— **Regular Article** —

Rates of Change in Tissue Fatty Acid Composition When Dietary Soybean Oil Is Switched to Olive Oil

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This experiment characterizes the time-dependent changes of phospholipids of liver microsomes, plasma, and triglycerides of adipose tissue when olive oil is substituted for soybean oil in the American Institute of Nutrition 93 Growth (AIN 93G) diet. After 2 weeks of acclimation with 7% soybean oil diet, 108 male Sprague-Dawley rats were randomly assigned to be fed diets with either 7% or 15% soybean oil or olive oil. Fatty acids of adipose, blood plasma, and liver microsomes were analyzed every four days for 4 weeks by gas chromatography. After 6 weeks of feeding, no differences in body weight or body composition were observed. The most profound changes occurred in the proportion of linoleic acid to oleic acid in the three tissues. The feeding regimens increased the ratio of oleic to linoleic acids by 6- to 14-fold in olive oil groups compared with soybean oil groups. The level of arachidonic acid declined in olive oil groups. In contrast, levels of n-3 series long chain fatty acids, eicosapentaenoic and docosahexaenoic acids, were higher in olive oil groups. The peroxidizability index, a measure of unsaturation, declined significantly in groups fed olive oil compared to soybean oil. These data indicate that substantial remodeling in fatty acid composition continues until 18-carbon precursors in adipose depots match the dietary pattern. The rate of change appears to be governed by the fractional exchange rate of adipose tissue, which is limited by the rates of lipolysis and daily fatty acid oxidation.

Key words —— dietary fat, fatty acid composition, liver microsome, plasma, adipose tissue

INTRODUCTION

Traditional human diets vary widely in their content of total fat, monounsaturated fat, and n-3 or n-6 polyunsaturated fat. Dietary fats affect physiological responses by influencing the fatty acid composition of tissue lipids including those of heart, brain, liver and blood. For humans and animals, the steady state composition of fatty acids in tissues is maintained by precursor supply and by the activity of different esterifying enzymes that act on specific sites. The saturated fatty acids (SFA) tend to be esterified in the sn-1 position of phospholipids, whereas the 16- and 18-carbon unsaturated fatty acids and the 20- and 22-carbon highly unsaturated fatty acids (HUFA) tend to be esterified in the sn-2 position during *de novo* synthesis and remodeling process, respectively. As a result, both the available fatty acids and enzymes selectivity can determine the fatty acid composition. Furthermore, the effect of dietary fat composition on tissue lipids can be predicted by a quantitative model.[1, 2)]

With few exceptions, biochemical or kinetic theory has been used little either in planning experiments or in developing dietary guidelines for fat intake. One exception is the ability to predict change in plasma cholesterol from change in intake of cholesterol, saturated fat, and unsaturated fat.[3)] In addition, the theory of fat balance may have contributed to the recommendation that fat intake should be reduced to less than 30% of total calories. If it is true that many effects of fatty acids stem from their ability to modify the composition of cellular membranes, then it seems logical to develop

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a more sophisticated theory of cause and effect. The predictive equations developed by Lands *et al.*[1,4] are a first step in this direction. However, a fully developed theory should include models of kinetic and effect. The prior equations were derived under steady state conditions and can not account for rates of change in tissue reservoirs.

Recent mean U.S.A. consumption of dietary fats as reported by National Health and Nutrition Examination Survey (NHANES) 1999–2000 was 33% of total energy from fat, 6.7% from polyunsaturated fatty acids (PUFA), 12.5% monounsaturated fatty acids (MUFA), and 11.3% SFA.[5] This is a very reasonable outcome, but results derived from analyzing human fat tissue suggest that many people maintain a much higher reserve of linoleic acid (18:2 n-6) in their adipose, and it is this reserve that provides the precursors for cellular membrane synthesis.

Because many dietary guidelines attempt to be disease-specific, some contradictions arise. Current Reference Daily Intake (RDI) emphasizes more PUFA and less SFA intake to reduce cardiovascular disease, but one third of U.S.A. and Korean populations have the risk of cardiovascular disease and the rest of them encounter the other problems. Under some experimental conditions, PUFA may produce adverse effects that presumably result from their susceptibility to peroxidation. For instance, PUFA from corn oil and fish oil augment oxidative stress, hepatic inflammation and pathology,[6–10] whereas dietary SFA in beef and pork are protective against alcohol-induced liver disease in men and animals.[6–8,10–12] However, a lipid profile similar to the "Mediterranean diet" (moderately low fat, high oleic acid content, lower PUFA and SFA) is protective against cardiovascular disease. Therefore, we decide to examine the rate of change and degree of representative dietary oils of PUFA and SFA. For PUFA-rich diet, we choose the American Institute of Nutrition 93 Growth (AIN 93G) rodent diet, which is formulated for laboratory rodents and contains 7% soybean oil by weight, which has 14.4 g SFA, 23.3 g MUFA, and 57.9 g PUFA per 100 g.[13] Thus, the AIN 93G rodent diet is a high linoleic acid diet that is not in balance with respect to these constituents. The rodent diet contains 16.7% of its total energy as fat, and would be low by human standards.

Lands and colleagues[1,4] have derived a quantitative saturation model of the response of membrane phospholipids and triglycerides to dietary fat composition. However, the kinetics of change was not included in this model. In addition, the model was not intended to predict changes in MUFA, which are important because they cause membranes to be less peroxidizable. Since we are interested in toxicity tests on animals that are fed different dietary fats thus resulting in different fatty acid compositions, a new dynamic model of fatty acid compositions is needed. The aim of this paper is to define the rate of change and degree of response of fatty acids in the tissues when a diet with a new fatty acid composition is substituted for the AIN 93G diet with 7 g per 100 g soybean oil. This study will help assess the effect of prior diet on potential for tissue injury during exposure to conditions or drugs that increase free radical production.

MATERIALS AND METHODS

Animals and Diets —— One hundred eight male Sprague-Dawley rats (Charles River, NC, USA) weighing 150–190 g were housed individually in hanging wire-mesh cages with feeding. Rats had free access to restricted experimental diets and tap water.

Rats were randomly assigned into four groups. All rats were fed the same AIN 93G diet containing 7% (wt/wt) soybean oil[13] for 2 weeks to equilibrate with the diet. Five rats were unfed overnight and killed to determine an initial fatty acid pattern. Rats in group 1 (n = 15) were fed the same AIN 93G diet and rats in group 2 (n = 18), 3 (n = 35), and 4 (n = 35) were switched to diets containing 15% soybean oil, 7% olive oil, or 15% olive oil for 4 more weeks, respectively. The low fat diets were made isocaloric by increasing Dyets PA, USA and the semipurified control diet was fed for one week to acclimate the animals to feed cups and individual caging. The fatty acid composition of the oils is shown in Table 1.

Table 1. Fatty Acid Composition of Soybean Oil and Olive Oil

Fatty Acid	Soybean oil	Olive oil
	(μmole %)	
C16:0	7.3	10.8
C18:0	3.7	2.9
C18:1 n-9	23.5	67.8
C18:2 n-6	62.1	9.7
C18:3 n-3	3.7	0.4
SFA	11.0	13.7
MUFA	23.5	67.8
PUFA	65.8	10.1

Samples were obtained every four days after the 2 week pre-feeding periods. Prior to autopsy, body composition was assessed by the Dual energy X-ray absorptiometry (DEXA) method for 5 rats in each group. They were unfed overnight and anesthetized with ketamine : acepronezine : xylazine (3 : 2 : 1) at a dose of 0.1 ml/100 g body weight and blood samples was collected by cardiac puncture in tubes containing EDTA and centrifuged at 2500 rpm for 20 min. The animals then were perfused transcardially with 0.9% NaCl and livers and epididymal adipose tissue were snap-frozen. All frozen samples were stored at −80°C for later experiments.

Preparation of Liver Microsomes —— The right lobe of liver was suspended in 25% homogenizing buffer which is 4°C, 1.15% KCl/50 mM Tris-HCl/1 mM EDTA (pH 7.4). Samples were homogenized and centrifuged at 10000 g at 4°C for 20 min. The resulting supernatant was centrifuged at 105000 g for 1 hr at 4°C and the microsomal pellet was suspended in 0.1 M potassium phosphate buffer (pH 7.4).

Fatty Acid Analysis —— Extraction of fatty acids in phospholipids of liver microsomes and plasma and triglycerides of adipose tissue was performed using the method of Folch *et al.*[14] Extracted fatty acids were applied to silica gel 60 Thin layer chromatography (TLC) plates (Sigma, MO, USA). After the application, solvent was allowed to dry and each plate was fully developed to 18 cm in acetone/acetic acid/water (100 : 2 : 1, by vol) for phospholipid and hexane/diethyl ether/acetic acid (80 : 20 : 2, by vol) for triacylglyceride. The lipid classes were visualized by spraying the plates with rhodamine 6G (0.02% in 95% ethanol) and exposing the plates to ultraviolet light. All of the silica in representative bands was scraped into 10-ml, screw-capped tubes (Teflon-lined caps). The silica was extracted with 3 ml 6% HCl in methanol containing 40 μl internal standard (heptadecanoic acid, C17:0, Nu Chek, MN, USA) and transmethylated at 75°C for 2 hr. The tubes were cooled on ice, 2 ml of hexane and 1 ml KCl were added. Samples were vortexed for 1 min and centrifuged at 1000 rpm for 10 min. The hexane extract (upper phase) was passed through a sodium sulfate column and concentrated under a stream of nitrogen for Gas chromatography (GC) analysis.

Fatty acid analysis was performed on a Hewlett-packard 5890 gas chromatograph using a flexible fused capillary column (30 m × 0.25 mm internal diameter (I.D.): thickness 0.25 μm, J & W Scientific, Folsom, CA). The column temperature was programmed to begin at 205°C for 20 min and then increase at a rate of 3°C/min to a final temperature of 220°C, which held for 3 min. The injection port temperature was 250°C and the detector was 260°C. Fatty acid values were presented as μmole % which represents the area under the chromatograph identified as a particular fatty acid based on its retention time when compared to the internal standard.

The peroxidizability index (PI) is calculated as an index of substrate availability for maximal rates of oxidation with two or more double bonds and is based on the formula of *in vitro* experiment.[15]

$$\begin{aligned}\mathrm{PI} = {} & (\text{Monoenoic \%} \times 0.025) + (\text{dienoic \%} \times 1) \\ & + (\text{Trienoic \%} \times 2) + (\text{Tetraenoic \%} \times 4) \\ & + (\text{Pentaenoic \%} \times 6) + (\text{Hexaenoic \%} \times 8)\end{aligned}$$

Statistical Analysis —— All data were presented as means ± S.D. The data were analyzed with non-parametric methods due to small sample sizes using Statistical Analysis System (SAS) computer-based statistics programs. The Kruskal-Wallis test and the Wilcoxon rank sum test were performed for one-way analysis of variance and statistical differences between two groups, respectively.

RESULTS

Body Weight and Body Composition

There were no significant differences in the initial and final body weights, which increased approximately from 150 g to 330 g after the 6 week feeding period. The mean body weight was high in the 15% olive oil group (342.8 g *vs*. on average 330 g of other groups). There were no significant differences in the body composition and body fat content ranged from 13 to 17% among the groups (data not shown).

Fatty Acid Composition of Phospholipids

The fatty acid composition of phospholipids of liver microsomes and plasma is shown in Tables 2 and 3. The major fatty acids were palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1 n-9), γ-linoleic acid (18:2 n-6), and arachidonic acid (20:4 n-6). The overall pattern of fatty acids was similar for the phospholipids of both tissues: values ranged from 42–46% SFA, 4.5–6.8% MUFA, and 35–38% PUFA in soybean oil groups and 47–50% SFA, 15–17% MUFA, and 26–29% PUFA in olive oil groups, respectively. There was no consistent

Table 2. Fatty Acid Composition of Phospholipids of Liver Microsomes in 7% or 15% Soybean Oil and Olive Oil-fed Rats

Fatty Acid	Group 1 (7% soybean oil)				Group 2 (15% soybean oil)				
	2wk	2wk8day	2wk16day	2wk28day	2wk[1)]	2wk8day	2wk16day	2wk24day	2wk28day
C14:0	0.44 ± 0.16[2)]	0.38 ± 0.15	0.32 ± 0.27	0.27 ± 0.32	0.44 ± 0.16	0.21 ± 0.16	0.41 ± 0.24	0.55 ± 0.27	0.49 ± 0.21
C16:0	23.0 ± 0.86	23.4 ± 2.27	23.1 ± 2.19	24.6 ± 2.59	23.0 ± 0.86	24.2 ± 4.68	22.6 ± 5.50	23.2 ± 3.23	24.5 ± 4.52
C16:1 n-7	0.82 ± 0.21^{a}	0.33 ± 0.28^{b}	0.32 ± 0.22^{b}	0.38 ± 0.37*	0.82 ± 0.21^{a}	0.32 ± 0.31	0.52 ± 0.52	0.47 ± 0.39	0.32 ± 0.24
C18:0	21.6 ± 1.07	24.2 ± 3.30	24.6 ± 1.85	24.6 ± 3.10	21.6 ± 1.07	22.9 ± 4.82	24.0 ± 4.70	20.4 ± 3.92	22.4 ± 3.98
C18:1 n-9	4.01 ± 1.40	4.47 ± 1.70	3.99 ± 1.41	4.20 ± 1.41	4.01 ± 1.40	4.57 ± 1.54	4.25 ± 1.65	3.77 ± 2.55	3.68 ± 1.85
C18:1 n-7	2.68 ± 1.19	2.02 ± 1.35	2.11 ± 1.38	2.01 ± 1.13	2.68 ± 1.19	2.26 ± 1.71	1.96 ± 1.73	2.10 ± 1.19	2.05 ± 1.51
C18:2 n-6	11.4 ± 1.64	10.8 ± 3.11	14.3 ± 2.12	13.2 ± 5.31	11.4 ± 1.64^{c}	11.3 ± 2.62^{c}	12.9 ± 1.27^{bc}	16.7 ± 2.25^{ab}	17.6 ± 5.79^{a*}
C18:2/C18:1[3)]	1.87 ± 0.74	1.66 ± 0.36	3.22 ± 1.68	2.27 ± 1.17	1.87 ± 0.74	1.84 ± 0.94	2.77 ± 1.09	4.00 ± 1.82	3.91 ± 1.48
C18:3	0.13 ± 0.16	0.22 ± 0.25	0.18 ± 0.14	0.25 ± 0.30	0.13 ± 0.16	0.17 ± 0.19	0.22 ± 0.23	0.23 ± 0.27	0.30 ± 0.30
C20:4 n-6	23.7 ± 1.17	22.5 ± 3.06	21.8 ± 6.51	18.8 ± 3.70	23.7 ± 1.17	18.6 ± 6.40	21.7 ± 2.90	18.2 ± 2.43	19.3 ± 4.73
C20:5 n-3	0.97 ± 0.79	0.72 ± 0.52	1.52 ± 0.45	1.07 ± 0.84	0.97 ± 0.79	0.54 ± 0.32	0.89 ± 0.44	0.78 ± 0.54	1.34 ± 1.02
C22:6 n-3	2.66 ± 1.06	2.58 ± 1.30	2.50 ± 1.08	2.66 ± 1.37	2.66 ± 1.06	2.24 ± 1.05	2.94 ± 0.74	2.43 ± 1.38	2.39 ± 1.27
PI[4)]	133.8	126.5	131.2	116.7	133.8	107.3	129.2	114.3	122.7

Fatty Acid	Group 3 (7% olive oil)							
	2wk[1)]	2wk4day	2wk8day	2wk12day	2wk16day	2wk20day	2wk24day	2wk28day
C14:0	0.44 ± 0.16[2)]	0.27 ± 0.36	0.25 ± 0.26	0.40 ± 0.35	0.49 ± 0.39	0.33 ± 0.27	0.40 ± 0.26	0.45 ± 0.50
C16:0	23.0 ± 0.86^{c}	24.9 ± 2.02^{bc}	26.2 ± 4.33^{bc}	27.5 ± 3.67^{abc}	27.4 ± 3.47^{abc}	29.4 ± 3.73^{ab}	30.3 ± 6.09^{a}	30.4 ± 2.94^{a**}
C16:1 n-7	0.82 ± 0.21	0.57 ± 0.26	0.56 ± 0.45	0.70 ± 0.47	0.75 ± 0.34	1.39 ± 0.88	1.32 ± 1.22	1.34 ± 0.86
C18:0	21.6 ± 1.07	23.1 ± 3.04	23.1 ± 3.10	24.8 ± 3.74	22.7 ± 4.27	22.7 ± 4.39	21.5 ± 7.35	21.0 ± 3.54
C18:1 n-9	4.01 ± 1.40^{c}	5.66 ± 1.97^{c}	10.9 ± 3.27^{b}	14.0 ± 5.42^{ab}	16.0 ± 2.93^{ab}	14.7 ± 4.19^{ab}	17.8 ± 4.40^{a}	17.6 ± 5.40^{a***}
C18:1 n-7	2.68 ± 1.19	3.01 ± 1.12	2.46 ± 1.12	3.15 ± 1.24	3.42 ± 1.90	3.90 ± 2.48	3.96 ± 1.67	3.14 ± 2.35
C18:2 n-6	11.4 ± 1.64^{a}	8.18 ± 1.52^{b}	8.82 ± 1.38^{b}	7.44 ± 2.48^{b}	7.50 ± 2.34^{b}	6.97 ± 1.96^{b}	7.00 ± 2.15^{b}	7.19 ± 0.63^{b*}
C18:2/C18:1[3)]	1.87 ± 0.74^{a}	0.98 ± 0.27^{b}	0.67 ± 0.13^{bc}	0.55 ± 0.36^{bc}	0.39 ± 0.11^{c}	0.40 ± 0.20^{c}	0.32 ± 0.03^{c}	0.35 ± 0.10^{c***}
C20:4 n-6	23.7 ± 1.17^{a}	24.2 ± 3.22^{a}	19.6 ± 3.27^{ab}	16.4 ± 3.74^{bc}	14.0 ± 3.88^{c}	12.3 ± 2.45^{c}	11.2 ± 4.28^{c}	12.0 ± 5.81^{c***}
C20:5 n-3	0.97 ± 0.79^{b}	0.61 ± 0.49^{b}	1.25 ± 0.77^{ab}	2.21 ± 1.22^{ab}	1.88 ± 1.98^{a}	2.38 ± 1.89^{a}	2.09 ± 1.40^{a}	2.34 ± 2.13^{a*}
C22:6 n-3	2.66 ± 1.06	3.02 ± 1.73	3.37 ± 2.71	2.53 ± 1.84	3.80 ± 2.02	3.62 ± 1.84	3.63 ± 2.39	3.85 ± 1.77
PI[4)]	133.8	133.1	122.1	107.0	105.8	99.8	94.1	100.5

Fatty Acid	Group 4 (15% olive oil)							
	2wk[1)]	2wk4day	2wk8day	2wk12day	2wk16day	2wk20day	2wk24day	2wk28day
C14:0	0.44 ± 0.16[2)]	0.24 ± 0.14	0.28 ± 0.31	0.33 ± 0.33	0.39 ± 0.34	0.38 ± 0.32	0.41 ± 0.34	0.40 ± 0.34
C16:0	23.0 ± 0.86^{c}	24.3 ± 1.35^{b}	26.9 ± 7.28^{ab}	28.9 ± 2.29^{ab}	28.4 ± 3.09^{ab}	30.1 ± 7.32^{ab}	29.3 ± 6.00^{a}	29.1 ± 4.12^{a*}
C16:1 n-7	0.82 ± 0.21^{ab}	0.60 ± 0.46^{b}	0.56 ± 0.29^{b}	0.70 ± 0.27^{b}	1.04 ± 0.28^{ab}	1.38 ± 0.19^{a}	1.42 ± 0.58^{a}	1.41 ± 0.85^{a*}
C18:0	21.6 ± 1.07	24.3 ± 2.58	24.0 ± 4.87	24.4 ± 6.80	22.9 ± 6.44	22.0 ± 7.26	20.5 ± 5.33	22.1 ± 5.33
C18:1 n-9	4.01 ± 1.40^{d}	6.45 ± 0.38^{d}	9.26 ± 4.42^{cd}	12.8 ± 3.83^{bc}	16.8 ± 6.55^{ab}	17.6 ± 2.64^{a}	19.7 ± 4.78^{a}	18.3 ± 5.58^{a***}
C18:1 n-7	2.68 ± 1.19	3.05 ± 1.66	3.91 ± 1.96	4.33 ± 3.02	3.72 ± 2.20	4.40 ± 3.96	4.57 ± 2.15	4.29 ± 3.54
C18:2 n-6	11.4 ± 1.64^{a}	8.60 ± 0.89^{ab}	7.62 ± 2.34^{b}	7.25 ± 2.88^{b}	7.30 ± 1.81^{b}	7.07 ± 1.59^{b}	7.03 ± 2.28^{b}	6.91 ± 2.40^{b*}
C18:2/C18:1[3)]	1.87 ± 0.74^{a}	0.92 ± 0.14^{b}	0.70 ± 0.35^{bc}	0.49 ± 0.14^{bc}	0.36 ± 0.11^{c}	0.32 ± 0.03^{c}	0.29 ± 0.11^{c}	0.31 ± 0.11^{c***}
C20:4 n-6	23.7 ± 1.17^{a}	23.3 ± 4.17^{a}	17.6 ± 6.78^{ab}	14.7 ± 2.22^{bc}	13.0 ± 4.41^{bc}	9.27 ± 1.89^{c}	10.6 ± 4.87^{c}	10.8 ± 7.07^{c***}
C20:5 n-3	0.97 ± 0.79	0.34 ± 0.38	0.72 ± 0.47	1.13 ± 1.24	1.90 ± 1.60	2.30 ± 1.50	1.93 ± 1.81	2.01 ± 0.94
C22:6 n-3	2.66 ± 1.06	2.96 ± 1.79	2.51 ± 1.61	3.13 ± 2.49	4.37 ± 1.55	3.91 ± 2.26	4.36 ± 2.11	4.53 ± 2.28
PI[4)]	133.8	127.7	102.9	98.2	106.0	89.8	96.5	99.2

1) 7% soybean oil-fed for 2 weeks, which was indicated for comparison between groups. 2) Values are means ± S.D. The unit is μmole %. 3) The ratio of linoleic acid to oleic acid. 4) Peroxidizability Index = (Monoenoic % × 0.025) + (Dienoic % × 1) + (Trienoic % × 2) + (Tetraenoic % × 4) + (Pentaenoic % × 6) + (Hexaenoic % × 8). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$ by Kruskal-Wallis test. Different superscripts (a, b, c, & d) for each fatty acid mean significantly different levels between dates.

change in SFA. The MUFA were less abundant in phospholipids of soybean oil-fed groups than olive oil fed groups, whereas PUFA were elevated in response to the soybean oil diet pattern. No significant differences in the total percentages of fatty acids were noticed in soybean oil-fed groups, except that the linoleic acid increased in 15% soybean oil-fed groups. A doubling of linoleic acid consumption increased the content in phospholipids by approximately 50%.

However, in olive oil-fed groups there were remarkable inverse changes in the quantities of oleic

Table 3. Fatty Acid Composition of Phospholipids of Plasma in 7% or 15% Soybean Oil and Olive Oil-fed Rats

Fatty Acid	Group 1 (7% soybean oil)				Group 2 (15% soybean oil)				
	2wk[1)]	2wk8day	2wk16day	2wk28day	2wk	2wk8day	2wk16day	2wk24day	2wk28day
C14:0	0.27 ± 0.35[2)]	0.24 ± 0.17	0.19 ± 0.21	0.22 ± 0.16	0.27 ± 0.35	0.24 ± 0.25	0.17 ± 0.21	0.12 ± 0.10	0.14 ± 0.18
C16:0	23.7 ± 2.61	25.4 ± 3.54	25.5 ± 7.38	28.0 ± 4.11	23.7 ± 2.61	24.0 ± 6.19	27.7 ± 4.29	27.1 ± 3.25	26.57 ± 3.41
C16:1 n-7	0.91 ± 0.28	0.56 ± 0.45	0.63 ± 0.44	0.50 ± 0.32	0.91 ± 0.28	0.53 ± 0.42	0.68 ± 0.39	0.39 ± 0.49	0.40 ± 0.37
C18:0	18.3 ± 3.16	17.9 ± 5.59	15.9 ± 6.68	16.1 ± 2.31	18.3 ± 3.16	19.7 ± 3.40	17.1 ± 6.45	13.1 ± 2.53	13.89 ± 3.77
C18:1 n-9	2.97 ± 0.26	2.40 ± 1.67	2.38 ± 1.36	2.35 ± 1.37	2.97 ± 0.26	2.92 ± 1.94	2.31 ± 2.07	2.39 ± 1.60	2.43 ± 1.62
C18:1 n-7	1.56 ± 0.48	1.46 ± 1.20	1.22 ± 0.48	1.24 ± 1.07	1.56 ± 0.48	1.50 ± 0.96	1.40 ± 0.98	1.30 ± 1.22	0.90 ± 0.35
C18:2 n-6	14.9 ± 3.05	15.5 ± 2.87	15.6 ± 1.61	15.0 ± 2.87	14.9 ± 3.05^{a}	15.7 ± 2.35^{b}	16.2 ± 4.38^{b}	17.1 ± 3.46^{b}	17.16 ± 3.87^{b**}
C18:2/C18:1[3)]	3.39 ± 1.11	4.38 ± 1.95	5.21 ± 1.53	4.17 ± 2.12	3.39 ± 1.11	4.03 ± 1.68	7.56 ± 1.28	6.96 ± 1.99	6.13 ± 1.92
C20:4 n-6	21.5 ± 3.12	18.9 ± 1.42	16.8 ± 5.20	16.2 ± 5.08	21.5 ± 3.12	15.3 ± 3.81	14.7 ± 2.19	12.5 ± 0.95	12.31 ± 4.66
C20:5 n-3	0.79 ± 1.02	1.38 ± 0.51	1.09 ± 0.68	0.97 ± 0.48	0.79 ± 1.02	0.54 ± 0.42	1.39 ± 1.04	0.92 ± 0.47	1.23 ± 0.78
C22:6 n-3	0.94 ± 0.67	1.97 ± 0.61	1.69 ± 0.63	1.70 ± 0.25	0.94 ± 0.67	1.70 ± 0.71	1.41 ± 0.50	1.29 ± 0.27	1.40 ± 0.47
PI[4)]	121.1	115.4	103.1	99.1	121.1	94.0	94.9	83.1	85.1

Fatty Acid	Group 3 (7% olive oil)							
	2wk[1)]	2wk4day	2wk8day	2wk12day	2wk16day	2wk20day	2wk24day	2wk28day
C14:0	0.27 ± 0.35[2)]	0.28 ± 0.29	0.24 ± 0.25	0.31 ± 0.34	0.38 ± 0.19	0.45 ± 0.26	0.56 ± 0.25	0.44 ± 0.17
C16:0	23.7 ± 2.61^{c}	24.3 ± 2.63^{c}	25.0 ± 4.18^{bc}	26.7 ± 3.43^{abc}	29.1 ± 5.77^{abc}	31.1 ± 4.02^{ab}	31.4 ± 6.53^{a}	29.8 ± 4.33^{abc*}
C16:1 n-7	0.91 ± 0.28	1.32 ± 0.56	1.08 ± 0.66	1.11 ± 0.53	0.81 ± 0.31	1.31 ± 1.12	1.43 ± 0.67	1.40 ± 1.44
C18:0	18.3 ± 3.16	18.9 ± 2.95	20.4 ± 3.01	18.5 ± 1.44	22.4 ± 5.36	17.9 ± 9.18	20.1 ± 3.74	20.8 ± 4.09
C18:1 n-9	2.97 ± 0.26^{d}	7.78 ± 1.13^{c}	10.3 ± 3.63^{c}	15.5 ± 2.73^{b}	14.8 ± 1.55^{b}	18.9 ± 3.42^{ab}	17.3 ± 5.28^{ab}	20.4 ± 4.59^{a***}
C18:1 n-7	1.56 ± 0.48	2.06 ± 1.16	1.69 ± 1.26	2.08 ± 1.40	2.40 ± 1.35	2.58 ± 2.24	2.19 ± 1.38	2.33 ± 1.00
C18:2 n-6	14.9 ± 3.05^{a}	11.0 ± 1.76^{b}	11.8 ± 1.47^{ab}	10.5 ± 3.21^{b}	9.57 ± 2.79^{b}	8.49 ± 2.42^{b}	9.37 ± 2.22^{b}	8.84 ± 3.34^{b*}
C18:2/C18:1[3)]	3.39 ± 1.11^{a}	1.14 ± 0.24^{b}	1.03 ± 0.22^{b}	0.61 ± 0.21^{bc}	0.56 ± 0.14^{bcd}	0.43 ± 0.20^{cd}	0.50 ± 0.15^{cd}	0.38 ± 0.12^{d***}
C20:4 n-6	21.5 ± 3.12^{a}	20.4 ± 4.62^{a}	15.3 ± 3.88^{b}	13.3 ± 3.54^{b}	8.48 ± 2.34^{c}	7.45 ± 3.00^{c}	7.74 ± 3.69^{c}	7.78 ± 1.69^{c***}
C20:5 n-3	0.79 ± 1.02	1.29 ± 1.66	1.35 ± 0.57	1.87 ± 2.58	1.47 ± 1.20	1.74 ± 1.14	1.94 ± 0.94	1.70 ± 1.31
C22:6 n-3	0.94 ± 0.67	1.24 ± 0.79	1.62 ± 0.42	1.57 ± 0.86	1.50 ± 1.14	1.69 ± 1.18	1.89 ± 1.41	1.83 ± 1.13
PI[4)]	121.1	110.6	94.3	87.7	64.8	62.8	67.6	65.4

Fatty Acid	Group 4 (15% olive oil)							
	2wk[1)]	2wk4day	2wk8day	2wk12day	2wk16day	2wk20day	2wk24day	2wk28day
C14:0	0.27 ± 0.35[2)]	0.36 ± 0.15	0.44 ± 0.34	0.44 ± 0.24	0.47 ± 0.29	0.33 ± 0.19	0.47 ± 0.30	0.37 ± 0.20
C16:0	23.7 ± 2.61^{c}	26.1 ± 3.22^{bc}	26.8 ± 2.99^{abc}	28.5 ± 3.84^{abc}	31.0 ± 3.38^{ab}	32.5 ± 4.56^{a}	31.8 ± 5.43^{ab}	31.0 ± 4.63^{ab*}
C16:1 n-7	0.91 ± 0.28	0.95 ± 0.34	0.99 ± 0.78	1.23 ± 0.75	0.85 ± 0.45	0.86 ± 0.25	1.30 ± 0.78	1.45 ± 1.11
C18:0	18.3 ± 3.16	22.4 ± 5.38	22.3 ± 3.19	18.8 ± 3.61	22.5 ± 11.43	17.2 ± 4.15	17.5 ± 2.30	19.4 ± 6.34
C18:1 n-9	2.97 ± 0.26^{c}	8.66 ± 2.35^{de}	12.2 ± 4.40^{ad}	18.7 ± 5.39^{ab}	16.2 ± 4.28^{bc}	20.1 ± 2.87^{ab}	23.1 ± 7.82^{a}	22.3 ± 5.18^{ab***}
C18:1 n-7	1.56 ± 0.48	1.83 ± 1.08	2.26 ± 1.82	2.43 ± 1.05	2.48 ± 2.33	2.64 ± 1.45	2.76 ± 1.15	2.99 ± 1.72
C18:2 n-6	14.9 ± 3.05^{a}	11.0 ± 1.70^{abc}	11.7 ± 1.85^{ab}	9.52 ± 1.12^{bc}	8.65 ± 0.58^{bc}	7.99 ± 3.09^{bc}	6.27 ± 5.11^{c}	6.45 ± 4.66^{c**}
C18:2/C18:1[3)]	3.39 ± 1.11^{a}	1.08 ± 0.28^{b}	0.84 ± 0.18^{bc}	0.48 ± 0.14^{bc}	0.48 ± 0.10^{bc}	0.36 ± 0.14^{c}	0.25 ± 0.11^{c}	0.27 ± 0.11^{c***}
C20:4 n-6	21.5 ± 3.12^{a}	20.6 ± 3.31^{ab}	14.4 ± 3.72^{bcd}	15.6 ± 6.17^{abc}	9.47 ± 3.72^{de}	8.31 ± 2.21^{de}	6.30 ± 7.00^{e}	6.26 ± 4.77^{e***}
C20:5 n-3	0.79 ± 1.02	0.33 ± 0.39	0.89 ± 0.50	1.18 ± 0.45	1.20 ± 0.98	1.12 ± 0.74	1.43 ± 0.60	1.28 ± 1.01
C22:6 n-3	0.94 ± 0.67	1.09 ± 0.80	1.43 ± 1.13	1.08 ± 0.49	1.34 ± 2.08	1.49 ± 1.23	1.60 ± 1.49	1.43 ± 1.56
PI[4)]	121.1	104.3	86.5	88.3	64.9	60.5	53.5	51.3

1) 7% soybean oil-fed for 2 weeks, which was indicated for comparison between groups. 2) Values are means ± S.D. The unit is μmole %. 3) The ratio of linoleic acid to oleic acid. 4) Peroxidizability Index = (Monoenoic % × 0.025) + (Dienoic % × 1) + (Trienoic % × 2) + (Tetraenoic % × 4) + (Pentaenoic % × 6) + (Hexaenoic % × 8). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$ by Kruskal-Wallis test. Different superscripts (a, b, c, d, & e) for each fatty acid mean significantly different levels between dates.

and linoleic acid (Figs. 1 and 2). Oleic acid (18:1 n-9) increased gradually from 4.0 to 17.6 or from 2.97 to 20.4 μmole % in phospholipid of liver microsomes and plasma in 7% olive oil-fed groups, respectively. Changes in oleic acid were similar in 15% olive oil-fed group. In contrast, linoleic acid decreased significantly from 11.4 to 7.2 or 8.8 μmole % in phospholipids of liver microsomes and plasma in the 7% olive oil-fed group, respectively.

Fatty acid profiles in phospholipids of the 15% olive oil-fed groups were almost identical to those of the 7% olive oil group. Therefore, the feeding regimens decreased the ratio of linoleic acid to oleic

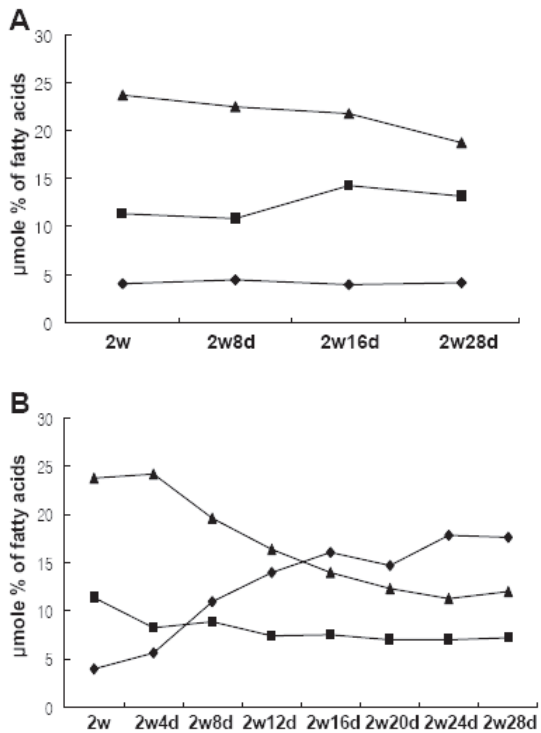

Fig. 1. Major Fatty Acid Composition of Membrane Phospholipid from Liver Microsomes of Rats Fed 7% Soybean Oil (A) and Olive Oil (B) Diets

In olive oil groups, there were progressive changes in the quantity of oleic and linoleic acids. Oleic acid increased gradually and significantly from 4.01 to 17.57 µmole % in 7% olive oil groups after 28 days. In contrast, linoleic acid decreased significantly from 11.35 to 7.19 µmole % in olive oil groups. The fatty acid composition in olive oil groups appears to have equilibrated with the diet after approximately 24 days. Symbols indicate; –◆– C18:1 n-9, –■– C18:2 n-6, –▲– C20:4 n-6. See Table 2 for S.D. and statistical significance.

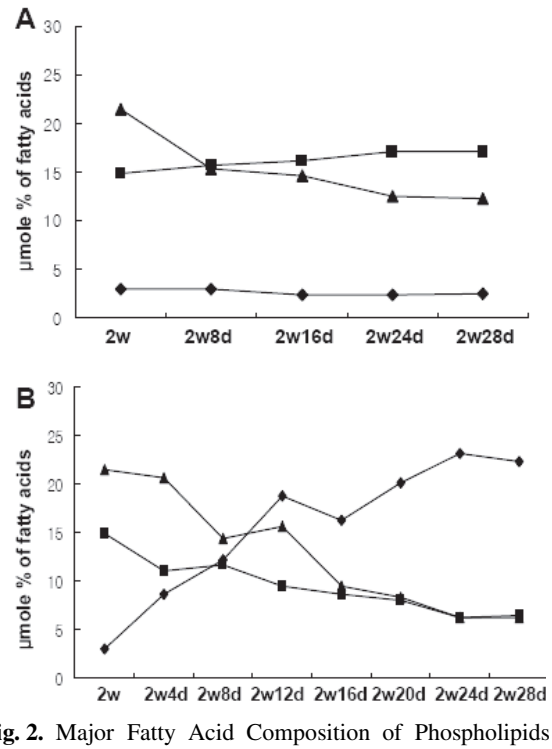

Fig. 2. Major Fatty Acid Composition of Phospholipids in Plasma of Rats Fed 15% Soybean (A) and Olive Oil (B) Diets

The level of each fatty acid in plasma follows a similar trend to the level in phospholipids of liver microsomes. The most striking change in fatty acid composition was linoleic and oleic acids in olive oil groups. There were almost no differences between 7% and 15% levels of dietary fats on the fatty acid composition of phospholipids of liver microsomes and plasma. Symbols indicate; –◆– C18:1 n-9, –■– C18:2 n-6, –▲– C20:4 n-6. See Table 3 for S.D. and statistical significance.

acid in liver phospholipid approximately 5- to 6-fold after feeding period. These ratios were 6- to 14-fold higher in groups fed soybean oil (Fig. 4). Olive oil diets produced significant reduction in arachidonic acid which varied from 23.7 to 10.8 µmole % and from 21.5 to 6.26 µmole % in phospholipids of liver microsomes and plasma, respectively. In contrast, both of 7% and 15% olive oil diets tended to increase eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels in phospholipids of liver microsomes and plasma (Tables 2 and 3). In general, olive oil groups had higher levels of EPA and DHA than soybean oil groups. The fatty acid composition in olive oil groups appeared to plateau after approximately 16–20 days of change in diet. These results suggested that fatty acid compositions of phospholipid reflected those of dietary fats in approximately 4 weeks of feeding.

The PI was much higher in soybean oil groups than olive oil groups, showing markedly decreased values in approximately 10 days after the switch to a new diet pattern (Fig. 5).

Fatty Acid Composition of Triglycerides in Adipose Tissue

The average fatty acid composition in adipose triglycerides was 29% SFA, 28% MUFA, and 29% PUFA in soybean group and 32% SFA, 42% MUFA, and 18% PUFA in olive oil groups, respectively (Table 4). Changes in the 15% soybean oil group were not significant compared to the 7% soybean oil group. A small decrease in oleic acid and increase in linoleic acid indicated that two week of acclimation soybean oil diet was not sufficient for full equilibration. As with the change in phospholipid, 4 weeks of feeding was required to achieve full equilibration. In 15% olive oil group, oleic acid increased from 25.5 to approximately 40.4 µmole % and linoleic acid decreased 25 to 11.4 µmole % (Table 4). In 7% olive oil group, there were similar but a little bit less contents of oleic and arachidonic

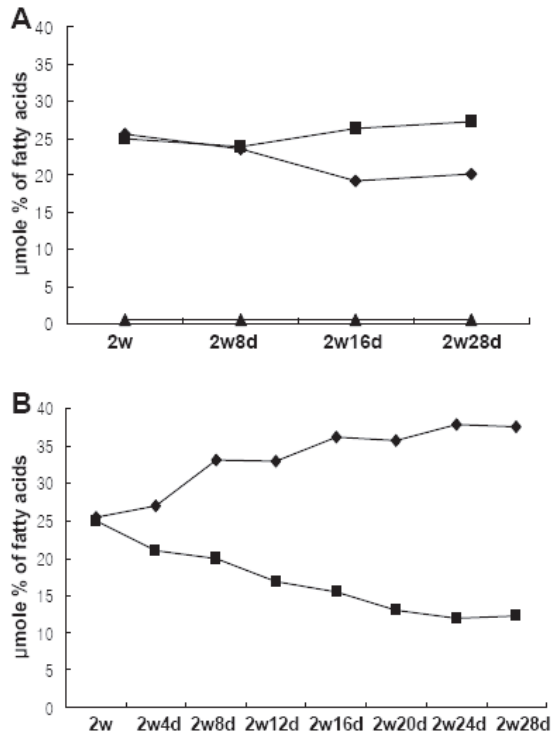

Fig. 3. Major Fatty Acid Composition of Triglycerides in Adipose Tissue of Rats Fed 7% Soybean Oil (A) and Olive Oil (B) Diets

After 28 days of feeding the new diets, oleic acid increased from 25.5 to 37.6 μmole % and linoleic acid decreased from 25.0 to 12.2 μmole % in the olive oil group. Symbols indicate; –◆– C18:1 n-9, –■– C18:2 n-6, –▲– C20:4 n-6. See Table 4 for S.D. and statistical significance.

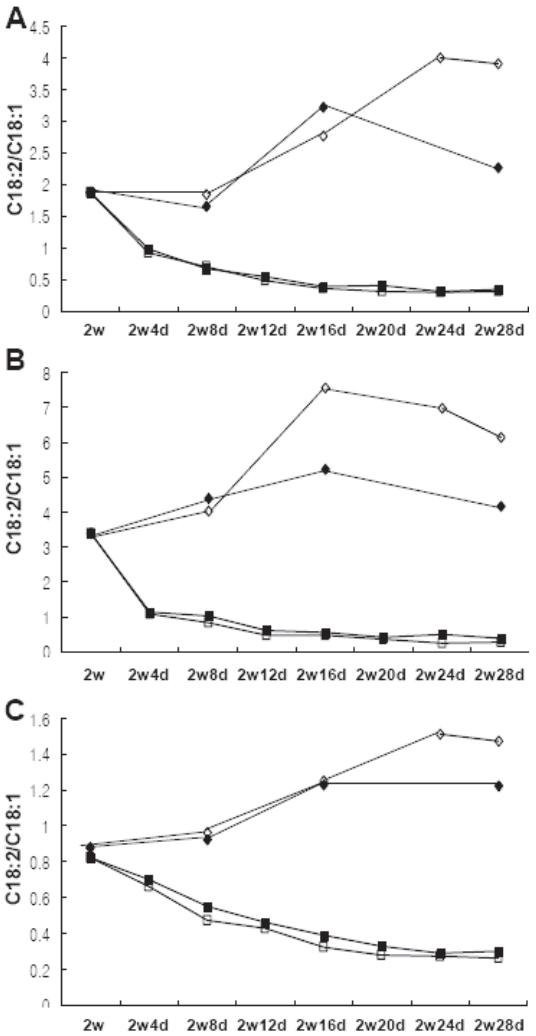

Fig. 4. The Ratios of Linoleic Acid and Oleic Acid in Phospholipids of Liver Microsomes (A) and Plasma (B) and in Triglycerides of Adipose Tissue (C)

The feeding regimens of olive oil increased these ratios approximately 5.3- to 12.5-fold in phospholipid composition and these ratios were 6- to 20-fold higher compared with soybean oil groups after 28 days feeding period. The ratios of linoleic to oleic acids in adipose were approximately 1.3-fold higher in soybean oil groups after 8 days and then 4- to 7-fold higher after 28 days, compared with soybean oil groups. Symbols indicate; –◆– 7% soybean oil, –◇– 15% soybean oil, –■– 7% olive oil, –□– 15% olive oil. See Tables 2–4 for S.D. and statistical significance.

acids (Table 3 and Fig. 3). The ratios of linoleic to oleic acids in adipose were approximately 1.3-fold higher in soybean oil groups after 8 days and then 4- to 7-fold higher after 28 days, compared with olive oil groups (Fig. 4). The proportions of oleic and linoleic acids in adipose of animals fed on different diets changed much more than the SFA, reflecting the fatty acid compositions of the diets.

Only small amounts of stearic, α-linolenic, and arachidonic acids were detectable in adipose and arachidonic acid declined to undetectable levels in olive oil groups. The proportions of palmitic and stearic acids in the phospholipids of the tissues did not vary widely, although the quantity taken in differed depending on the diet fed.

DISCUSSION

The present work was conducted to study the time-dependent effects of changing dietary fat sources on fatty acid composition in three different tissues. Our results show that the rate of change of 18-carbon precursors for membrane lipids is limited by the fractional exchange rate within adipose tissue. Thus, tissues required about 4 weeks to equilibrate with a dietary change initiated after 2 weeks of acclimation to an AIN 93G diet with 7% soybean oil. During the period of equilibration with dietary oleic acid, the ratios of linoleic acid to oleic acid in membrane phospholipids declined from about 2–4 to about 0.3. In addition, the concentration of

Table 4. Fatty Acid Composition of Triglycerides of Adipose Tissue in 7% or 15% Soybean Oil and Olive Oil-fed Rats

Fatty Acid	Group 1 (7% soybean oil)				Group 2 (15% soybean oil)				
	2wk[1)]	2wk8day	2wk16day	2wk28day	2wk	2wk8day	2wk16day	2wk24day	2wk28day
C14:0	0.89 ± 0.60[2)]	0.77 ± 0.46	0.80 ± 0.40	0.85 ± 0.59	0.89 ± 0.60	0.98 ± 0.59	0.88 ± 0.35	0.94 ± 0.75	0.84 ± 0.54
C16:0	24.8 ± 2.18	25.2 ± 4.44	26.3 ± 1.97	24.9 ± 3.49	24.8 ± 2.18	24.6 ± 3.78	24.5 ± 3.45	25.6 ± 5.67	24.3 ± 2.09
C16:1 n-7	3.34 ± 0.83	3.24 ± 1.56	3.52 ± 1.87	3.46 ± 2.23	3.34 ± 0.83	3.13 ± 1.69	3.27 ± 2.04	3.55 ± 2.08	3.39 ± 1.86
C18:0	3.88 ± 1.30	3.11 ± 2.21	2.53 ± 1.38	2.51 ± 1.43	3.88 ± 1.30	3.36 ± 1.91	3.45 ± 2.01	2.76 ± 1.57	2.92 ± 1.25
C18:1 n-9	25.5 ± 1.90^{a}	23.5 ± 6.15^{ab}	19.2 ± 3.21^{c}	20.2 ± 4.56^{bc*}	25.5 ± 1.90^{a}	24.5 ± 4.37^{a}	19.6 ± 3.71^{b}	17.7 ± 4.65^{b}	19.0 ± 5.26^{b**}
C18:1 n-7	2.91 ± 2.09	2.54 ± 1.51	2.09 ± 1.77	1.97 ± 1.05	2.91 ± 2.09	2.74 ± 1.18	2.64 ± 1.31	2.36 ± 1.21	2.19 ± 0.98
C18:2 n-6	25.0 ± 2.53^{b}	23.9 ± 1.74^{b}	26.3 ± 3.01^{ab}	27.3 ± 1.60^{a*}	25.0 ± 2.53^{b}	25.3 ± 5.71^{bc}	27.9 ± 2.69^{ab}	30.2 ± 2.60^{a}	31.2 ± 0.96^{a**}
C18:2/C18:1[3)]	0.88 ± 0.13^{b}	0.92 ± 0.32^{b}	1.23 ± 0.19^{a}	1.22 ± 0.33^{a**}	0.88 ± 0.13^{b}	0.96 ± 0.22^{b}	1.25 ± 0.44^{a}	1.51 ± 0.41^{a}	1.47 ± 0.37^{a***}
C18:3	1.29 ± 1.13	2.22 ± 1.36	2.59 ± 0.96	2.47 ± 1.38	1.29 ± 1.13	2.17 ± 0.96	2.24 ± 1.27	2.27 ± 1.13	2.15 ± 1.24
C20:4 n-6	0.39 ± 0.12	0.46 ± 0.37	0.48 ± 0.33	0.47 ± 0.34	0.39 ± 0.12	0.49 ± 0.37	0.51 ± 0.25	0.45 ± 0.26	0.55 ± 0.26

Fatty Acid	Group 3 (7% olive oil)							
	2wk[1)]	2wk4day	2wk8day	2wk12day	2wk16day	2wk20day	2wk24day	2wk28day
C14:0	0.89 ± 0.60[2)]	0.64 ± 0.28	0.69 ± 0.44	0.94 ± 0.50	1.00 ± 0.57	0.95 ± 0.57	1.27 ± 0.59	0.98 ± 0.91
C16:0	24.8 ± 2.18^{b}	24.8 ± 3.56^{b}	27.7 ± 6.86^{ab}	29.2 ± 2.40^{ab}	31.5 ± 6.77^{a}	33.1 ± 2.59^{a}	33.8 ± 4.18^{a}	32.5 ± 3.91^{a*}
C16:1 n-7	3.34 ± 0.83	4.68 ± 1.24	4.66 ± 2.45	5.38 ± 2.18	5.42 ± 2.33	6.10 ± 3.14	6.31 ± 2.86	6.28 ± 2.51
C18:0	3.88 ± 1.30	2.89 ± 0.73	2.66 ± 1.28	2.48 ± 1.15	2.42 ± 0.85	2.51 ± 1.39	2.48 ± 0.87	2.52 ± 0.80
C18:1 n-9	25.5 ± 1.90^{b}	27.0 ± 4.63^{b}	33.1 ± 3.81^{a}	32.9 ± 1.64^{a}	36.1 ± 5.59^{a}	35.7 ± 5.66^{a}	37.9 ± 5.07^{a}	37.6 ± 5.03^{a**}
C18:1 n-7	2.91 ± 2.09	3.17 ± 1.98	3.15 ± 1.49	3.55 ± 1.34	3.46 ± 1.75	3.54 ± 1.75	3.35 ± 2.03	3.78 ± 1.99
C18:2 n-6	25.0 ± 2.53^{a}	21.0 ± 1.12^{ab}	19.9 ± 3.54^{ab}	16.9 ± 3.08^{bc}	15.5 ± 2.88^{cd}	13.1 ± 2.13^{d}	12.0 ± 6.54^{d}	12.2 ± 2.73^{d***}
C18:2/C18:1[3)]	0.82 ± 0.13^{a}	0.70 ± 0.06^{ab}	0.55 ± 0.12^{bc}	0.46 ± 0.08^{cd}	0.39 ± 0.12^{cd}	0.33 ± 0.12^{d}	0.29 ± 0.09^{d}	0.30 ± 0.08^{d***}
C18:3 n-3	1.29 ± 1.13	1.94 ± 0.98	1.57 ± 0.38	2.04 ± 0.73	1.02 ± 1.36	1.67 ± 1.62	0.92 ± 0.78	1.07 ± 0.84

Fatty Acid	Group 4 (15% olive oil)							
	2wk[1)]	2wk4day	2wk8day	2wk12day	2wk16day	2wk20day	2wk24day	2wk28day
C14:0	0.89 ± 0.60[2)]	0.71 ± 0.58	0.77 ± 0.68	1.14 ± 0.56	1.02 ± 0.93	1.14 ± 1.10	1.40 ± 0.89	1.23 ± 0.82
C16:0	24.8 ± 2.18^{b}	24.5 ± 1.45^{b}	27.8 ± 4.94^{ab}	29.4 ± 2.67^{ab}	34.7 ± 10.16^{a}	32.6 ± 12.78^{ab}	33.0 ± 4.59^{ab}	31.5 ± 3.61^{ab*}
C16:1 n-7	3.34 ± 0.83	4.82 ± 1.52	4.48 ± 2.61	5.14 ± 2.74	5.04 ± 1.32	5.86 ± 3.39	6.05 ± 3.02	6.20 ± 2.67
C18:0	3.88 ± 1.30	3.54 ± 2.32	3.27 ± 1.55	3.35 ± 2.00	3.31 ± 1.33	2.85 ± 1.26	3.18 ± 1.57	3.20 ± 1.28
C18:1 n-9	25.5 ± 1.90^{d}	29.6 ± 2.17^{cd}	34.9 ± 4.01^{bc}	32.0 ± 2.97^{abc}	37.1 ± 2.01^{ab}	38.2 ± 4.43^{a}	39.4 ± 3.44^{a}	40.4 ± 6.62^{a***}
C18:1 n-7	2.91 ± 2.09	2.94 ± 1.59	3.24 ± 2.07	3.40 ± 2.41	3.53 ± 2.30	3.57 ± 1.92	4.04 ± 2.21	4.05 ± 2.81
C18:2 n-6	25.0 ± 2.53^{a}	21.4 ± 4.45^{ab}	18.0 ± 3.32^{bc}	15.1 ± 2.74^{c}	13.1 ± 3.66^{e}	11.8 ± 3.88^{cd}	11.9 ± 1.66^{cd}	11.4 ± 1.62^{cd***}
C18:2/C18:1[3)]	0.82 ± 0.13^{a}	0.66 ± 0.13^{b}	0.47 ± 0.10^{c}	0.43 ± 0.10^{cd}	0.32 ± 0.09^{cd}	0.28 ± 0.10^{de}	0.27 ± 0.05^{de}	0.26 ± 0.04^{de***}
C18:3 n-3	1.29 ± 1.13	1.89 ± 1.25	1.43 ± 0.46	1.60 ± 1.26	1.31 ± 1.17	1.45 ± 1.64	1.08 ± 0.51	1.10 ± 0.76

1) 7% soybean oil-fed for 2 weeks, which was indicated for comparison between groups. 2) Values are means ± S.D. The unit is μmole %. 3) The ratio of linoleic acid to oleic acid. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$ by Kruskal-Wallis test. Different superscripts (a, b, c, d, & e) for each fatty acid mean significantly different levels between dates.

arachidonic acid (20:4 n-6) in liver phospjolipids declined by about 50%. As a result, the predicted degree of peroxidizability of liver membranes and plasma phospholipids decreased significantly. In theory, these changes are consistent with a lower tendency to oxidation by free radicals. Because the free fatty acids mostly come from lipolysis in adipose tissue, the composition of plasma phospholipids is a reliable measure of lipid composition.[2)] Also, blood sample is more accessible than tissue biopsies for the ultimately resulting studies with humans.

Dietary fatty acids have a great effect on the fatty acid composition of phospholipids, which comprise 95% of the total membrane lipids. However, daily intakes of fatty acids are about 1–2 grams for a rat. This compares with 30–50 g of triglyceride in the adipose tissue, which serves as a temporary storage site for phospholipid precursors. Fatty acid compositions of three different tissues and, in particular, the MUFA and PUFA content are highly dependent on the fat composition of the diet by *de novo* synthesis or by elongation from fatty acids provided by the dietary fats.[16–18)] This was profoundly significant with linoleic acid and oleic acid in our data. Therefore, the steady state composition of fatty acids in tissues, *i.e.*, the proportions of SFA, MUFA, and PUFA could be maintained by different esterifying enzymes actions, *i.e.*, on site remodeling of fatty acid esters in accordance with the

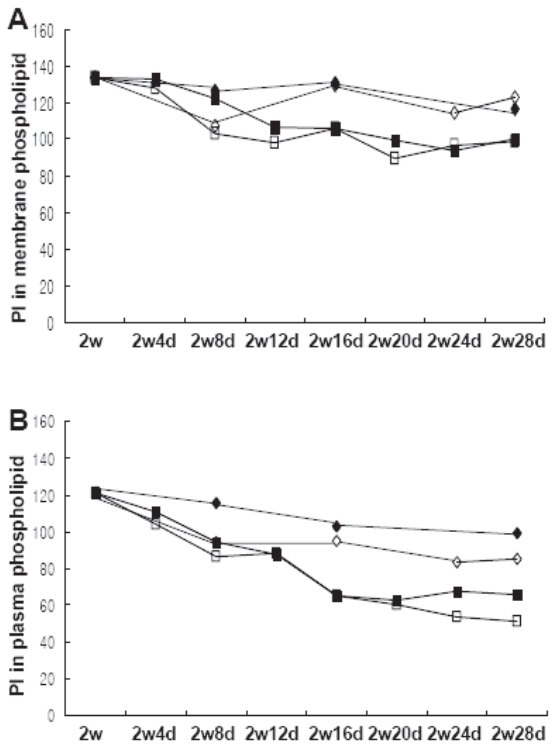

Fig. 5. The PI in Phospholipids of Liver Microsomes (A) and Plasma (B)

PI is calculated as an index of substrate availability for maximal rates of oxidation with two or more double bonds and is based on the formula of in vitro experiment.[15] In olive oil groups, PI decreased markedly approximately after 10 days in liver microsomes and after 16 days in plasma due to decrease in linoleic and arachidonic acids and increase in oleic acid. Symbols indicate; –◆– 7% soybean oil, –◇– 15% soybean oil, –■– 7% olive oil, –□– 15% olive oil. See Tables 2 and 3 for S.D. and statistical significance.

preferred selectivities for synthesis and degradation during lipid metabolism.[1, 2]

MUFA include mostly palmitoleic acid (16:1 n-9) and oleic acid (18:1 n-9). Intakes of MUFA in the U.S.A. diet have decreased from 21% to 12% of total calories since the 1950's.[5, 19] This represents a decrease of > 30 g MUFA per day. Oleic acid is the most abundant fatty acid, comprising 35–55% of the fatty acid in human adipose,[20–22] and averages 4–7 kg in depot fat. In Spain with a purely Mediterranean diet, oleic acid and linoleic acid stores in adipose were 46–49% and 11–15%, respectively.[23] Oleic acid deposition is site-specific[20] and region-specific.[21] Calder *et al.*[20] reported that ranges of 18:1 n-9 and 18:1 n-7 were 36–42% and 6–8% in regional adipose depots, respectively. When MUFA contributed 12.3% of total calories, the gluteal depot contained 58.7% MUFA and the waist had 53% MUFA.[22] Adipose levels of oleic acid were significantly higher in the Spanish (55%) than in German (46%) subjects, and the content of oleic acid was inversely related to breast cancer in Spanish.[21] Recently mounting evidences have shown that diets with high oleic acid and low linoleic acid may be protective effects against diseases.[18, 24–26]

Human adipose contains a range of 6–20% linoleic acid.[22, 27] The average adult U.S.A. citizen has a reserve of approximately 2 kg of linoleic acid in depot fat, and there is evidence that the reserve of linoleic acid has approximately doubled since the 1960's, with recent average values closer to 18%.[27] In England, Calder *et al.*[20] found a range of 6–16% linoleic acid in regional adipose depots, and Feunekes *et al.*[28] measured an average of 14% linoleic acid among subjects in the Netherlands. The trend towards increased intake of PUFA and decreased intake of SFA[19] appears to be stabilizing, based on the cholesterol-lowering effects of PUFA. Although it is not clear why the tissue content of linoleic and oleic acids seems to be elevated two-fold relative to data from food consumption, this higher reserve of linoleic and oleic acids in their adipose provides the precursors for cellular membrane synthesis.

Because linoleic acid is not synthesized in the body, the amount present in adipose and in cell membranes is closely related to dietary supply.[2] On changing intake of linoleic acid, the reserve approaches a new steady state within about 5 years, so that the effective half-time for turnover is about a year in sedentary individuals.[29] Daily need for essential fatty acids is not more than 1–2% of calories. Therefore, daily need is ≈ 0.02 times 2400 kcal/9 kcal per gram ≈ 5 g. This means that the 2 kg reserve would suffice for over 1 year. In our study, the fatty acid composition in olive oil groups appeared to be equilibrated after approximately 16–20 days of diet change and those in soybean oil groups appeared to be the plateau earlier than in olive oil groups due to the 2 weeks acclimation of soybean oil diet. Therefore, in the rats, approximately 3 weeks appear to be enough for the changes of fatty acid composition in regard to relatively short life span.

In our study, the increase in MUFA content (18:1 n-9 in particular) occurred at the expense of the PUFA content (18:2 n-6) in animals switched to olive oil. Actually, rats in the soybean oil groups accumulated more linoleic acid and less oleic acid than rats in olive oil groups. Low levels of α-linolenic acid (18:3 n-3) were found in different tissues, which suggests that α-linolenic acid is rapidly metabolized.[30] As time progressed, the ratio of

oleic acid to linoleic acid increased from 6- to 14-fold. The synthesis of oleic acid could be suppressed by n-6 PUFA rich diet.[17,31] Another possible mechanism is that Δ9-desaturase might be inhibited by an excess of linolenyl-CoA, which is metabolized from linoleic acid. Using deuterium isotopes, Emken *et al.*[32] demonstrated that dietary linoleic acid reduced Δ9-desaturase activity by 30–50%. These changes are also in good accordance with Rioux *et al.*,[33] who found that MUFA in the liver phospholipids were highly subject to dietary fats. From this evidence, the change of these ratios could be attributed to the high oleic acid content of olive oil diet as well as a lower Δ9-desaturase activity in the soybean oil groups.

Plasma arachidonic acid may arise from hepatic synthesis or release from the adipose tissue. Arachidonic acid level of all tissues in animals fed olive oil decreased to nearly one-half the level of formed in soybean oil groups. This higher proportion of arachidonic acid in soybean oil groups may be attributed to the high content of substrates, *i.e.* linoleic acid, for desaturation and elongation in soybean oil. Linoleic acid is rapidly incorporated into tissues and complex lipids and elongated and desaturated to arachidonic acid.[1,2] Conversely, higher intake of oleic acid may displace linoleic acid from membranes and favor oxidation rather than conversion to arachidonic acid. After being released from liver microsomes, PUFA activate phospholipase A2 and favor oxidation of arachidonic acid by cytochrome P-450 system.[34] Olive oil promoted an increase in EPA and DHA in phospholipids. These long chain fatty acids are derived from α-linolenic acid through Δ6-desaturation and elongation steps. The differences in the fatty acid composition may be attributed to increased hepatic turnover and the subsequent release of long chain PUFA. It is possible that animals fed soybean oil have constitutively low Δ6-desaturase activities because desaturation, not elongation, is the rate-limiting step in the n-3 biosynthetic pathway.[35]

In our study, SFA were unchanged during the feeding period, whereas MUFA and PUFA were continuously mobilized. Apparently more SFA are more likely to be stored but less likely to be mobilized. Nuclear magnetic resonance data suggested that it is energetically favorable to store more SFA and mobilize and oxidize PUFA from a molecular standpoint.[36]

The overall patterns of fatty acid composition are similar among different tissues. This may be attributed to the similarities of competitive interactions and the general esterification selectivity among fatty acids and the close metabolic interactions of these three pools. For example, PUFA are selectively mobilized from adipose tissue. Movement of fatty acids from adipose to liver occurs rapidly via plasma free fatty acids. The return flow is provided by apolipoproteins secreted by the liver. The studies of Lands[1,2] supported this pattern by showing the similarity of fatty acid composition of plasma and adipose tissues for individuals from all over the world. In particular, the similar proportions of endogenous fatty acids were attributed to the fatty acid synthesis from carbohydrates and amino acid precursors.

Adult humans require only 1–2% of total calories from linoleic acid and a trace of α-linolenic acid,[1,4] which can be supplied by one-half tablespoon of plant oil. The single instance in which any public agency has made a recommendation to increase the level of dietary fat is for PUFA and a recommendation of 10% of total calories corresponds to 5 times the amount required if heart disease is not present. If the aim is to reduce total fat so that energy balance is maintained, and to minimize risks for all-cause mortality including heart disease, cancer, atherosclerosis, stroke, and immunological disorders, then it may be a mistake to recommend consumption of 10% PUFA. There is no biological process that operates at 5–10 times saturation,[1,2,4] and most natural human diets contain 3–7% linoleic acid.[3]

As PUFA consumption has increased, consumption of SFA and MUFA has decreased. This is due to decreased consumption of animal fats as less red meat is consumed and as plant oils have replaced lard and shortening in cooking. To the extent that this reflects a decrease in total fat consumption, it is regarded as highly desirable, yet it raises a second question. Would it now be worthwhile to determine more appropriate ratios between MUFA and PUFA, particularly given the evidence that a "Mediterranean" style diet, with most fat calories derived from olive oil, is consistent with excellent long-term health?[3,26,37] It is possible that the tendency to decrease MUFA along with SFA is not necessary beyond present levels, and that it is important to determine appropriate ratios of MUFA to PUFA. For example, the diet in southern Italy typically includes about 73 g of lipid per day, of which about 49 g is from olive oil.[37] In many traditional Mediterranean diets, olive oil accounts for

about 80% of all fat calories. Whereas it is unlikely that any health agency will recommend a decrease in the P : S ratio, it is feasible that the protective effect of MUFA could be obtained in diets that do not elevate cholesterol. Stearic acid is not hypercholesterolemic because it is rapidly converted to oleic acid.[38] Neither stearic acid nor oleic acid is thought to elevate cholesterol, and these lipids are not readily peroxidizable. Therefore, they may be relatively innocuous considering peroxidative membrane damage and free radical propagation during conditions of oxidative stress.

In conclusion, we characterize the time-dependent changes in tissue fatty acid composition with different types and levels of dietary fats. The results indicate that substantial remodeling occurs in fatty acid compositions, especially linoleic and oleic acids for approximately 4 weeks of each diet pattern. Further studies are needed to build a new saturation kinetic model to predict the rate and extent of change in fatty acid compositions according to dietary fats. This approach may be useful in providing further information for future experimental design in order to predict when to perform functional tests concerning the effect of diet on effects of exposure to chemical agents, toxins, or other stimuli.

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