In vivo ^{13}C NMR analysis of acyl chain composition and organization of perirenal triacylglycerides in rats fed vegetable and fish oils

Teresa W-M. Fan,[1,*] Andrew J. Clifford,[†] and Richard M. Higashi[§]

Department of Land, Air and Water Resources* and Department of Nutrition,† University of California, Davis, CA 95616; and University of California Bodega Marine Laboratory,§ Bodega Bay, CA 94923

Abstract Lipid composition of body fat can be a key indicator of nutritional status and a number of human disorders. In vivo ^{13}C NMR provides for repeated, noninvasive analysis of fatty acyl chain composition on individuals, which circumvents classical problems of individual variation and repetitive invasive sampling. It also offers a unique opportunity to examine acyl chain organization in situ. This approach was used to examine the fatty acyl chain composition in the perirenal fat pads of rats fed olive, safflower, and menhaden oil-containing diets. These changes were then monitored during a diet switch between olive and menhaden oil-fed rats. The fatty acid composition of perirenal fat pads and livers was also analyzed using gas chromatography for comparison with the in vivo NMR analysis. Both tissues assumed the general characteristics of diet fatty acyl chain and fatty acid composition and the diet switch induced a switchover of the perirenal composition in 30–45 days. These results indicate that a large portion of the diet fatty acyl chains were incorporated directly into adipose and liver tissues although some were also metabolized, particularly in menhaden oil-fed rats. Furthermore, changes in the in vivo spin-lattice relaxation times (T_1) of fatty acyl carbons in the perirenal fat pads and their lipid extracts were followed and effective correlation times (τ_{eff}) were calculated from the T_1 data. The result indicated that the in vivo segmental mobility of acyl carbons was sensitive to changes in diet-derived fatty acyl chain composition and that the central region of the acyl chain was more sensitive to these changes. There was a qualitative similarity but quantitative differences in the τ_{eff} of acyl carbons acquired in vivo and from extracts. These results suggest that adipose triacylglycerides experience an overall liquid-like microenvironment in vivo but with more restriction in their mobility, and that different factors may exist in governing their organization in situ versus in extracts.—**Fan, T. W-M., A. J. Clifford, and R. M. Higashi.** In vivo ^{13}C NMR analysis of acyl chain composition and organization of perirenal triacylglycerides in rats fed vegetable and fish oils. *J. Lipid Res.* 1994. **35:** 678–689.

Supplementary key words rat perirenal fat • lipid • spin-lattice relaxation time • surface coil • olive oil • safflower oil • menhaden oil

Body fat is histologically classified as essential (lipid constituents of cells) and nonessential (fat stored in subcutaneous tissue, yellow bone marrow, and abdominal cavity). The mass of body fat increases from about 10% of the body weight in newborn humans to as much as 45% of the body weight in adults. It has recently become clear that, in addition to the amount of body fat, its pattern of distribution has been shown to play an important role in the risk of cardiovascular disease, developing diabetes, hypertension, gallbladder disease, stroke, and overall mortality (1).

Despite the importance of body fat, knowledge about its composition in relation to disease states is limited and even less is known about its molecular organization in tissues. Until recently it was necessary to obtain body tissue specimens through surgery or liposuction, followed by solvent extraction of the lipids from the specimen, in order to study body lipid composition and metabolism. Because of the invasive nature of these procedures, relatively few studies have been reported. In addition, biopsy inevitably destroys information on molecular organization of body fat, which may hold clues to its metabolism.

Most importantly, processes involving changes in body fat composition tend to be chronic in nature. For example, the fatty acid composition of tissue lipids can change drastically—but only over the long term—to mimic that of the fat ingested from the diet (2, 3). The turnover rate of the constituent fatty acids of body fat may be very slow (2, 3), and even after large weight gain and loss, the fatty acid composition of adipose lipids changes minimally (4). To detect these gradual changes, long-term studies with multiple sampling points are necessary but are difficult to implement in animal models using any invasive procedure; for human subjects, the invasive methods can be very difficult to justify for research purposes.

Abbreviations: T_1, spin-lattice relaxation time; τ_{eff}, effective correlation time; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; r^2, correlation coefficient; NOE, nuclear Overhauser enhancement.

[1]To whom correspondence should be addressed.

Downloaded from www.jlr.org by guest, on June 18, 2012

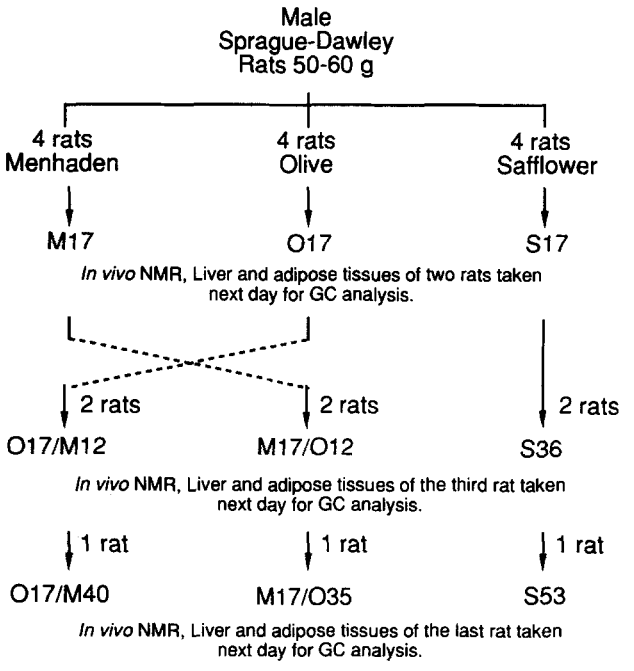
The family of noninvasive nuclear magnetic resonance spectroscopy (NMR) techniques provide an outstanding means to circumvent the problems of biopsy. In particular, in vivo ^{13}C NMR has been applied to monitor fatty acyl chain composition in excised or exposed organs of laboratory animals (5, 6), in isolated adipocytes (7), and in situ (8, 9) including humans (10). Its capability of long-term, repetitive monitoring on individuals helps to circumvent interferences arising from individual differences in chronic studies. In several of these reports, the in vivo ^{13}C spin-lattice relaxation time (T_1) was also investigated, which yielded information on the in situ acyl chain dynamics and organization in intact organs (8, 10) or cells (7). While these reports (5–10) illustrated the utility of in vivo ^{13}C NMR for noninvasive fatty acyl chain analysis, long-term time course studies of individuals have not yet been demonstrated, nor has the molecular organization of fatty acyl chains been probed in live animals.

Clearly the use of NMR provides a unique opportunity to identify and follow in situ alterations in the composition and organization of body fat in relation to factors such as changes in dietary fat, prolonged weight loss, aging, and onset of lipid-related diseases. In this report, we conducted a laboratory model study employing surface probe-localized ^{13}C NMR to examine in situ the fatty acyl chain composition of perirenal fat pads from rats fed three defined oil (olive, safflower, and menhaden) diets. Then, rats from the olive and menhaden oil diet groups were switched diets and monitoring of the time course changes in perirenal acyl chain composition was continued after such a switch. In addition, results from in vivo ^{13}C NMR analysis were compared with the fatty acid composition acquired from gas chromatographic analysis of the corresponding lipid extracts. Finally, the changes in ^{13}C T_1 of fatty acyl carbons induced by the dietary switch were determined, which provided information on diet-induced changes in the in situ adipose triacylglyceride organization.

MATERIALS AND METHODS

Animals and diets

Twelve weanling male Sprague-Dawley rats weighing 50–60 g were obtained from Bantin and Kingman (Gilroy, CA), housed in individual cages, and fed a commercial rat diet (Ralston Purina Co., St. Louis, MO) for a 2-day adjustment period. As outlined in **Scheme 1,** animals were systematically divided into three groups (4 rats/group) of equal mean weight (72.3 ± 0.3 g) and fed semipurified diets containing menhanden, olive, or safflower oil as the main lipid source. These diets were maintained for 17 d, after which two rats each from the three diet groups (M17, O17, and S17) were treated as follows: *a)* two rats from the menhaden oil diet group were switched to the olive oil-containing diet, with one rat fed for 12 d (M17/O12) and the other one fed for 35 d (M17/O35); *b)* two rats from the olive oil group were switched to the diet containing menhaden oil, with one rat fed for 12 d (O17/M12) and the other one fed for 40 d (O17/M40); *c)* two rats from the safflower oil group were continued on that diet, with one fed for 19 d (S36) and the other for 36 d (S53).



Scheme 1.

The composition of the semipurified diets were as follows: casein, 15 g; starch, 42 g; glucose, 22 g; cellulose, 5 g; safflower oil, 0.2 g (for providing essential fatty acids); mineral mix (11), 6 g; vitamin mix, 120 mg (11); and either 7.2 g of menhaden (ICN Biochemicals Inc., Costa Mesa, CA), olive (Pompeian, Inc., Baltimore, MD), or safflower (Saffola Quality Foods Inc., Los Angeles, CA) oil.

Extraction and gas chromatographic analysis of tissue triacylglyceride

Rats were killed on the day following in vivo ^{13}C NMR measurement. Perirenal fat pads and livers were removed, freeze-clamped in liquid N_2, and lyophilized.

Total lipid extracts were prepared as follows. Tissues were individually homogenized with a Polytron™ (Brinkman Instruments, Westbury, NY) in at least 10 volumes (w/v) of $CHCl_3$–CH_3OH 2:1 (v/v) for 1 min, the homogenate was centrifuged for 5 min at 3000 *g* at room temperature, and the supernatant was transferred to a vacuum flask. The pellet was re-extracted by the same procedure, the supernatants from both extractions were combined and dried in a rotary evaporator under vacuum at 40°C. The lipid residue was solubilized in $CHCl_3$, an

Downloaded from www.jlr.org by guest, on June 18, 2012

aliquot was dried under N_2 stream and transesterified as follows (12). The dried extract was dissolved in CH_3OH–C_6H_6 3:2 (v/v) and mixed with one volume of freshly prepared 5% acetyl chloride in CH_3OH in a crimped 1-ml vial at 100°C for at least 1 h. After cooling, one volume of hexane plus one volume of water were added and the vial was shaken and centrifuged to separate the two solvent layers. The methyl ester of C15:0 and butylated hydroxytoluene (BHT) was added to the top hexane layer as internal standards.

Fatty acid methyl esters were analyzed by gas–liquid chromatography on a Varian (Palo Alto, CA) model 3300 gas chromatograph equipped with a flame ionization detector (GC-FID) and a 40 m × 0.18 mm i.d. open-tubular column with DB-1 phase of 0.4 μm film thickness (J&W Scientific, Folsom, CA). Conditions for chromatography were: H_2 carrier gas velocity = 60 cm/s; injector = 260°C; detector = 320°C; column = 60°C held for 2 min, ramped to 150°C at 20°C/min, then ramped to 300°C at 6°C/min; splitless injector purge valve held for 1.0 min, then valve open at 100 ml/min for the duration of the run. Fatty acid methyl esters were identified by comparing retention times with authentic standards and by fragmentation patterns acquired from GC–mass spectrometry (GC–MS) using a Hewlett-Packard (Hewlett-Packard Analytical Products, Palo Alto, CA) model 5890 gas chromatograph equipped with a 0.25 mm i.d. × 30 m DB-5 open-tubular column (0.25 μm film; J&W Scientific, Folsom, CA) and interfaced to a VG (VG Masslab, Altrincham, UK) model Trio-2 quadrupole mass spectrometer. Analysis conditions for GC–MS were as described above, except that: carrier gas was He of velocity 35 cm/sec; maximum column temperature at 280°C; transfer line at 250°C; electron impact ionization at 70 eV; source temperature at 200°C. Quantification of fatty acid methyl esters were by comparison of GC-FID peak areas with standards of known concentrations.

^{13}C NMR spectroscopy

Diet oils. A 2.5-cm diameter solenoid ^{13}C probe was constructed with balanced-match circuitry to acquire broad-band decoupled ^{13}C NMR spectra of the three diet oils using a one-pulse sequence and a General Electric™ CSI-2 Tesla imaging spectrometer (GE NMR Instruments, Fremont, CA); broad-band decoupling was implemented using an MLEV-64 scheme and a 15.24 cm diameter ^{1}H birdcage probe (13). The pulse parameters included a pulse width of 15 μs, a pulse delay of 1 s, a spectral width of ± 2500 Hz, 2048 digital points, and 256 transients. A 1 Hz line broadening was applied to the free induction decays before Fourier transformation.

Rat perirenal fat pads in vivo. Localized in vivo ^{13}C NMR measurements of the perirenal fat pads were made on two rats from each diet group at 17 d (O17, S17, and M17). The measurements were repeated at 12 d (M17/O12) and 35 d (M17/O35) for the menhaden oil group after the switch to the olive oil diet, at 12 d (O17/M12) and 40 d (O17/M40) for the olive oil group after the switch to the menhaden oil diet, and at 19 d (S36) and 35 d (S53) for the safflower oil group.

Rats were first immobilized with an intramuscular injection of Ketamine-HCl (VetalarR, Parke-Davis, Morris Plains, NJ) at 50 mg/kg body wt in the gastrocnemius region and then sedated with 10 mg/kg Xylazine (Rompun™, Haver, Miles Laboratory, Shawnee, KS) subcutaneously in the lumbar region. Rats were then positioned such that a 2 turn, 3.5 cm diameter foil surface probe constructed by photoetching (14) was centered directly beneath the perirenal fat pad. The rat and the surface probe assembly was then placed in the ^{1}H birdcage probe (described above) which provided ^{1}H broad-band decoupling using the high efficiency WALTZ-16 sequence. This decoupling scheme minimized heat production and no significant temperature change was observed in a phantom sample with comparable tissue salinity. In vivo ^{1}H-decoupled ^{13}C NMR spectra of the fat pad were acquired using the same pulse scheme as described above, a spectral width of ± 2500 Hz, a pulse width of 15 μs, a pulse delay of 0.3 s, 2048 digital points, and 1024 transients. The nuclear Overhauser enhancement (NOE) was determined from two one-pulse experiments with decoupling either on or off during the interpulse delay, a 90° pulse width (35 μs), a 5 s interpulse delay, and 144 transients.

Measurements of ^{13}C spin-lattice relaxation times and effective correlation times. In vivo ^{13}C spin-lattice relaxation times (T_1s) of the perirenal fat pads were measured as described above, except that the pulse sequence was a modified inversion recovery (composite 230°–τ–90°–acquire) with decoupling only during acquisition (15), ±2500 Hz spectral width, a 6 s interpulse delay, 2048 digital points, and 128–144 transients. Composite 230° instead of 180° pulse was used for a better inversion of in vivo NMR signals using surface probes (15). A three-parameter exponential fit (16) of six to eight time points was used to calculate the in vivo T_1s. In vitro T_1s were determined using the lipid extracts of perirenal fat pads from the same rats, the solenoid probe arrangement described above, and nine time points for the three-parameter exponential fit.

Effective correlation times (τ_{eff}) were calculated from measured T_1 values by interpolation from a graph plotted as $1/T_1$ versus correlation time according to the following equation (17):

$$1/(NT_1) = (0.1\ h^2\gamma_H^2\gamma_C^2/4\pi^2r^6)[J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)] \quad \textit{Equation 1)}$$

where the spectral density function, $J(\omega) = \tau_{eff}/(1 + \omega^2\tau_{eff}^2)$, N is the number of directly bonded hydrogens, h is Planck's constant, γ_H and γ_C are the gyromagnetic ratios

Downloaded from www.jlr.org by guest, on June 18, 2012

of ^{1}H and ^{13}C nuclei, respectively, and r is the C-H bond length (1.095 Å) (17). For a given T_1 value, there can be two τ_{eff} values, each representing a long or a short correlation time. Only the short correlation time was applicable here due to the extreme narrowing conditions as deduced from the large NOE and narrow linewidth data for the fatty acyl carbons.

RESULTS

^{13}C NMR spectra of the three dietary oils and perirenal fat pads

The ^{13}C NMR spectra of menhaden, safflower, and olive oils are shown in **Fig. 1** (see also Table 1 for the fatty acid content of these three oils). These spectra were characteristic of triacylglycerides and contained resonances arising from the ω-methyl, methylenes, glycerol backbone, and olefinic carbons of polyunsaturated fatty acids (PUFAs) and of monounsaturated fatty acids (MUFAs), and carbonyl carbons (8). In addition, a peak (*X*) at 132.50 ppm was present in the menhaden oil spectrum only (Fig. 1), the identity of which was undetermined. The PUFA peak (peak *10* at 128.89 ppm in Fig. 1) was derived from PUFA-containing triacylglyceride only while both MUFA and PUFA-containing triacylglyceride contributed equally to peak *11* (at 130.68 ppm in Fig. 1) (18). The ratio of peaks *10:11*, or PUFA/(PUFA+MUFA), represents the degree of polyunsaturation which is of the order menhaden > safflower > olive. This data was confirmed by the fatty acid composition of the three dietary oils, as determined by GC-FID (see **Table 1**).

In the methylene carbon region, the ratio of peak *3* (25.66 ppm) to peak *4* (28.00 ppm) (Fig. 1) was also of the order menhaden > safflower > olive. This relationship was to be expected since peak *3* was mostly derived from the $-CH_2-$ between two olefinic carbons from PUFA and peak *4* corresponded to $-CH_2-$ adjacent to one olefinic carbon from both MUFA and PUFA. Thus, both ratios of peaks *10:11* and *3:4* reflected the extent of polyunsaturation of the fatty acyl chains in the three dietary oils.

Fig. 2 shows in vivo ^{13}C NMR spectra of the perirenal fat pads of rats fed menhaden, safflower, and olive oil for 17 d (designated M17, S17, and O17, respectively). These spectra were dominated by carbon resonances arising from triacylglyceride and they had appearances similar to those of the corresponding dietary oils (Fig. 1). In particular, the unidentified peak at 132.50 ppm (*X*) from the menhaden oil spectrum (Fig. 1) was also present in the M17 spectrum (Fig. 2) but was absent in the spectra of S17 and O17.

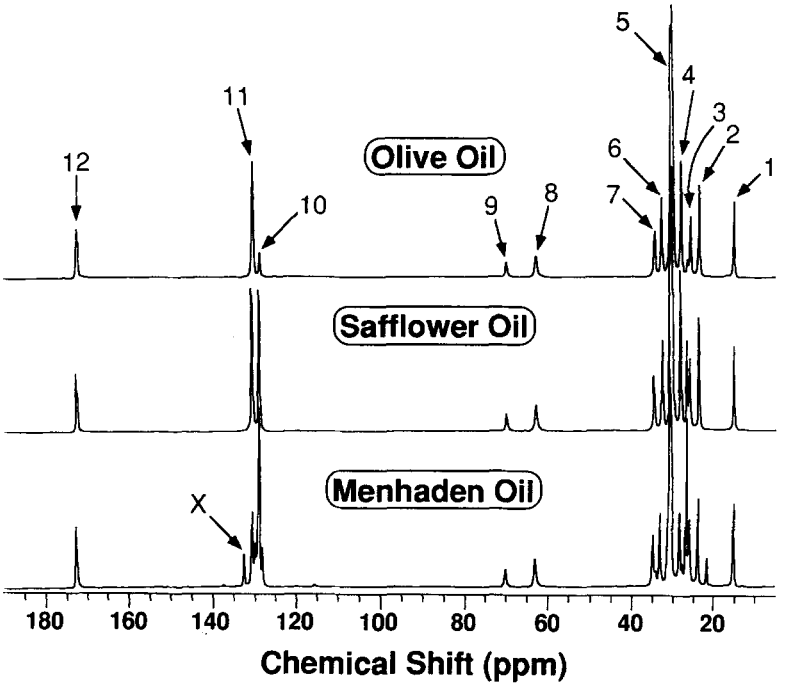


Fig. 1. ^{13}C NMR spectra of menhaden, safflower, and olive oils. Spectra were obtained as described in Materials and Methods. The peak (indicated by *) assignments are as follows: *1* (14.93 ppm), ω-$^{*}CH_3$; *2* (23.49 ppm), CH_3-$^{*}CH_2$-; *3* (25.66 ppm), $-CH_2-^{*}CH_2-CH_2-C=O$ + $-CH=CH-^{*}CH_2-CH=CH-$; *4* (28.00 ppm), $-CH_2-^{*}CH_2-CH=CH-$; *5* (30.41 ppm), $-(^{*}CH_2)_n-$; *6* (32.71 ppm), $CH_3-CH_2-^{*}CH_2-CH_2-$; *7* (34.84 ppm), $-^{*}CH_2-C=O$; *8* (62.90 ppm), $C_{1,3}$-glycerol backbone; *9* (70.20 ppm), C_2-glycerol backbone; *10* (128.89 ppm), $-CH=^{*}CH-CH_2-^{*}CH=CH-$; *11* (130.68 ppm), $-CH_2-CH_2-^{*}CH=^{*}CH-CH_2-CH_2-$ + $-^{*}CH=CH-CH_2-CH=^{*}CH-$; X (132.70 ppm), unidentified; *12* (172.61 ppm), $-^{*}C=O$.

Downloaded from www.jlr.org by guest, on June 18, 2012

TABLE 1. Fatty acid composition of three dietary oils and of perirenal fat pads and livers excised from rats fed these oils[a]

	Dietary Oil			Perirenal Fat			Liver		
Fatty Acid	Menhaden	Olive	Safflower	(M17)[b]	(O17)[b]	(S17, S36, S53)[c]	(M17)[b]	(O17)[b]	(S17)[c]
				mole %					
C14:0	7.37	5.72	0.08	4.24 (0.68)	1.66 (0.17)	1.83 (0.21)	0.55 (0.08)	0.73 (0.01)	0.61 (0.04)
C15:0	0.36	ND	ND	0.23 (0.03)	ND	ND	0.20 (0.05)	ND	ND
C16:0	16.72	11.24	6.43	32.74 (1.84)	25.77 (0.34)	24.80 (1.02)	19.49 (1.11)	27.55 (0.43)	26.53 (2.25)
C16:1	10.65	0.89	0.07	11.35 (0.01)	6.18 (0.07)	4.80 (0.63)	2.69 (0.35)	2.82 (0.03)	1.33 (0.29)
C18:0	2.61	2.89	2.45	4.26 (0.04)	3.48 (0.42)	4.21 (0.81)	13.02 (0.72)	14.17 (0.57)	15.26 (0.08)
C18:1	12.86	68.30	13.24	26.25 (3.04)	52.99 (0.38)	18.18 (1.42)	9.53 (0.39)	30.59 (0.21)	8.16 (1.17)
C18:2	1.40	10.34	77.35	9.37 (0.28)	9.19 (0.40)	45.31 (1.54)	8.21 (0.73)	7.46 (0.57)	30.24 (0.89)
C18:3	0.68	ND	ND	0.19 (0.19)	0.51 (0.06)	0.28 (0.04)	0.13 (0.03)	0.50 (0.17)	0.32 (0.07)
C18:4	3.50	ND	ND	1.54 (0.06)	ND	ND	0.28 (0.04)	ND	ND
C20:0	0.12	0.55	0.39	0.13 (0.05)	0.08 (0.08)	0.03 (0.06)	0.04 (0.00)	ND	0.22 (0.22)
C20:4	0.71	0.07	ND	0.40 (0.01)	0.14 (0.01)	0.60 (0.09)	7.66 (0.46)	13.23 (1.04)	16.02 (3.61)
C20:5	21.44	ND	ND	4.62 (0.43)	ND	ND	12.99 (1.74)	0.13 (0.13)	0.09 (0.09)
C22:6	21.57	ND	ND	4.68 (0.01)	ND	ND	25.22 (4.92)	2.83 (0.26)	1.23 (0.47)

Numbers in parentheses are SEM; ND, none detected.
[a]Determined from GC analysis; C13:0, C22:1, and C22:4 were not detected.
[b]Diet group designations are the same as in Scheme 1; average of two measurements.
[c]Diet group designations are the same as in Scheme 1; average of four measurements.

The in vivo ^{13}C NMR spectra of the perirenal fat pads from S17 and O17 rats (Fig. 2) closely resembled the spectra of the dietary safflower and olive oils (Fig. 1), respectively. For example, the ratios of peaks *10:11* and peaks *3:4*, both of which correspond to the degree of polyunsaturation in the acyl carbons, were much higher for S17 than for O17 rat (Fig. 2 and **Table 2**). It should be noted that this relationship was confirmed with in vitro GC analyses as shown in Table 2. In addition, the two ratios were highest in the perirenal fat pad of M17 rat (Fig. 2 and Table 2), which was also confirmed by GC analyses (Table 2). However, these ratios for the M17 rats were significantly lower than for the dietary menhaden oil (Fig. 1). It is possible that this difference was due to the metabolism of some of the PUFAs in menhaden oil prior to incorporation into the perirenal fat pads.

Acyl chain and fatty acid composition of fatty tissues from rats fed the three oil diets

In vivo NMR determination of degree of triacylglyceride unsaturation. To acquire the degree of unsaturation in the perirenal fat pad acyl chains of the three diet groups, the partially overlapping peaks *10* and *11* from the in vivo ^{13}C NMR spectra (e.g., Fig. 2) were deconvoluted using a curve-fit routine from the GE ™CSI program before their areas were integrated. Table 2 shows the two peak areas along with their ratios for rats fed the three dietary oils. The ratios for M17 and S17 rats were 6- and 4-fold greater, respectively, than for O17 rats. The values obtained by in vivo NMR analysis were comparable to those calculated from the fatty acid composition of the corresponding lipid extracts acquired by GC–FID (Table 2). Thus, this in vivo ^{13}C NMR protocol for the noninvasive determination of extent of polyunsaturation in perirenal fat pad triacylglycerides appears to be reasonable.

GC analysis of dietary oils, perirenal fat pads, and liver. Table 1 presents a detailed fatty acid composition of the three dietary oils, as well as of the lipid extracts of liver and perirenal fat pads of rats fed these oils. The fatty acid composition of the dietary oils agreed well with previous reports (19), confirming that the diets were as intended and that the GC analytical protocols were appropriate.

Downloaded from www.jlr.org by guest, on June 18, 2012

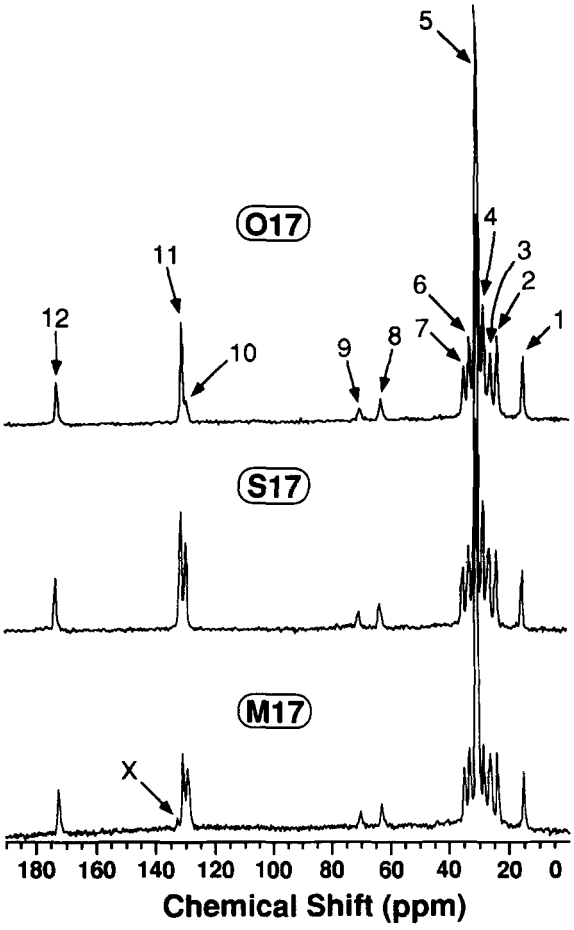


Fig. 2. Localized in vivo ^{13}C NMR spectra of perirenal fat pads of live rats fed on menhaden, safflower, and olive oil diets. These spectra were acquired as described in Materials and Methods. A 5 Hz of line-broadening was included in the processing of the free induction decays and chemical shifts were referenced indirectly to that of tetramethylsilane at 0.0 ppm. The designations for dietary treatments are the same as in Scheme 1 and the peak assignments are the same as in Fig. 1.

The perirenal fat pads of all three diet groups were abundant in C16:0, C16:1, C18:0, C18:1, and C18:2, with the menhaden oil-fed rat pads additionally enriched in C20:5 and C22:6. More importantly, the content of many major fat pad fatty acids reflected the fatty acid profile of the dietary oils. For example, C18:1 was dominant in both dietary olive oil and fat pads from olive oil-fed rats (O17); likewise, C18:2 was dominant in dietary safflower oil and fat pads from safflower oil-fed rats (S17) (Table 1). In addition, fat pads of menhaden oil-fed rats contained the highest amounts of C18:4, C20:5 and C22:6, as did the dietary oil itself (Table 1).

Despite the high correspondence of fatty acid profiles, there were also differences in fatty acid profiles between fat pads and the dietary oils. The fat pads from M17 rats had disproportionately lesser amounts of C20:5 and C22:6 and greater amounts of C16:0, C18:1 and C18:2 than did the dietary menhaden oil (Table 1). In addition, the amounts of C16:0, C16:1, and C18:1 in the fat pads of O17 rats were significantly different from those in the dietary olive oil, or the amounts of C16:0, C16:1, and C18:2 in the fat pads of safflower oil-fed rats deviated from those in the dietary safflower oil (Table 1).

As with the fat pads, the fatty acid profiles of the livers retained some of characteristics of the dietary oil. This was illustrated by the abundance of n-3 PUFA (C20:5 and C22:6), C18:1, and C18:2 in the livers of M17, O17, and S17 rats, respectively (Table 1). Several of the most abundant fatty acids in the liver (C16:0, C18:0, C18:1, and C18:2) were also among the most abundant in the fat pad; an exception was C20:4 which was much more abundant in the liver irrespective of the dietary oil (Table 1). Moreover, as with the fat pad, discrepancies in fatty acid profiles between liver and dietary oils were evident, particularly for C18:0 and C20:4 in all three cases.

TABLE 2. Lipid composition of perirenal fat pads of rats fed different oils as measured in vivo by ^{13}C NMR and in corresponding solvent extracts by GC analysis

	In Vivo ^{13}C NMR Peak Areas[a]			Peak Areas Calculated from GC Data[b]		
Dietary Group[c]	MUFA + PUFA (Peak *11*)	PUFA (Peak *10*)	PUFA/MUFA + PUFA (Peaks *10/11*)	MUFA + PUFA	PUFA	PUFA/MUFA + PUFA
M17	50.6	49.4	0.98	50.4	49.6	0.98
	(0.5)	(0.5)				
M17/O12	60.2	39.8	0.66	76.4	23.6	0.31
M17/O35	73.5	26.5	0.36	82.0	18.0	0.22
O17	85.7	14.3	0.17	86.6	13.4	0.16
	(1.8)	(1.8)				
O17/M12	65.2	34.8	0.53			
O17/M40	52.7	47.3	0.90	62.8	37.2	0.59
S17	57.2	42.8	0.75	59.2	40.8	0.69
	(0.4)	(0.4)				

Values are averages of two measurements and SEMs are indicated in parentheses below; remaining values represent single measurement.

[a]NMR peak assignments are as in Fig. 2; peak areas were determined with a curve-fit routine provided with the GE ™CSI program.

[b]Calculated from the fatty acid composition in Tables 1 and 3; MUFA + PUFA = 2 × content (MUFA + PUFA); PUFA = 2 × content C18:2 + 4 × content C18:3 + 6 × content (C18:4 + C20:4) + 8 × content C20:5 + 10 × content C22:6 (Bachelor et al., 1979; Canioni et al., 1983).

[c]Diet group designations are the same as in Scheme 1.

Downloaded from www.jlr.org by guest, on June 18, 2012

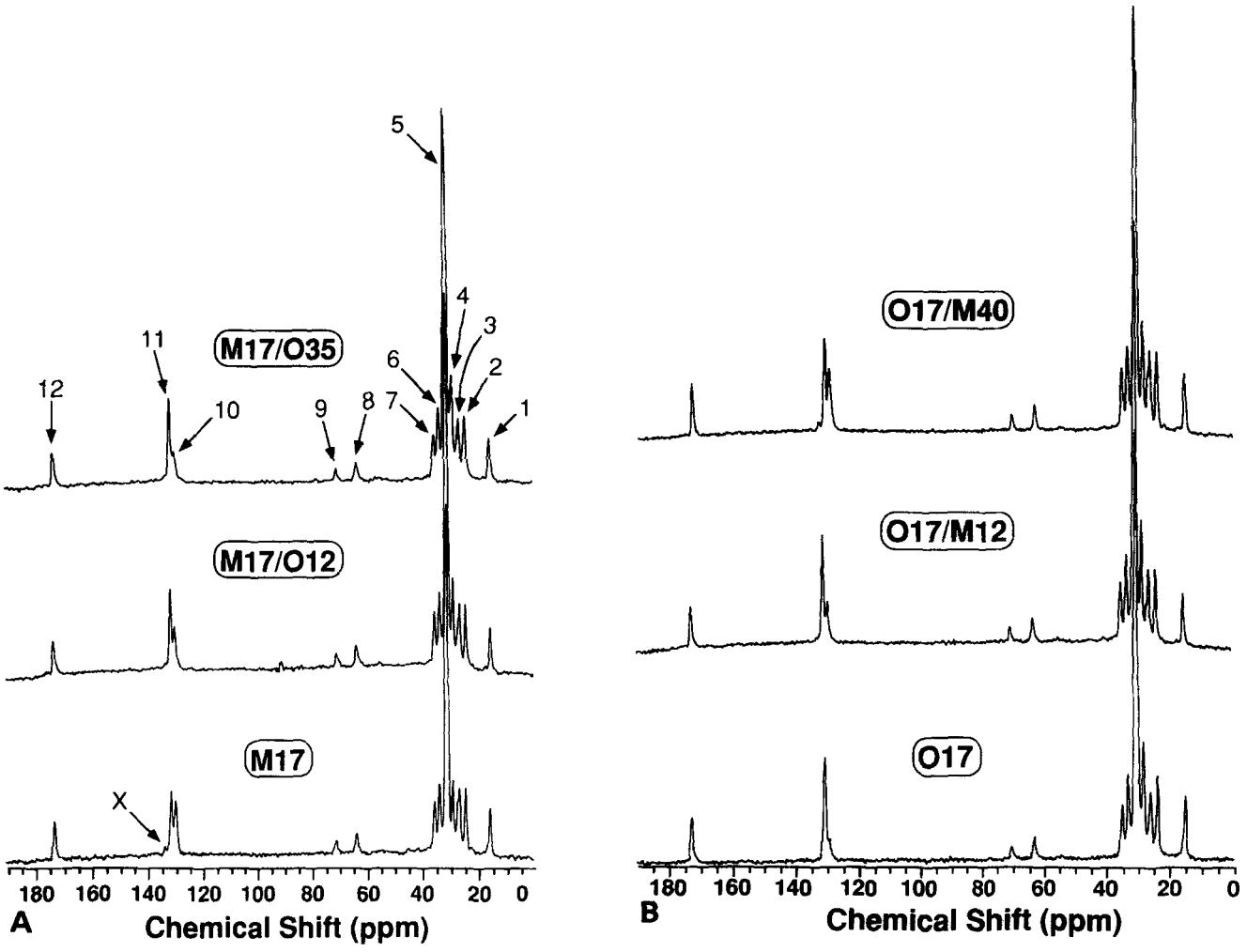


Fig. 3. Changes in in vivo ^{14}C NMR spectra of rat perirenal fat pads induced by dietary switch. Spectral acquisition, processing, and assignments are the same as in Fig. 2. Panels A and B illustrate spectra acquired from rats subjected to dietary switch from menhaden to olive and olive to menhaden oil, respectively. The designations for dietary treatments are the same as in Scheme 1.

Effects of diet switch on fatty acyl chain composition of rat perirenal fat pads and livers

^{13}C NMR monitoring. After the initial 17 d of feeding with the purified oil diets, the diets of menhaden and olive oil fed rats were switched in the manner illustrated in Scheme 1. Changes in the in vivo ^{13}C NMR spectra of perirenal fat pads that occurred after these switches is shown in **Fig. 3A and B.** It is evident that a decrease in the ratios of peaks *10:11* and peaks *3:4* (Fig. 3A and Table 2) occurred for M17 rats after 12 d of switch to the olive oil diet (M17/O12). Opposite changes were observed for O17 versus O17/M12 rats (Fig. 3B and Table 2). These changes progressed over time and after 35–40 d of the switch, the ratios of peaks *10:11* and peaks *3:4* for M17/O35 and O17/M40 rats approached the values for O17 and M17 rats, respectively (Fig. 3 and Table 2). Concurrently, the menhaden oil "marker," peak *X*, disappeared in M17/O35 rat (Fig. 3A), while it appeared in O17/M40 rat (Fig. 3B). These results demonstrated that the fatty acyl chain composition of perirenal fat pads in young rats can be modified extensively by diet over 1–1.5 months, and that these changes can be monitored in vivo by ^{13}C NMR spectroscopy.

GC analysis. The effect of diet switching from menhaden to olive oil (Scheme 1) on the fatty acid composition of liver and perirenal fat pads is shown in **Table 3.** A progressive decline in the PUFA (C18:4, C20:4, C20:5, and C22:6) content of the fat pad lipids was observed, which was accompanied by an increase in the MUFA (C18:1) content. These changes were consistent with those observed by in vivo ^{13}C NMR monitoring (Table 2 and Fig. 3A). Two other changes also occurred after the switch, including a decrease in C16:0 and C16:1 contents. All of these changes reflected the compositional differences between the dietary menhaden and olive oils (Table 1) and were indicative of a transition from menhaden to

Downloaded from www.jlr.org by guest, on June 18, 2012

TABLE 3. Changes in fatty acid composition of perirenal fat pad and liver extracts from rats fed menhaden oil and after switch to olive oil diets[a]

Fatty Acid	Perirenal Fat Pads			Liver		
	M17[b]	M17/O12[b]	M17/O35[b]	M17[b]	M17/O12[b]	M17/O35[b]
C14:0	4.91	4.11	2.36	1.00	0.59	0.71
C15:0	0.27	ND	ND	ND	ND	ND
C16:0	34.58	35.21	27.99	31.89	27.87	24.11
C16:1	11.35	9.18	5.03	4.59	2.36	1.32
C18:0	4.29	4.95	4.51	17.34	15.81	19.55
C18:1	23.21	37.22	48.20	10.38	24.05	25.81
C18:2	9.09	6.58	10.72	3.84	9.55	11.58
C18:3	0.38	0.59	0.58	0.58	0.44	0.38
C18:4	1.60	0.33	0.09	ND	ND	ND
C20:0	0.19	0.10	ND	0.36	0.29	0.66
C20:4	0.40	0.24	0.17	7.26	9.24	12.03
C20:5	5.05	0.81	0.19	12.28	1.85	0.37
C22:6	4.68	0.67	0.16	10.50	7.97	3.48

ND, none detected.
[a]Analyzed by GC; C13:0, C22:1, and C22:4 were not detected; single measurement.
[b]Diet group designations are the same as in Scheme 1.

olive oil-fed rats (Tables 1 and 3). The effect of the switch on the liver was similar except that it caused an increase instead of a decrease in the C20:4 content. Lastly, opposite changes were noted for both fat pads and livers from rats switched from olive to menhaden oil diet for 12 to 40 d (data not shown).

Effects of diet switch on ^{13}C T_1s and effective correlation times of fatty acyl carbons of rat perirenal lipids

To probe the physical state of the perirenal lipids, we have measured the ^{13}C T_1 values of their fatty acyl carbons both in vivo and in extracts using a modified inversion recovery method (15). **Fig. 4A** shows a typical inversion recovery curve for peak *11* along with the fitted three-parameter regression line (see Materials and Methods). For all T_1 measurements, the correlation coefficient (r^2) was ≥ 0.99, except for the T_1 (0.19 s) of the methylene carbon at 34.84 ppm for O17/M12 rat (Fig. 3B) with an r^2 of 0.90. Due to a signal-to-noise limitation, the in vivo T_1 measurements (Fig. 4B) were performed on older rats with larger perirenal fat pads; in the case of the menhaden and olive diet groups, first measurements were made 12 d after the diet switch. Nevertheless, the fat pad acyl chains from these rats retained to some extent the characteristics of the original diets (see Table 3).

For all cases, the in vivo T_1s of the acyl carbons were longer at the ω than at the carbonyl end except for the carbonyl and olefinic carbons (Fig. 4B). A qualitatively similar result was observed for the fat pad extracts (Fig. 4C). However, the in vivo T_1s (Fig. 4B) were generally shorter than the T_1s acquired from the corresponding extracts (Fig. 4C); such findings have been reported for isolated adipocytes (7). It should also be noted that the in vivo T_1 values acquired in this study were comparable to those for human adipose tissue (10), excised rat adipose tissue (8), and isolated rat epididymal adipocytes (7).

When comparing among different treatments, the in vivo T_1s of peaks *2* (23.49 ppm), *10* (128.89 ppm), and *11* (130.68 ppm) (Fig. 4B) were dependent on the extent of polyunsaturation in the fat pad acyl chains. This is illustrated by the result that M17/O12, O17/M40, and S36 rats (all enriched in PUFA at the time of measurement) exhibited longer in vivo T_1s of these peaks than O17/M12 and M17/O35 rats (all enriched in MUFA at the time of measurement).

To compare in vivo and in vitro segmental mobility of fat pad acyl chains, the effective correlation times (τ_{eff}) of fatty acyl carbons were calculated from the T_1 data and the result is shown in **Table 4.** As expected, τ_{eff} showed an opposite relationship to T_1 for all acyl carbons measured in vivo and in extracts. For example, the τ_{eff} was shorter at the ω-end than at the carbonyl end and most τ_{eff}s obtained in vivo were at least twice those derived from the corresponding extracts.

DISCUSSION

Dietary impact on fatty acyl chain composition in rat tissues

In vivo ^{13}C NMR provided a unique means to examine the impact of diet on tissue fatty acyl chain composition in rats. Using this technique, the progression of such an impact can be measured without interference from intrinsic individual heterogeneities as repeated measurements were performed on the same animal. This approach also opened a window on the largely unexplored area of tri-

Downloaded from www.jlr.org by guest, on June 18, 2012

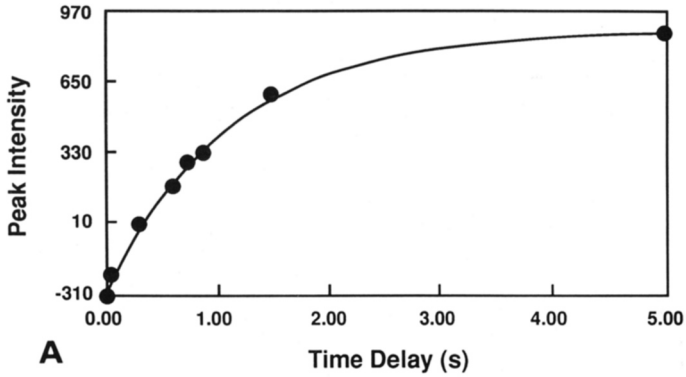
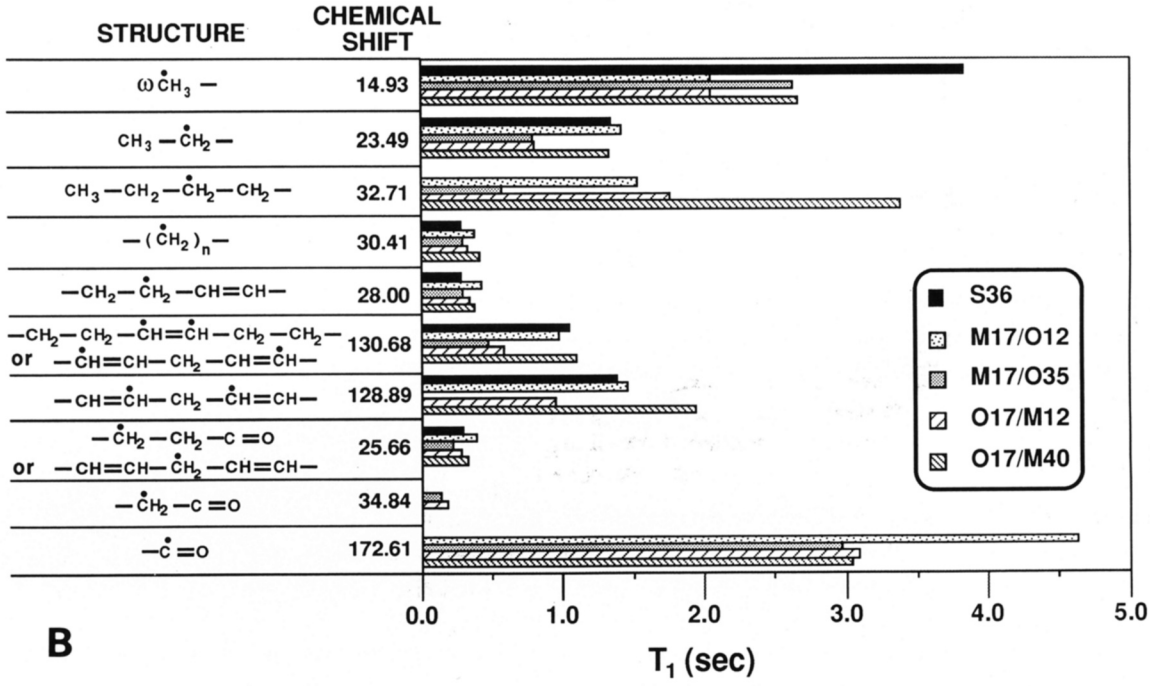
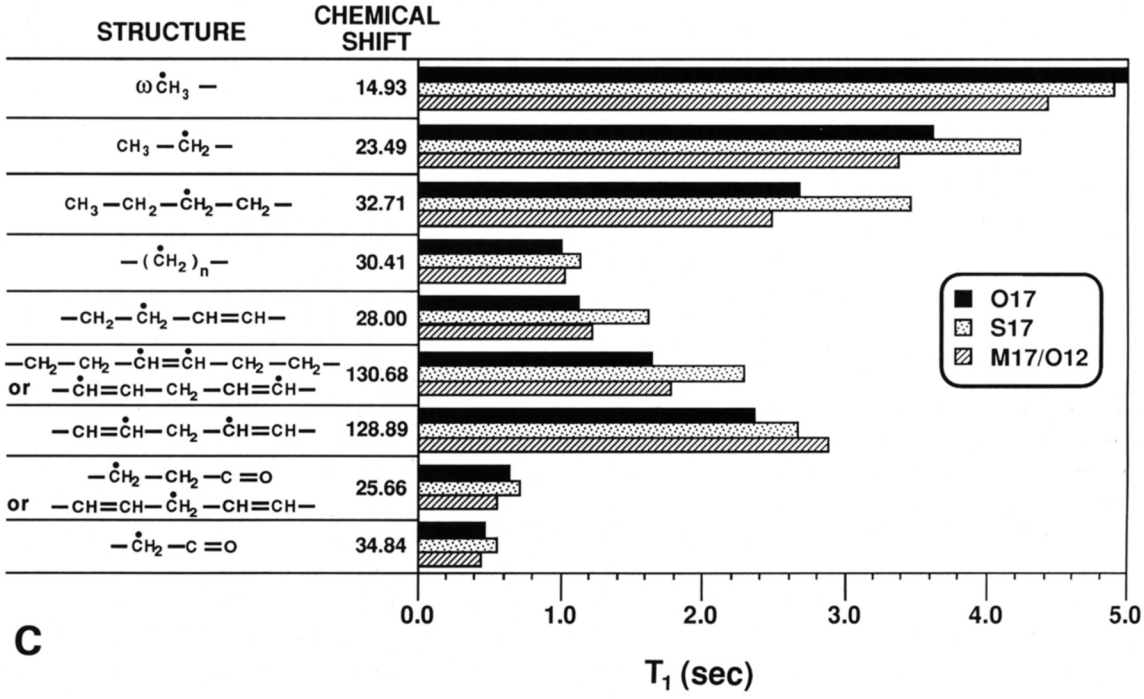


Fig. 4. In vivo and in vitro ^{13}C spin-lattice relaxation times of rat perirenal fat pad lipids. The example in vivo T_1 measurement (A) was performed on the O17/M40 rat (see Scheme 1 for detailed treatments). A three-parameter exponential fit (solid line) of eight inversion recovery time points (filled circles) for the MUFA peak (peak *11*, Fig. 2) gave a T_1 value of 1.11s with a correlation coefficient of 0.998. All in vivo (B) and extract (C) T_1s of perirenal fat pad lipids were determined similarly from rats fed with O17, S17, M17, M17/O12, M17/O35, O17/M12, O17/M40, and S36 diets (see Scheme 1 for detailed treatments).





Downloaded from www.jlr.org by guest, on June 18, 2012

TABLE 4. Effective correlation times (τ_{eff}) of fatty acyl carbons of perirenal lipids calculated from in vivo and extract T_1s

		In Vivo τ_{eff} (ps)					In Vitro Extract τ_{eff} (ps)		
δ (ppm)	Carbon Type[a]	S36[b]	M17/O12[b]	M17/O35[b]	O17/M12[b]	O17/M40[b]	S17[c]	M17/O12[c]	O17[c]
14.93	ω-CH_3	4	8	7	8	7	4	4	4
23.49	CH_2	17	17	30	30	18	6	7	7
32.71	CH_2	nd[d]	16	70	14	7	7	10	10
30.41	CH_2	80	63	80	71	54	21	23	23
28.00	CH_2	80	54	80	70	61	15	20	21
130.68	C=H	46	50	100	80	43	28	27	29
128.89	C=H	35	32	nd	49	23	17	17	20
25.66	CH_2	80	60	105	85	70	33	43	38
34.84	CH_2	nd	nd	170	120	nd	43	50	50

nd, not determined.
[a]Assignments are as in Fig. 1 legend.
[b]Same set of rats as in Fig. 4B; diet group designations are the same as in Scheme 1.
[c]Same set of rats as in Fig. 4C; diet group designations are the same as in Scheme 1.

acylglyceride organization in vivo. In terms of dietary effects on fatty acyl chain composition, the ^{13}C NMR data revealed that the extent of acyl chain unsaturation in the diet had a profound impact on that in rat adipose tissues. This is evidenced by the close resemblance between the ^{13}C NMR spectra of the diet oils (Fig. 1) and of the tissues acquired in vivo (Fig. 2). Also, in conjunction with the fatty acid analysis (Table 1), it is reasonable to conclude that the MUFA peak (peak *11*) of the in vivo ^{13}C NMR spectra of olive oil-fed rats was mainly attributed to the C18:1-containing triacylglycerides. Likewise, the C18:2-containing triacylglycerides in safflower oil were primarily responsible for the high PUFA (peak *10*) peak for safflower oil-fed rats. This dietary influence on tissue fatty acyl chain composition is further demonstrated by the conversion of adipose triacylglyceride profile of menhaden oil-fed rats to that of olive oil-fed rats (cf. Tables 1 and 3) through a switch to the olive oil diet and vice versa. These results suggest that fatty acid moieties of dietary triacylglycerides can be directly incorporated into adipose tissues without metabolism. Such direct incorporation appeared to operate but to less an extent for n-3 PUFA (C18:4, C20:5 and C22:6) in adipose tissues of menhaden oil-fed rats (cf. Table 1; Fig. 1 and 2).

On the other hand, a deviation of the fatty acid profile of the adipose tissue from that of the diet also occurred in all three diet cases (Table 1) and is most likely mediated through regulation of triacylglyceride uptake or metabolism or both. In addition, since n-3 PUFA are particularly susceptible to lipid peroxidation (20–22), reduction of these fatty acids in the menhaden oil-fed rat through peroxidation mechanism cannot be excluded. Moreover, the enrichment of C16:0 above the diet content and to a comparable level in all diet cases indicates that C16:0 turnover is highly regulated in rat adipose tissues.

The fatty acid composition of the liver was also influenced by diet but to a lesser extent than in the adipose tissue (Table 2). This is consistent with a more active metabolism of triacylglycerides and possibly regulation of their uptake in the liver. Again, the selective enrichment of C16:0 as well as C18:0 and C20:4 to comparable levels in all diet cases suggests a strong regulation of their turnover in the liver. The enrichment of C20:4 (arachidonate) was to be expected based on its role in liver prostaglandin metabolism (23). However, the unusually high content of C20:5 and C22:6 in the livers of menhaden oil-fed rats warrants special attention, considering the sensitivity of these long-chain PUFA to lipid peroxidation and prostaglandin metabolism (24, 25). Further investigation will be needed to determine whether this enrichment has any bearing on the benefit of dietary supplement with menhaden oil.

Effects of diet on triacylglyceride organization in rat adipose tissues

The ^{13}C spin-lattice relaxation of triacylglycerides is mainly mediated through the dipole interaction of individual carbon with its directly bonded hydrogens (17, 26); therefore, the T_1s of the fatty acyl carbons are sensitive to the rate of the C–H rotational reorientation. The rotational correlation of triacylglyceride molecules is difficult to calculate as their molecular motion is most likely anisotropic. However, under extreme narrowing or fast motion limit, the correlation time characteristic of the motion of the C–H vector can be approximated as τ_{eff}, which is calculated from T_1 according to Equation 1, assuming isotropic motion. Since large NOEs (>2) and narrow linewidths (Fig. 2) were observed in the present study, relaxation via paramagnetic constituents was excluded and the extreme narrowing approximation applied. Therefore, only one of the two solutions to Eqn. 1 was valid and the value of τ_{eff} so obtained is characteristic of the degree of mobility of each carbon in the fatty acyl chain of the triacylglycerides.

In all diet cases, the adipose acyl chains, whether in vivo or in extracts, exhibited a higher degree of rotational

Downloaded from www.jlr.org by guest, on June 18, 2012

freedom at the ω-end than at the carbonyl end, as judged by their τ_{eff} (Table 4) or T_1 (Fig. 4). However, the olefinic carbons in the central region of the acyl chain were exceptions and showed higher mobility (shorter τ_{eff}) than the ω-end CH_2s. This can be related to the *cis* configuration of the acyl double bond that increases internal motion by disrupting the packing of acyl chains. The shorter τ_{eff} for poly- than for mono-olefinic carbons (Table 4) is consistent with this notion because the former contain more *cis*-double bonds which cause additional degree(s) of freedom between acyl chains. The general trend of shorter in vivo τ_{eff} for safflower (S36) and menhaden oil-impacted rats (M17/O12 and O17/M40) than for olive oil-impacted rats (M17/O35 and O17/M12) also supports this conclusion as the PUFA content of adipose triacylglycerides from the two former groups was higher than that from the latter group. It should be noted that the shorter τ_{eff} or higher in vivo mobility of the olefinic carbons for the PUFA-enriched adipose tissue may be associated with its reported sensitivity to lipid peroxidation (20–22). The disrupting effect of *cis*-double bonds is further consistent with the finding that in vivo τ_{eff} became shorter with increasing PUFA content (O17/M12 vs. O17/M40) or vice versa (M17/O12 vs. M17/O35) (Table 4). Interestingly, the in vivo τ_{eff} of the ω-end methylene carbons (23.49 and 32.71 ppm) appeared to have the strongest dependence on the extent of polyunsaturation in adipose triacylglycerides, which suggests that polyunsaturation of the acyl chain can have effects on triacylglyceride organization in situ.

Because of the qualitative similarity in the in vivo and extract τ_{eff} as a function of the acyl carbon position, adipose triacylglyceride molecules in vivo may be organized in a liquid-like microenvironment similar to that reported for extracts (27). However, quantitative differences existed between the two. For example, the overall longer in vivo τ_{eff}s of the acyl carbons (Table 4) indicate that adipose triacylglycerides in vivo experience a more restricted microenvironment than those dissolved in solvents. This restricted motion may be related to a tighter packing or higher microviscosity (17) that can exist in vivo. In addition, the difference in the mobility along the acyl chain appeared to be amplified in vivo as the τ_{eff}s of internal CH_2s (28.00, 30.41, and 25.66 ppm) were disproportionally longer in vivo than in extracts (Table 4). This suggests that the segmental mobility of the acyl chain towards the central region is more sensitive to the packing force imposed in vivo. Furthermore, the dependence of τ_{eff}s on the extent of polyunsaturation observed in vivo was not noted in extracts, indicating that different factors exist in governing triacylglyceride organization in vivo versus in extracts.

In conclusion, this ^{13}C NMR study demonstrated a noninvasive means to evaluate the impact of different oil diets on adipose fatty acyl chain composition in situ. At the same time, it allowed investigations on largely unexplored questions of how triacylglyceride molecules are organized in vivo, how diet can alter this organization, and how this organization may affect the development of physiological disorders. As a number of disorders including lipid peroxidation and cancers have been associated with tissue lipids, this noninvasive analysis of fatty acyl chain content and organization on the same individual prior to and during the progression of disorders can greatly improve our ability to reveal the metabolic factors underlying these disorders (9). It is also valuable for investigating the long-term impact of diet on lipid-related disorders in human subjects.

We wish to thank Dave Wilson for his assistance on preparation of animals, Dr. Andrew N. Lane for consultation on internal motions of lipids, and Dr. A. Daniel Jones for general discussions, and some of the reviewers' comments. We also acknowledge the financial support from NIH Grant no. DK38637, DK43098.

Manuscript received 21 May 1993 and in revised form 10 November 1993.

REFERENCES

1. Bouchard, C., G. A. Bray, and V. S. Hubbard. 1990. Basic and clinical aspects of regional fat distribution. *Am. J. Clin. Nutr.* **52:** 946–950.
2. Hirsch, J., J. W. Farquhar, E. H. Ahrens, Jr., M. L. Peterson, and W. Stoffel. 1960. Studies of adipose tissue in man. *Am. J. Clin. Nutr.* **8:** 499–511.
3. Dayton, S., S. Hashimoto, W. Dixon, and M. L. Pearce. 1966. Composition of lipids in human serum and adipose tissue during prolonged feeding of a diet high in unsaturated fat. *J. Lipid Res.* **7:** 103–111.
4. Hudgins, L. C., and J. Hirsch. 1991. Changes in abdominal and gluteal adipose-tissue fatty acid compositions in obese subjects after weight gain and weight loss. *Am. J. Clin. Nutr.* **53:** 1372–1377.
5. Reo, N. V., C. S. Ewy, B. A. Siegfried, and J. J. H. Ackerman. 1984. High-field ^{13}C NMR spectroscopy of tissue in vivo. A double-resonance surface-coil probe. *J. Magn. Reson.* **58:** 76–84.
6. Alger, J. R., K. L. Behar, D. L. Rothman, and R. G. Shulman. 1984. Natural-abundance ^{13}C NMR measurement of hepatic glycogen in the living rabbit. *J. Magn. Reson.* **56:** 334–337.
7. Sillerud, L. O., C. H. Han, M. W. Bitensky, and A. A. Francendese. 1986. Metabolism and structure of triacylglycerols in rat epididymal fat pad adipocytes determined by ^{13}C nuclear magnetic resonance. *J. Biol. Chem.* **261:** 4380–4388.
8. Canioni, P., J. R. Alger, and R. G. Shulman. 1983. Natural abundance carbon-13 nuclear magnetic resonance spectroscopy of liver and adipose tissue of the living rat. *Biochemistry.* **22:** 4974–4980.
9. Cerdan, S., B. Künnecke, A. Dölle, and J. Seelig. 1988. In situ metabolism of 1,ω medium chain dicarboxylic acids in the liver of intact rats as detected by ^{13}C and ^{1}H NMR. *J. Biol. Chem.* **263:** 11664–11674.
10. Moonen, C. T. W., R. J. Dimand, and K. L. Cox. 1988. The noninvasive determination of linoleic acid content of

Downloaded from www.jlr.org by guest, on June 18, 2012

human adipose tissue by natural abundance carbon-13 nuclear magnetic resonance. *Magn. Reson. Med.* **6:** 140–157.
11. Allen, L. H., and F. J. Zeman. 1973. Influence of increased postnatal nutrient intake on kidney cellular development in progeny of protein-deficient rats. *J. Nutr.* **103:** 929–936.
12. Lepage, G., and C. Roy. 1984. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *J. Lipid Res.* **25:** 1391–1396.
13. Hayes, C. E., W. A. Edelstein, J. F. Schenck, O. M. Mueller, and M. Eash. 1985. An efficient, highly homogeneous radiofrequency coil for whole-body NMR imaging at 1.5 T. *J. Magn. Reson.* **63:** 622–628.
14. Fan, T. W-M., and R. M. Higashi. 1989. Reproducible nuclear magnetic resonance surface coil fabrication by combining computer-aided design and a photoresist process. *Anal. Chem.* **61:** 636–638.
15. Matson, G. B., T. Schleich, C. Serdahl, G. Acosta, and J. A. Willis. 1984. Measurement of longitudinal relaxation times using surface coils. *J. Magn. Reson.* **56:** 200–206.
16. Gupta, R. K., J. A. Ferretti, E. D. Becker, and G. H. Weiss. 1980. A modified fast inversion-recovery technique for spin-lattice relaxation measurements. *J. Magn. Reson.* **38:** 447–452.
17. Hamilton, J. A., C. Talkowski, R. F. Childers, E. Williams, A. Allerhand, and E. H. Cordes. 1974. Rotational and segmental motions in the lipids of human plasma lipoproteins. *J. Biol. Chem.* **249:** 4872–4878.
18. Batchelor, J. G., R. J. Cushley, and J. H. Prestegard. 1974. Carbon-13 Fourier transform nuclear magnetic resonance. VIII. Role of steric and electric field effects in fatty acid spectra. *J. Org. Chem.* **39:** 1698–1705.
19. Bradley, F. P., F. D. Gunstone, and J. L. Harwood. 1986. Occurrence and characteristics of oils and fats. *In* The Lipid Handbook. F. D. Gunstone, J. L. Harwood, and F. B. Padley, editors. Chapman and Hall, New York. 49–170.
20. Mouri, K., H. Ikesu, T. Esaka, and O. Igarashi. 1984. The influence of marine oil intake upon levels of lipids, α-tocopherol and lipid peroxidation in serum and liver of rats. *J. Nutr. Sci. Vitaminol.* **30:** 307–318.
21. Mounié, J., B. Faye, J. Magdalou, H. Goudonnet, R. Truchot, and G. Siest. 1986. Modulation of UDPglucuronosyltransferase activity in rats by dietary lipids. *J. Nutr.* **116:** 2034–2043.
22. Gower, J. D., and E. D. Wills. 1987. The oxidation of benzo[a]pyrene-7,8-dihydrodiol mediated by lipid peroxidation in the rat intestine and the effect of dietary lipids. *Chem. Biol. Interact.* **63:** 63–74.
23. Samuelsson, B., M. Goldyn, E. Granstrom, M. Hamberg, S. Hammarstrom, and C. Malmsten. 1978. Prostaglandins and thromboxanes. *Annu. Rev. Biochem.* **47:** 997–1029.
24. Lokesh, B. R., G. Bruckner, and J. E. Kinsella. 1984. Reduction in thromboxane formation by n-3 fatty acids enriched lung microsomes from rat and guinea pig following the ingestion of dietary menhaden oil. *Prostaglandins Leukotrienes Med.* **15:** 337–348.
25. Hwang, D. H., M. Boudreau, and P. Chanmugam. 1988. Dietary linolenic acid and longer-chain n-3 fatty acids: Comparison of effects on arachidonic acid metabolism in rats. *J. Nutr.* **118:** 427–437.
26. Allerhand, A., D. Doddrell, and R. Komoroski. 1971. Natural abundance carbon-13 partially relaxed Fourier transform nuclear magnetic resonance spectra of complex molecules. *J. Chem. Phys.* **55:** 189–198.
27. Williams, E., J. A. Hamilton, M. K. Jain, A. Allerhand, E. H. Cordes, and S. Ochs. 1973. Natural abundance carbon-13 nuclear magnetic resonance spectra of the canine sciatic nerve. *Science.* **181:** 869–871.

Downloaded from www.jlr.org by guest, on June 18, 2012