and presents data to evaluate some of the changes in Pufa metabolism attributable to high levels of isomeric octadecenoic acids in dietary fat.

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MATERIALS AND METHODS

Male weanling Sprague–Dawley rats were housed individually and fed semipurified diets and water ad lib. The diets were a modified Efa-deficient diet (6) with fat substitutions made at the expense of sucrose. They contained, in addition to fats, (g/kg) vitamin-free casein, 250; nonnutritive fiber, 40; Williams–Briggs salts, 35; vitamin mix, 10; choline chloride mix, 10; DL-methionine, 3; sucrose/fat supplement to make 1000. In experiment 1, three groups of 12 rats each were used. Group 1 was fed a diet containing 20% partially hydrogenated soybean oil (Phso) providing 10.5% total *trans* fatty acid isomers and 4.6% total *cis* 18:1 isomers (1.38% oleic acid and 3.22% other *cis* isomers). Group 2 was fed a diet containing 20% fully hydrogenated coconut oil (Hcno), providing only saturated acids, and 0.03% corn oil. Group 3 was fed a low-fat diet containing 0.15% corn oil as the sole source of fat. Corn oil was added to the diets of groups 2 and 3 to equalize linoleic acid content in all diets at 0.09%, to provide 18% of the linoleic acid requirement (11, 12), at which rats should be highly responsive to perturbations in Efa metabolism. The experiment was designed to test the effects of Phso and saturated fat on Efa metabolism. Rats were weighed periodically and dermatitis was scored at 31 weeks (11, 12) when they were sacrificed under ether anesthesia and livers and hearts were taken for analysis. Microsomes were prepared from individual livers and stored briefly at −70°C until used for desaturase assays (6, 7, 13). Tissue lipids were extracted, total phospholipids were isolated, and methyl esters were prepared and analyzed by gas/liquid chromatography (6) using a 20 ft × 1/8 in. (6.10 m × 3.18 mm) aluminum column packed with 15% OV-275 and operated isothermally at 240°C. Data were entered into disc memory by a FOCAL program and a PDP-12 computer. Another program calculated means and SEMs for 23 fatty acids ranging from 12:0 through 22:6ω3 and 13 other parameters calculated from these data. An example of complete computer printout of similar primary data has been published (14). Sums of products resulting from Δ6, Δ5, Δ4, and Δ9 desaturation reactions and from chain elongations to C_{20} and to

Abbreviations: Pufa, polyunsaturated fatty acids; Efa, essential fatty acids; Phso, partially hydrogenated soybean oil; Hcno, hydrogenated coconut oil.

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† The ω system of abbreviated nomenclature of fatty acids is as follows—chain length, number of double bonds; ω, number of carbon atoms beyond the last double bond, including the terminal methyl group (i.e., 20:4ω6 is arachidonic acid). This system is used to indicate metabolic interrelationship, for metabolic change does not alter the terminal (ω) structure.

Table 1. Pufa metabolic products produced in the ω3, ω6, and ω9 series by individual desaturation and elongation steps

Step	ω3	ω6	ω9
Δ9 desaturation			9–18:1
Δ6 desaturation	6,9,12,15–18:4	6,9,12–18:3	6,9–18:2
C_{20} elongation	8,11,14,17–20:4	11,14–20:2	11–20:1
		8,11,14–20:3	8,11–20:2
Δ5 desaturation	5,8,11,14,17–20:5	5,8,11,14–20:4	5,8,11–20:3
C_{22} elongation	10,13,16,19–22:4	10,13,16–22:3	10,13–22:2
	7,10,13,16,19–22:5	7,10,13,16–22:4	7,10,13–22:3
Δ4 desaturation	4,7,10,13,16,19–22:6	4,7,10,13,16–22:5	4,7,10,13–22:4

C_{22} were also calculated. Individual metabolic products within these groups are given in Table 1. For ease of graphic presentation, the normalcy ratio was also calculated: the experimental value divided by the control value.

Experiment 2 used eight rats per group, and the effect was compared of seven dietary levels of 18:2ω6 at each of two dietary levels of Phso (5% and 10%). Safflower oil supplied 0.5%, 1.0%, 1.5%, 2.0%, 3.0%, 5.0%, and 7.5% of calories of 18:2ω6, providing 0.5–7.5 times the minimum Efa requirement. Diets were made isocaloric at 15% fat by adjustment with Hcno. One group received 18:2ω6 at 1% of calories but no Phso to serve as a low-fat control. In other respects, the diets were similar to those used in experiment 1. After 14 weeks, the rats were sacrificed under ether anesthesia; hearts were excised, frozen, and extracted; and phospholipids were analyzed.

Although OV-275 stainless steel columns are suboptimal for Pufa analysis (15), we have had 93–97% recovery of 18:2ω6 and 20:4ω6 and 60–80% recovery of longer chain Pufa with OV-275 in aluminum columns 20 ft × 1/8 in., which separate most of the individual Pufa. Recoveries were acceptable for purposes of comparison.

RESULTS AND DISCUSSION

Experiment 1 showed that all groups were, as expected, moderately Efa deficient judged by growth, dermatitis, and elevated 20:3ω9 and reduced 20:4ω6 levels (Table 2). Although all three groups received the same low level of 18:2ω6 in the diet, the Phso-fed group and the Hcno-fed group were more Efa deficient than the low-fat-diet group. Levels of 18:2ω6 in liver phospholipids of the Phso-fed and low-fat-diet groups were equal but total metabolic products derived from 18:2ω6 were lower in the Phso-fed group than in the Hcno-fed and low-fat-diet groups. Growth and dermal scores confirmed that saturated fat (16) and isomeric unsaturated fatty acids (6) intensified Efa deficiency. Phso (68% isomeric 18:1 acids) had a stronger detrimental effect on Efa metabolism than did fully saturated Hcno. Thus, isomeric octadecenoic acids cannot be considered nutritionally equivalent to saturated acids.

Table 2. Major liver fatty acids, body weights, and dermal scores of rats fed Phso, Hcno, or a low level of corn oil (experiment 1)

	Liver phospholipid fatty acids, % total fatty acids		
	Phso	Hcno	Low fat
16:0	13.1	14.1	18.1
trans-16:1	0.8	0	0
cis-16:1	4.5	6.3	7.9
18:0	20.2	25.7	25.4
trans-18:1	11.4	0	0
cis-18:1	23.7	18.0	18.9
cis, trans-18:2ω6	1.2	0.2	0
cis, cis-18:2ω6	3.3	2.8	3.3
20:3ω9	8.7	19.4	11.8
20:4ω6	4.6	5.8	9.4
Total *trans* fatty acids	13.3	0.2	0
Total ω6 fatty acids	10.0	12.3	16.4
ω6 metabolites	6.6	9.5	13.1
Body weights,* g	317 ± 10.3	291 ± 6.1	371 ± 12.2
Dermal scores*	1.17 ± 0.17	1.50 ± 0.28	0.54 ± 0.22

* Mean ± SEM.

In vitro desaturase activities of liver microsomes were measured for comparison with the analytical data because dietary isomeric acids have been shown to affect liver desaturases (6, 7, 9, 17–19). Specific activities of liver microsomes from the three groups of rats are given for Δ6 desaturase, Δ5 desaturase, and Δ9 desaturase in Table 3. The appropriate labeled substrate for measurement of Δ4 desaturase activity was unavailable. *In vitro* Δ5 desaturase assay showed no significant differences among the three dietary groups. Δ6 desaturase activities of liver microsomes of the Phso-fed group were lower than those of the Hcno-fed group and Δ9 desaturase activities in Phso-fed rats were significantly lower than in Hcno-fed and low-fat-diet rats. Some differences were apparent between results of this study using assays on individual livers under optimal assay conditions (7) and those of our preliminary study with pooled samples (6).

Normalcy ratios comparing groups of Hcno-fed vs. low-fat-diet and Phso-fed vs. low-fat-diet, showing the effects of dietary saturated fatty acids or of isomeric 18:1 acids, respectively, on the pattern of Pufa in liver phospholipids are presented in Fig. 1. All ω6 fatty acids except 20:3ω6 were less in the Hcno-fed group than in the low-fat-diet group, confirming that Efa deficiency was enhanced by dietary saturated fat (17). This was evidenced also by the highly significant elevations of both 20:2ω9 and 20:3ω9. Saturated fat enhanced the elongation to C_{20} and desaturation at Δ5.

Table 3. *In vitro* desaturase activities of rat liver microsomes (experiment 1)

	Specific activity		
	Phso	Hcno	Low fat
18:2ω6 → 18:3ω6 Δ6 desaturase*	0.30 ± 0.02	0.39 ± 0.02	0.21 ± 0.04
20:3ω6 → 20:4ω6 Δ5 desaturase†	0.25 ± 0.01	0.24 ± 0.01	0.25 ± 0.01
16:0 → 16:1ω7 Δ9 desaturase‡	0.84 ± 0.06	1.00 ± 0.08	1.24 ± 0.08

Results (mean ± SEM) are expressed as nanomoles of substrate converted per minute per milligram of protein.
* Phso vs. Hcno fed, $P < 0.05$.
† No significant differences among groups.
‡ Phso vs. Hcno fed, $P < 0.05$; Phso fed vs. low-fat diet, $P < 0.001$.

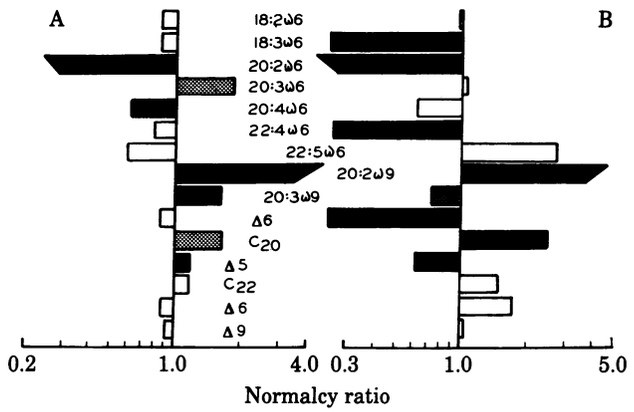

FIG. 1. Normalcy ratios of Pufa in liver phospholipids comparing Hcno-fed vs. low-fat-diet rats (*A*) and Phso- vs. corn oil-fed rats (*B*) (experiment 1). □, Not significant; ▨, $P < 0.05$; ■, $P < 0.01$; ■, $P < 0.001$. Truncated bar, value outside range of the chart.

Dietary isomeric 18:1 acids had other effects. The proportions of 18:3ω6 and 20:2ω6 were diminished very significantly, indicating impairment of Δ6 desaturase activity and elongation of 18:2ω6. 20:2ω9 was elevated significantly, indicating that elongation of 18:2ω9 was not impaired. 20:4ω6 and 22:4ω6 were likewise subnormal, indicating that Δ5 desaturation was impaired. The subnormal level of 20:3ω9 and the elevation of its precursor indicated that, for this family of acids too, the Δ5 desaturation was impaired.

Normalcy ratios for direct comparison of Phso- and Hcno-fed groups for heart and liver phospholipids are shown in Fig. 2. This comparison showed the enhancement of Efa deficiency by a fat containing isomeric 18:1 fatty acids to be greater than the enhancement by saturated fat. Linoleic acid was present in liver phospholipids in nearly equal amounts in the two groups, so isomeric 18:1 acids in the Phso did not interfere with its digestion, absorption, and incorporation into phospholipids more than did the saturated acids in Hcno. Δ6 desaturation of 18:2ω6 to 18:3ω6 and Δ5 desaturations to form 20:4ω6, 20:5ω3, and 20:3ω9 were suppressed more by the isomeric 18:1 acids than by saturated fat. The elongation product of arachidonic acid, 22:4ω6, was only 32% of the value ($P < 0.01$) for the Hcno-fed group. 22:5ω6, formed by Δ4 desaturation of 22:4ω6, was elevated nonsignificantly. Many of the changes in Pufa were <1% of total fatty acids and thus the differences in mass were small, but they represented significant changes in proportions of available substrates for formation of prostaglandins, which could have profound effects on metabolism.

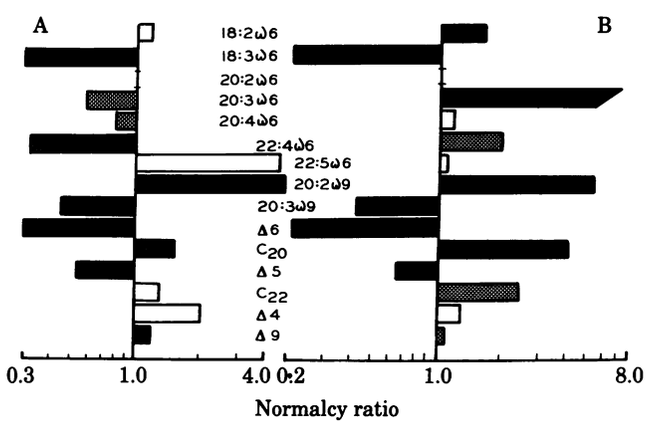

FIG. 2. Normalcy ratios of Pufa in liver (*A*) and heart (*B*) phospholipids comparing Phso- vs. Hcno-fed groups (experiment 1). Bars are as in Fig. 1.

The 20:3ω9 content of liver phospholipids in Phso-fed rats was 8.7 ± 0.4% of total fatty acids; in Hcno-fed rats, it was 19.4 ± 0.4% and, in low-fat-diet rats, it was 11.8 ± 0.8%; the 20:4ω6 contents were 4.6 ± 0.4%, 5.8 ± 0.3%, and 9.4 ± 0.4%, respectively. These high levels of 20:3ω9 and low levels of 20:4ω6 confirmed the Efa deficiency in all groups and indicated that the deficiency was most severe in the Phso-fed group. In liver phospholipids of Phso-fed rats, the low 20:4ω6 was compensated only partially by another ω5 desaturase product, 20:3ω9, indicating inhibition of Δ5 desaturase by isomeric 18:1 acids. This inhibition was confirmed by the 5-fold accumulation of the Δ5 desaturase substrate, 20:2ω9, in the Phso-fed group. The same 5-fold accumulation of 20:2ω9 and the suppression of 20:3ω9 to 42% of the Hcno value also occurred in heart phospholipids.

Judging from growth, dermal score, and low content of the functional Pufa in phospholipids, Efa deficiency was exaggerated by Phso. However, the triene/tetraene ratio (20:3ω9/20:4ω6) for liver phospholipids from Phso-fed rats was 1.3 ± 0.10; for Hcno-fed rats, it was 3.42 ± 0.22; and for low-fat-diet rats, it was 1.28 ± 0.09. Thus the triene/tetraene ratio did not reflect the true Efa status of the Phso-fed rats. In this instance, the triene/tetraene ratio is not useful because isomeric 18:1 acids may partially substitute for the functional arachidonic acid or otherwise affect the synthesis of 20:3ω9 or 20:4ω6. The triene/tetraene ratio should also be used with caution when linolenic acid is a dietary variable because it and its metabolic products strongly suppress the synthesis of both 20:3ω9 and 20:4ω6 (20). We prefer to evaluate Efa status by individual and collective contents of ω6 acids rather than by triene/tetraene ratio.

Analysis of Pufa by a discriminating gas/liquid chromatography method showed the status of a desaturase more precisely than did its enzymatic assay. The SEM of Δ6, Δ5, and Δ9 desaturase activities on replicate samples was relatively large (Table 3), and real but small differences in rate of reaction may not be discernable. Undetectable small differences in rate of reaction over extended times may lead to larger more easily detectable differences in the products incorporated and accumulated in structural and functional lipids. The superiority of the analytical approach to desaturase activity is shown in Table 4.

Data from experiment 2, involving adequate intake of dietary linoleate, confirm findings from experiment 1. Body weights

Table 4. Comparison of desaturation reactions measured by enzyme assay and by analysis of Pufa in liver phospholipids (experiment 1)

Desaturation	Assay: Enzyme	Assay: GLC
Δ5		
Phso fed vs. low fat	NS	↓ $P < 0.001$
Phso vs. Hcno fed	NS	↓ $P < 0.001$
Hcno fed vs. low fat,	NS	↑ $P < 0.001$
Δ6		
Phso fed vs. low fat	NS	↓ $P < 0.001$
Phso vs. Hcno fed	↓ $P < 0.05$	↓ $P < 0.001$
Hcno fed vs. low fat	NS	NS
Δ9		
Phso vs. corn oil fed	↓ $P < 0.001$	NS
Phso vs. Hcno fed	NS	↑ $P < 0.01$
Hcno vs. corn oil fed	↓ $P < 0.05$	NS

GLC, gas/liquid chromatography; NS, not significant.

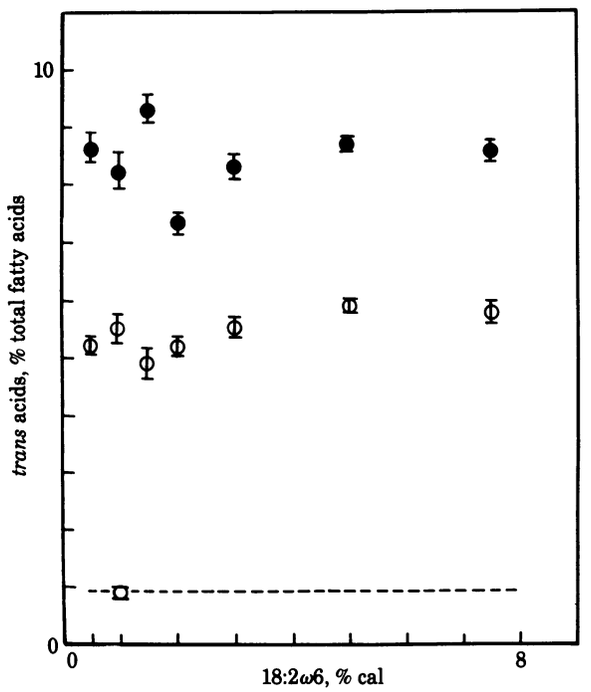

FIG. 3. Incorporation of *trans* fatty acids into heart phospholipids of rats fed 5% (○) and 10% (●) Phso and various levels of dietary 18:2ω6 (experiment 2). ----, No dietary Phso. (1 cal = 4.18 J.)

of the rats increased stepwise as the level of 18:2ω6 increased, with the group fed 5% Phso always nonsignificantly higher than the group fed 10% Phso. At each level of dietary Phso, incorporation of the *trans* fatty acids into heart phospholipids was almost equal regardless of the intake of 18:2ω6 (Fig. 3). The ratio of *trans* acids at the 10% Phso level to that at the 5% level (1.56) indicated less than proportional increase in tissue *trans* acids with increased dietary *trans* acids. Increased dietary 18:2ω6 did not significantly displace the *trans* acids from structural lipid, even at the lower intake of Phso.

The effect of dietary level of Phso on total ω6 fatty acids in heart phospholipids is shown in Fig. 4. In the control group, receiving 1% of calories as 18:2ω6 but no Phso, the total ω6 acid content of heart phospholipid was 46.8%, which was above the values for the groups fed 5% or 10% Phso and 1% of calories as 18:2ω6. The total ω6 acids were significantly greater at the 5% Phso level than at the 10% level in six of the seven levels of 18:2ω6, indicating proportional reduction of ω6 metabolism by Phso. Significantly lower levels of 20:2ω9 and 20:3ω9 in the groups fed 5% Phso at the two lower levels of dietary 18:2ω6 (Fig. 5) indicated reduced elongation to C_{20} and Δ5 desaturase, respectively.

The results described above reveal an overall impairment of the Pufa metabolism by isomeric octadecenoic acids. Enhancement of physical symptoms of Efa deficiency occurred at levels of 18:2ω6 below the dietary requirement (1% of calories), but ω6 metabolism was inhibited by Phso, even at higher dietary 18:2ω6 levels. Using the analytical approach, we found that dietary Phso induced modifications in Efa metabolism and consequent changes in Pufa pattern of structural lipids of liver and heart. Accentuation of Efa deficiency by Phso is greater than that induced by an equivalent proportion of dietary fully saturated fat. Because the Phso used was only 68% isomeric unsaturated acids, the enhancing effects of the latter on Efa deficiency would be 1.5 times greater than those reported here.

The analytical approach described here is also being used to reveal abnormalities of Pufa in human disease states (21). This method has revealed an instance of nutritional linolenic acid deficiency (22), and abnormal Efa metabolism has been ob-

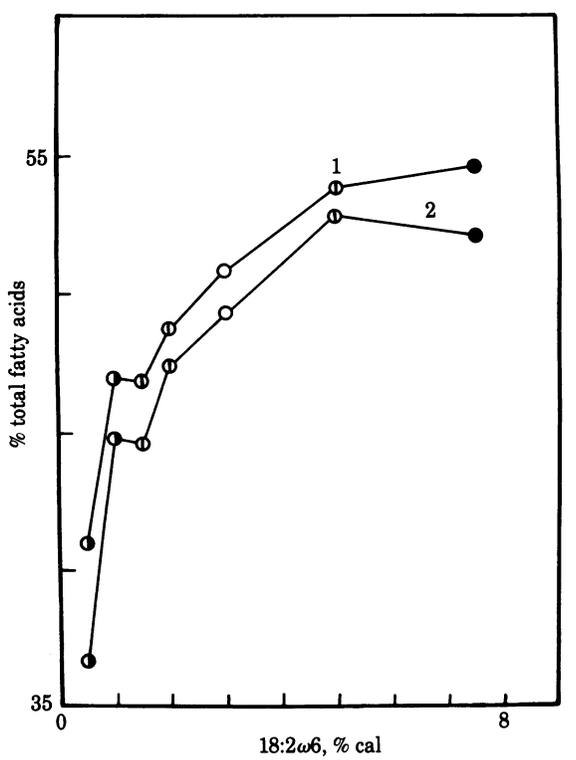

FIG. 4. Total ω6 fatty acids in heart phospholipids of rats fed 5% (curve 1) and 10% (curve 2) Phso at various levels of 18:2ω6 (experiment 2). ○, No significant difference; ◔, $P < 0.05$; ◑, $P < 0.01$; ●, $P < 0.001$.

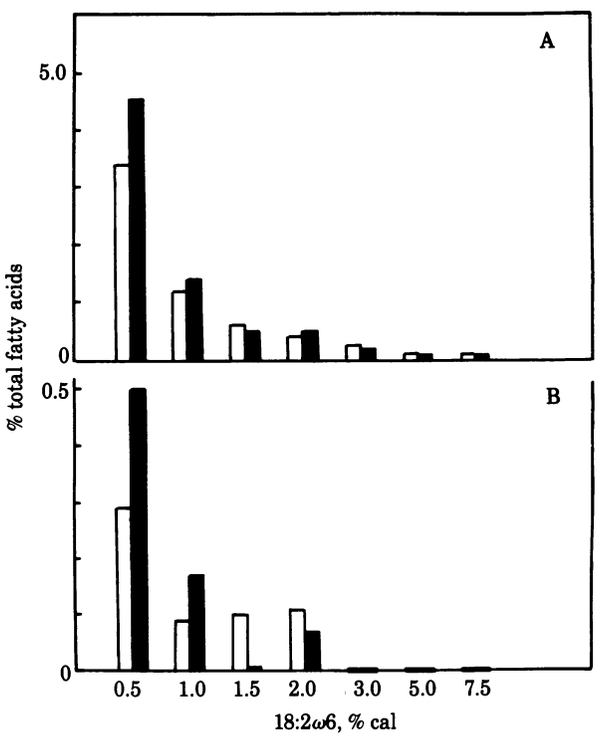

FIG. 5. 20:2ω9 (*B*) and 20:3ω9 (*A*) fatty acids in heart phospholipids of rats fed various levels of dietary 18:2ω6 (experiment 2). Differences were significant only at the 0.5% and 1.0% calorie levels. □, 5% Phso; ■, 10% Phso.

served in cystic fibrosis (23), chronic malnutrition (24), syndromes of genetic origin involving neuropathy (25, 26), and Reye syndrome (27).

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