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Natural Abundance Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Liver and Adipose Tissue of the Living Rat†

Paul Canioni,* Jeffry R. Alger, and Robert G. Shulman

ABSTRACT: We have employed the topical magnetic resonance (TMR) technique to obtain natural abundance ^{13}C nuclear magnetic resonance (NMR) spectra from liver and adipose tissue in the living rat. Experiments were performed in a TMR magnet (20-cm diameter) with a two-turn radio-frequency coil ("surface" coil) combined with a focused static magnetic field. The in vivo spectra that were obtained at 20.2 MHz have been assigned by comparison with those from excised organs obtained in a conventional spectrometer operating at 90.5 MHz. Signals in the TMR spectra corresponding to carbons of the carbohydrates, glucose and glycogen, and of the lipids, triglycerides and phospholipids, have been resolved in vivo and assigned. The effects of chronic modification of dietary fat and carbohydrate on the in vivo spectra have been investigated. The levels of carbohydrates and of saturated and unsaturated fats in the liver as measured by ^{13}C TMR reflect the relative amounts of these compounds in the long-term diet.

High-resolution nuclear magnetic resonance (NMR)[1] spectroscopy is presently making significant contributions in physiology, biochemistry, and clinical diagnosis due to its ability to measure noninvasively the concentrations and the dynamics of metabolites in living tissue (Shulman et al., 1979; Edwards et al., 1982; Gordon et al., 1982; Gadian et al., 1979; Ugurbil et al., 1979; Scott et al., 1981; Ross et al., 1982). Many reports have shown that ^{31}P NMR can be used to assay the energetic status of tissue by measuring the concentrations of phosphocreatine, inorganic phosphate, and nucleoside triphosphates. The potential usefulness of ^{13}C NMR has not been explored as extensively partly because the ^{13}C nucleus is only 1% abundant in nature whereas ^{31}P is 100%. However in a number of previous reports (Shulman et al., 1979; Ugurbil et al., 1979; Cohen et al., 1979, 1980, 1981a,b; den Hollander et al., 1981), we have shown that the low natural abundance, in fact, facilitates the ability to observe specific metabolic pathways by using ^{13}C-enriched substrates. Certain molecules have very high intracellular concentrations. For some of these molecules, it has been shown that detectable ^{13}C NMR signals can be obtained without isotopic enrichment (Norton, 1981). For instance, trehalose stored in encysted *Acanthamoeba castellanii* (Deslauriers et al., 1980) and in yeast spores (Barton et al., 1982) has been observed in natural abundance by ^{13}C NMR. Triglycerides and phospholipids in a number

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[1] Abbreviations: NMR, nuclear magnetic resonance; TMR, topical magnetic resonance.

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of systems (Norton, 1981; Doyle et al., 1981, Barany et al., 1982; Block, 1982) and catecholamines in chromaffin granules (Sharp & Richards, 1977a,b) and in excised adrenal glands (Alger, 1979) have also been observed by natural abundance ^{13}C NMR.

Recently, NMR measurements have been carried out on live animals with the aid of "surface" radio-frequency coils. High-resolution ^{31}P spectra have been obtained from the brain and skeletal muscle of the rat (Ackerman et al., 1980). The development of topical magnetic resonance (TMR) has allowed high-resolution NMR spectra to be recorded from a selected place deep within a living animal (Gordon et al., 1980, 1982). In TMR, the narrow lines are obtained from a localized volume where the external magnetic field is homogeneous, while in the surrounding regions the homogeneity is destroyed so that the NMR lines are broadened and they can be removed by data-processing techniques. Using a surface coil to select a volume, Alger et al. (1981) have shown that natural abundance ^{13}C NMR spectra from a human arm or from rat tissues give information on the content of fatty acids.

In the present study, we continue this line of investigation in more detail: the TMR technique is used to obtain natural abundance ^{13}C NMR spectra from rat liver and rat adipose tissue inside the living animal without recourse to surgery. The TMR spectra were similar to those obtained from the same excised organ or tissue in a conventional wide-bore spectrometer. This comparison permitted assignments of carbon resonances from carbohydrate metabolites (glucose and glycogen) and lipids (triglycerides and phospholipids) in the in vivo spectra. We have observed changes in these highly concentrated molecules in the living rat introduced by chronic modifications of the diet.

Materials and Methods

Synthetic diet materials were obtained from Teklad Test Diets, Madison, WI. Male rats (Wistar strain, 30–40 g) were selected randomly and caged in groups of three. Each group was fed ad libitum a synthetic diet composed of 20% casein (vitamin free), 50% sucrose, 1% vitamin mixture (Teklad 40060), 4% salt mixture (Teklad 170750), 10% nonnutritive fiber cellulose, and 3% methionine. This basic diet was supplemented with fat and carbohydrate for each group in the following manner: Group A served as the control; the basic diet was supplemented with 8% hydrogenated coconut oil, 2% choline, and 2% safflower oil to ensure adequate essential polyunsaturated fatty acids. The basic diet for group B was supplemented with 12% sucrose. This regime was high in carbohydrate and free of both essential and nonessential fats. The basic diet for group C was supplemented with 10% corn oil and 2% choline. It was high in polyunsaturated fat. After 16 weeks on the diets A, B, or C, a single rat from each group was subjected to ^{13}C TMR analysis during pentobarbitol anesthesia. The measurements were always performed 4 h after a meal.

The in vivo ^{13}C TMR spectra were acquired at 20.2 MHz by using an Oxford Research Systems TMR-32/200 spectrometer with a superconducting magnet having a 20-cm bore diameter. The NMR signals were obtained with a surface coil placed adjacent to the tissue to be studied. The surface coil was a two-turn coil, of 2-cm diameter, which had a 90° pulse width of 8 μs at its center. Pulses of 16 μs were repeated every 0.5 s for acquiring the data. A coplanar, concentric single-turn coil (of 6-cm diameter) was used for proton decoupling. Gated decoupling was employed with an approximate decoupling power of 20 W during the acquisition time of 0.2 s and no power during the remainder of the delay. For observation of liver signals, magnetic field profiling was used to create a spot of static magnetic field homogeneity, of approximately 2.0 cm in diameter. The animal was positioned so that this homogeneous volume was centered in the liver.

For the analysis of excised tissues, the liver and epididymal fat pads were excised under pentobarbitol anesthesia as quickly as possible, washed with Krebs/Henseleit bicarbonate buffer, pH 7.4 and 4 °C, and then immediately analyzed by natural abundance ^{13}C NMR. Spectra of excised livers were recorded in 20-mm sample tubes on a Bruker WB-360 spectrometer operating at 90.5 MHz. Radio-frequency pulses of 20 μs were used with a recycling time of 0.5 s. A gated broad-band decoupling procedure was employed: 10 W of decoupling power was used during the signal acquisition, and 2 W was used during the recycling delay period. The center of the noise-modulated decoupler bandwidth was set on the proton water resonance. The tissue was suspended in Krebs buffer containing 10% D_2O in order to observe a deuterium signal for the field-frequency lock. The sample temperature was maintained at 24 °C. Excised adipose tissue was analyzed in a similar manner except that a 10-mm sample tube was used.

Spin–lattice relaxation (T_1) measurements in excised liver and adipose tissue were carried out at 90.5 MHz by using the standard inversion–recovery method. The data were processed with a two-parameter-fit program (Bruker, DISNMR) by assuming a single exponential decay. Care was taken to ensure that the delay time between repeated pulse sequences was at least 5 times the T_1 value. The nuclear Overhauser effect (NOE) at 90.5 MHz was obtained by the gated decoupling method, in which the NOE-enhanced and decoupled spectrum was obtained with continuous ^{1}H irradiation, and the decoupled (but not NOE enhanced) spectrum was obtained by irradiating ^{1}H only during the acquisition time.

Results

High-Resolution ^{13}C NMR Spectra of Excised Liver and Adipose Tissue. The 90.5-MHz proton-decoupled ^{13}C NMR spectrum of an excised liver from a rat maintained on a standard diet is shown in Figure 1A. The spectrum consists of numerous sharp resonances that have been assigned on the basis of chemical shifts previously reported from various carbohydrate and lipid compounds (see Table I for assignments). Resonances 1–8 arise from saturated carbons of the fatty acyl chains; resonances 9 and 10 come from the unsaturated carbons; resonance 11 comes from the carboxyl groups at the ester linkages. The assignments of resonances 14 and 15 are less certain. They appear at positions characteristic of the methyl carbons of the phosphatidylcholine head groups and the methylene units from the head groups of both phosphatidylcholine and phosphatidylethanolamine. The presence of carbohydrate in the liver is shown by resonances 16 and 19–28. The glycogen C-1 carbon and the β and α anomers of glucose C-1 give rise to the three peaks numbered 28, 27, and 26, respectively, while peaks in the 60–80 ppm region (peaks 19–25) are from other carbons of the glucose ring either in free glucose or in glycogen. The normal cellular concentration of glucose is about 5 mM so that unlabeled free glucose should not be observed under these conditions. The glucose peaks observed must arise from hydrolysis of the glycogen during the excision and analysis. Proteins may contribute to the broad signals centered at 60 ppm (α-carbon region) and 20 ppm (aliphatic region of amino acid side chains) and to the peak at 158 ppm (ζ-carbons of arginine residues).

The spectrum of the excised adipose tissue (Figure 1b) has similarities to the liver spectrum; the two organs share many of the resonances from the fatty acyl chains. The striking

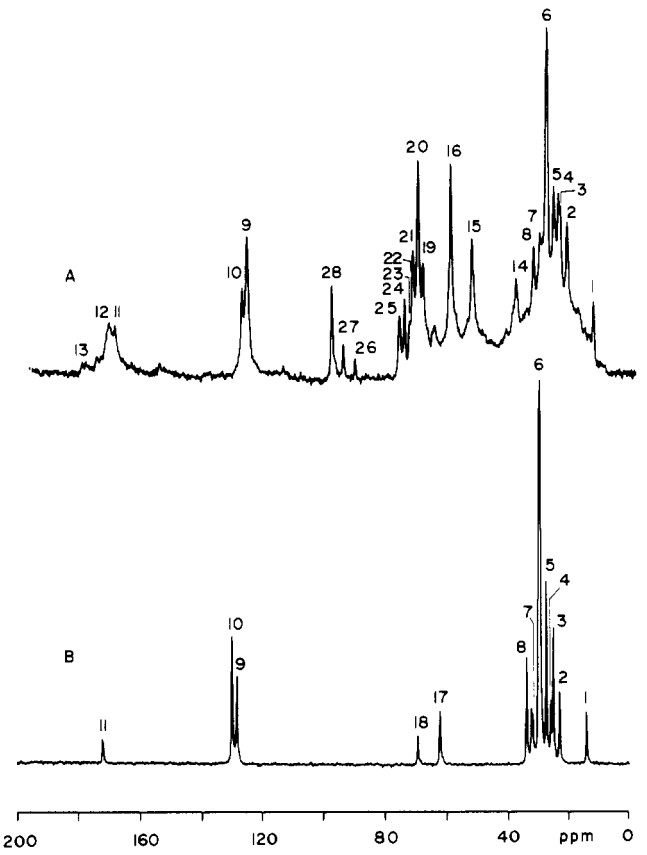

FIGURE 1: High-resolution ^{13}C NMR spectra of rat liver (A) and rat adipose tissue (B). The spectra recorded at 90.5 MHz represent 1024 free-induction decays accumulated at 24 °C in a 20- (liver) and 10-mm sample cell (adipose) with a 45° radio-frequency pulse and a delay of 0.5 s. Resonances have been numbered from 1 to 11 for carbons of fatty acyl chains and from 12 to 15 for phospholipids and free carboxylate groups. The region between 55 and 105 ppm shows the signals from carbohydrates (16 and 19–28) and the glycerol backbone in triglycerides and phospholipids (17 and 18). The assignments of resonances are reported in Table I. Chemical shifts are in ppm from tetramethylsilane.

Table I: Assignments and Chemical Shift Values of ^{13}C Resonances of Rat Liver and Rat Adipose Tissue Spectra[a]

resonance	assignment	chemical shift (ppm)
1	$*CH_3-CH_2-CH_2-$, fatty acyl chain	14.20
2	$CH_3-*CH_2-CH_2-$, fatty acyl chain	23.05
3	$-*CH_2-CH_2-CO-$, fatty acyl chain	25.20
4	$-CH=CH-*CH_2-CH=CH-$, fatty acyl chain	25.05
5	$-CH=CH-*CH_2-CH_2-$, fatty acyl chain	27.60
6	$-(CH_2)_n-$, fatty acyl chain	30.05
7	$CH_3-CH_2-*CH_2-$, fatty acyl chain	32.30
8	$-CH_2-*CH_2-CO-$, fatty acyl chain	34.15
9	$-CH=*CH-CH_2-*CH=CH-$, fatty acyl chain	128.40
10	$-*CH=CH-CH_2-CH=*CH-$, fatty acyl chain	130.00
11	$-CH_2-CH_2-*CO-$, fatty acyl chain	172.10
12	-CO-OR, protein, phospholipid	173.80
13	-COO-, Glu, free fatty acid	182.20
14	$-CH_2-NH_2$, ethanolamine (protein)	40.00
15	$(CH_3)_3N-$, choline	54.60
16	C-6, α,β-glucose, glycogen	61.40
17	C-1, C-3, glycerol (ester)	62.20
18	C-2, glycerol (ester)	69.50
19	C-4, α,β-glucose	70.40
20	C-2, C-5, α-glucose; C-5, glycogen	72.10
21	C-3, α-glucose	73.50
22	C-3, glycogen	73.95
23	C-2, β-glucose	79.90
24	C-3, C-5, β-glucose	76.50
25	C-4, glycogen	78.05
26	C-1, α-glucose	92.70
27	C-1, β-glucose	96.60
28	C-1, glycogen	100.50

[a] Chemical shifts are in parts per million downfield from tetramethylsilane. The methyl group of fatty acyl chain is used as an internal reference at 14.2 ppm downfield from tetramethylsilane (Batchelor et al., 1979; Hamilton et al., 1974). The resonances are numbered as indicated in Figure 1. (*) The specifically assigned carbon.

difference is the absence, in the adipose tissue spectrum, of signals from carbohydrates and from phospholipid head groups. From the absence of phospholipid head group signals, we conclude that in adipose tissue the ^{13}C fatty acid peaks come predominantly from triglycerides, as expected, whereas in the liver the phospholipid head group signals indicate a contribution from phospholipids.

The unique chemical structure of the polyunsaturated fatty acids permits them to be distinguished from the monounsaturated fatty acids in the ^{13}C NMR spectra. The monounsaturated fatty acids such as oleic acid have a single double bond, and both its olefinic carbons resonate at 130.0 ppm. The polyunsaturated fatty acids have two or more double bonds separated in pairs by a single methylene unit. In linoleic acid, which has two double bonds, the two olefinic carbons adjacent to the single methylene group resonate at 128.5 ppm, and the exterior two olefinic carbons resonate at 130.0 ppm. In linoleic acid, which possesses two double bonds separated by a single methylene group, carbons 10 and 12 ("interior" olefinic carbons) resonate at 128.5 ppm, and carbons 9 and 13 ("exterior" olefinic carbons) resonate at 130.0 ppm. In polyunsaturated fatty acids with three or four double bonds such as linolenic and arachidonic acids, there are two exterior olefinic carbons resonating also at 130.0 ppm, and there are four and six interior olefinic carbons, respectively, resonating around 128.5 ppm (Batchelor et al., 1979). Thus, the ^{13}C spectrum has a resonance at 128.5 ppm that comes only from polyunsaturated fatty acids and a signal at 130.0 ppm that comes from mono- and polyunsaturated fatty acids. The ratio of the intensity at 128.5 ppm to that at 130.0 ppm reflects the relative amounts of polyunsaturated fatty acids. For the data shown in Figure 1, the ratio is 1.6 for liver and 0.6 for adipose tissue. Although this ratio is determined by the fatty acid composition, it alone does not contain enough information to determine the mole fraction of the individual fatty acids.

We have attempted to determine if some of the fatty acyl resonances of the liver come from phospholipids by studying the temperature dependence of the spectrum. It is known that the phospholipid methylene carbon resonances (but not the methyl head group resonances) of model membranes broaden to an unobservable width at low temperatures (Doyle et al., 1981; Sears, 1975). We have compared rat liver spectra recorded at 10 and 40 °C. In these experiments (data not shown), the resonances at 30.0 and 128.5 ppm sharpened by 10–15% going from 10 to 40 °C, indicating that membranes may make a small contribution to the fatty acyl signals.

Values of the longitudinal relaxation time (T_1) and NOE for carbons in fatty acyl chains in tissue are reported in Table II. The T_1 values measured for carbon in adipose and liver fats are essentially the same and are very close to those found in very low density lipoprotein (Hamilton et al., 1974). In both tissues, we observed a decrease in T_1 values from the ω-carbon to the carboxyl ester group of the chain, suggesting a decrease

Table II: Longitudinal Relaxation Time (T_1) and Nuclear Overhauser Effect for ^{13}C Resonances of Fatty Acyl Chains and Carbohydrates in Rat Liver and Rat Adipose Tissue[a]

		liver		adipose	
resonance	assignment	T_1 (s)	NOE	T_1 (s)	NOE
1	*CH_3–CH_2–CH_2–	1.8	1.5	1.9	1.9
2	CH_3–*CH_2–CH_2–	0.8	1.3	0.8	1.5
3	–*CH_2–CH_2–CO–	0.4	1.1	0.3	1.1
4	–CH=CH–*CH_2–CH=CH–	0.5	1.3	0.5	1.4
5	–CH=CH–*CH_2–CH_2–	0.6	1.1	0.4	1.1
6	–$(CH_2)_n$–	0.4	1.1	0.3	1.1
7	CH_3–CH_2–*CH_2–	0.5	1.2	0.5	1.1
8	–CH_2–*CH_2–CO–	0.2	1.1	0.2	1.1
9	–CH=*CH–CH_2–*CH=CH–	0.8	1.2	0.5	1.2
10	–*CH=CH–CH_2–CH=*CH–	0.6	1.1	0.4	1.1
14	–CH_2–NH_2–, ethanolamine	0.2	1.1		
15	$(CH_3)_3$N–, choline	1.5	1.8		
		0.4	1.1		
26	C-1, α-glucose	1.8	1.5		
27	C-1, β-glucose	2.1	1.6		
28	C-1, glycogen	0.3	1.3		

[a] Measurements have been made at 90.5 MHz in excised rat tissues, as indicated under Materials and Methods. The numbering of resonances is the same as in Table I.

in chain mobility going from the terminal methyl group to the glycerol backbone.

In Vivo ^{13}C NMR Spectra of Rat Tissues. We have used the TMR spectrometer to obtain ^{13}C signals from liver in vivo. Gordon et al. have shown that a ^{31}P spectrum of the liver in a living rat could be obtained by using the TMR technique (Gordon et al., 1980). The absence of a phosphocreatine signal in the spectrum was used to monitor the focusing on the liver. We have obtained a similar spectrum lacking phosphocreatine by using the field-profiling method with a ^{31}P radio-frequency coil. When we used a ^{13}C coil of identical dimensions, positioned identically with the same static field gradients, we obtain the ^{13}C spectra of type shown in Figure 2. The spectra were obtained with 10 min of signal averaging under the best parameters for pulse length and field profiling as determined by ^{31}P NMR. For comparison, the liver measured in vivo in Figure 2C was excised at the end of the TMR experiment and its high-field NMR spectrum is presented in Figure 2D. The TMR spectra are not as well resolved as the high-field spectra because of the lower field strength. Nevertheless, some signals, characteristic of the liver, can be observed, and they are numbered as in Figure 1. The signals from C-1 (resonance 28), C-6 (resonance 16), and the unresolved group of C-2 and C-5 (resonance 20) of liver glycogen are clearly present in spectrum C. Contributions of phospholipids are also visible at 40 ppm (resonance 14) and 54.7 ppm (resonance 15). The C-1 and -3 and C-2 carbons of the glycerol moiety resonate at 62.2 ppm (resonance 17) and 69.5 ppm (resonance 18).

We have used natural abundance ^{13}C NMR to follow liver metabolism in the living animal in a series of comparative feeding experiments. The spectra were taken noninvasively, and it was possible to monitor the liver of the same animal for many weeks. Three groups of rats were maintained on different diets as described under Materials and Methods. Characteristic results are illustrated in Figure 2. Comparison of the spectrum from the regime B (high-carbohydrate) group with the regime A (control) group shows larger amounts of carbohydrate and monounsaturated fatty acids, although there is not a definite peak from the C-1 of glycogen observed in

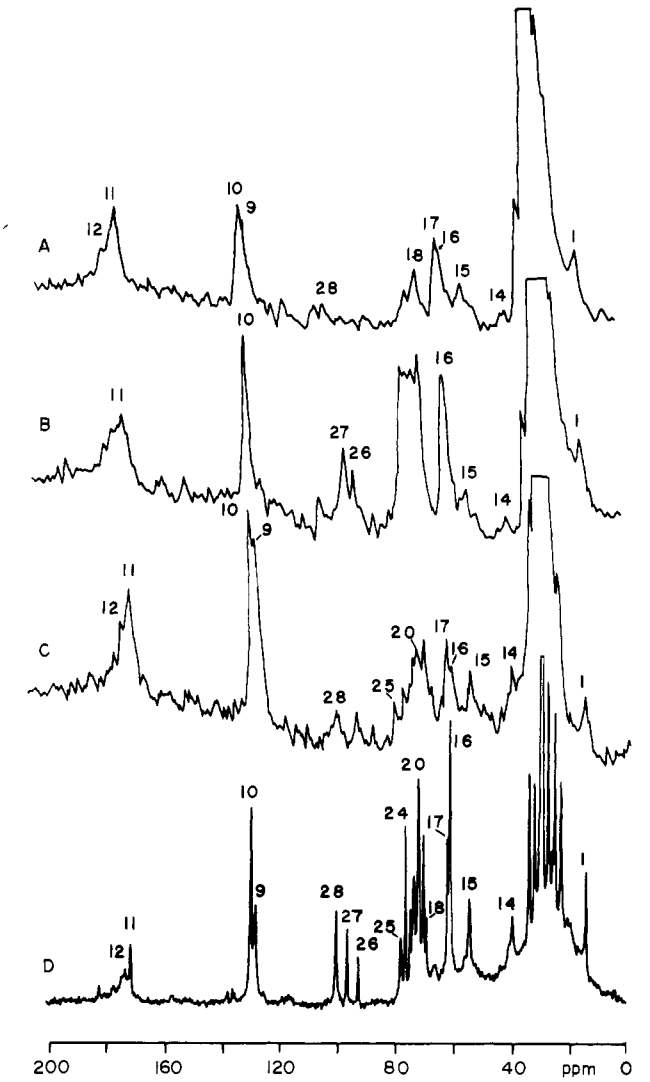

FIGURE 2: Effect of chronic modification of dietary fat and carbohydrate on ^{13}C TMR spectrum of rat liver. Rats have been fed during 16 weeks the specific synthetic diets described under Materials and Methods. 1024 scans have been accumulated for each spectrum. (Spectrum A) Rat from group A (control); (spectrum B) rat from group B (fat-free diet); (spectrum C) rat from group C (high polyunsaturated fat diet). Spectrum D has been measured at 90.5 MHz from the liver excised from the rat in (spectrum C).

Figure 2B. Glucose is expected to be at low concentration in the liver (5–7 mM), and consequently, natural abundance ^{13}C signals are not expected from this metabolite. The appearance in spectrum B of the glucose C-1 signals at 96.8 and 93.0 ppm may be coming from glucose in the gastrointestinal tract since the most recent meal had been only 4 h earlier.

Comparison of the spectrum from the regime C group with that from the regime B group shows the expected difference in the olefinic carbon peaks: In spectrum B (fat-free diet), the polyunsaturated peak at 128.5 is completely missing. In spectrum C (high polyunsaturated fat), it is relatively strong. In addition, spectrum C shows a rather strong peak from glycogen C-1 at 100.5 ppm.

We have also studied the dietary influences on the ^{13}C natural abundance spectra of adipose tissue in the living animal. These experiments were done with rats maintained on regimes A–C. The results presented in Figure 3 show that the diet has had a strong effect on the ^{13}C TMR spectra. In Figure 3, we have enlarged the region of the in vivo spectra around the olefinic region near 130 ppm. Rats maintained on a diet free of polyunsaturated fat (regime B) give an adipose

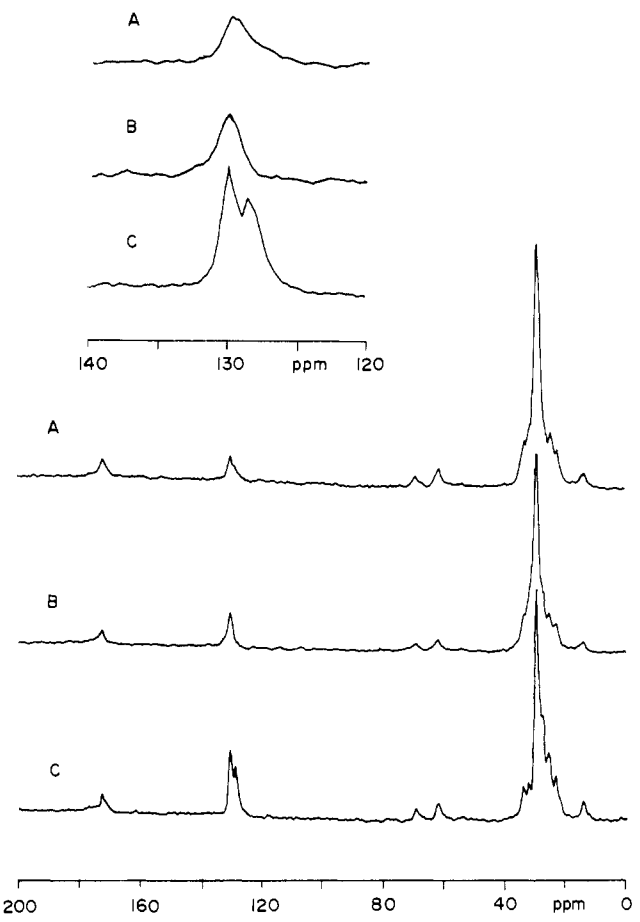

FIGURE 3: ^{13}C TMR spectrum of rat epididymal adipose tissue, in vivo. Same rats as in Figure 2 have been used for this experiment. (Upper spectrum) Regime A; (middle spectrum) regime B; (lower spectrum) regime C. The inset represents an expanded plot of the olefinic carbon region (130 ppm). 1024 scans have been accumulated for each spectrum.

tissue spectrum with no observable intensity at 128.5 ppm (Figure 3B), indicating relatively low levels of polyunsaturated fatty acids. When rats maintained on this restricted regime for 16 weeks were given polyunsaturated fat, a resonance at 128.5 ppm could be observed after 2–3 weeks (data not shown). For rats fed a high polyunsaturated fat diet (regime C), the ratios of the intensity at 128.5 ppm to the intensity of 130.0 ppm were high throughout the entire period, indicating an increased storage of polyunsaturated fatty acids in the adipose tissue. The in vivo and excised adipose tissue spectra show excellent agreement, as can be seen by comparing Figures 1B and 3.

Discussion

Topical magnetic resonance (TMR) uses static nonlinear magnetic field gradients to create a limited volume where the applied static field B_0 is homogeneous (Hanley & Gordon, 1981; Gordon et al., 1980, 1982). If this restricted volume is localized within an animal, the detected NMR signal will be composed of narrow lines from the selected volume and broad lines coming from the part of the sample located in the inhomogeneous field region. These two spectral components can be separated mathematically in the time domain. Localization in the topical NMR technique is also aided by the introduction of flat radio-frequency coils called surface coils, placed on the surface of the animal, close to the region of interest. In contrast to conventional radio-frequency coils (saddle shaped or solenoidal), surface coils produce a nonuniform radio-frequency field B_1, which decreases with increasing distance from the center of the coil.

Previously, we have shown that natural abundance ^{13}C NMR spectra could be acquired from mammalian tissue in vivo by using surface coils, and we have suggested that the spectra obtained might be useful in the study of specific metabolic pathways (Alger et al., 1981). In the present work, we have recorded natural abundance ^{13}C NMR spectra from the living animal and compared the data with high-resolution spectra obtained from analogous excised tissue. The ^{13}C resonances from the high concentrations of lipids and carbohydrates are resolved and easily identified in the high-field spectrum of an excised rat liver (Figure 1A). Some of these resonances clearly appear in the liver spectrum of living anesthetized rats as illustrated in Figure 2. Of particular interest is the resonance of the anomeric carbon (C-1) of the glucoside unit in glycogen. The intensity of this resonance reports the amount of glycogen that is present in liver, since a recent study has shown that all the carbons in the high molecular weight glycogen contribute to the NMR peak (Sillerud & Shulman, 1983). Other glycogen signals are also present and could be used for this purpose, but they are not as well resolved as the C-1 resonance. There is a discrepancy in Figure 2 in that the control regime A and the high-carbohydrate regim B did not show a glycogen C-1 peak, while the high-polyunsaturated regime C did show a C-1 peak with good signal to noise. Subsequent experiments (J. R. Alger, K. Rothman, and R. G. Shulman, unpublished results) show that the glycogen levels are very dependent upon hormonal release during the trauma of anesthesia. Hence, the amount of glycogen observed, in the rat, is somewhat variable. For the present, we wish to emphasize very strongly that it has been possible to observe the C-1 glycogen peak in the living rat (see Figure 2C) without isotopic enrichment.

Our comparisons of excised adipose and liver tissue have shown differences in their natural abundance ^{13}C NMR spectra (Figure 1). The liver spectrum displays carbohydrate resonances (mostly from glycogen). It also contains resonances that can be assigned to the choline and ethanolamine head groups, the presence of these signals suggesting, but not proving, that fatty acids in phospholipids are contributing to the spectra. Carbohydrate, choline, and ethanolamine resonances are completely absent from the adipose tissue spectrum (Figure 1B), and in contrast to the liver, strong peaks from glycerol are observed. Hence, the signals we observe in adipose tissue are without question entirely from triglycerides. However, in liver, the assignments are less certain. The amounts of both phospholipids and triglycerides in the liver have been reported. The triglycerides usually account for 9–30% of the total lipids, while the phospholipids account for about 65% (Connellan & Masters, 1965). Major portions of these liver phospholipids are not constituents of cellular membranes but are either from free phospholipids, lipoproteins, or relatively small vesicles. Therefore, the fatty acyl ^{13}C signals that we observe from the liver may come from the triglycerides, phospholipids in membranes, or phospholipids in these nonmembrane structures, and the present NMR signals from the liver do not allow us to clearly distinguish among these sources. The small temperature dependence between 10 and 40 °C suggests that phospholipid membranes make only a small contribution. It is planned to do ^{13}C-label experiments in these liver experiments to help in assigning the sources of the fatty acid peaks.

The differences observed between ^{13}C spectra of liver and adipose tissue can be used to assess the quality of the focusing

Table III: Line Widths (Hertz) for Fatty Acyl Chain and Carbohydrate ^{13}C Resonances of Liver and Adipose Tissue in Rat[a]

	liver		adipose	
	90.5 MHz	20.2 MHz	90.5 MHz	20.2 MHz
CH_3, fatty acyl chain	30	45	35	45
$-CH=CH-$	80	75	65	60
$-(CH_2)_n-$	80	55	75	50
C-1, glycogen	56	55		
C-1, glucose	30	25		

[a] Line widths have been measured as the full width at half-height. Exponential line broadening has been subtracted from the given values.

in the TMR spectra of the liver of the living rat. The amounts of polyunsaturated fatty acids relative to monounsaturated fatty acids are lower in our TMR spectra than in spectra from the excised liver. Thus, the in vivo ^{13}C liver spectra seem to have appreciable contributions from adipose tissue that vary somewhat among animals. Since ^{31}P NMR established our selectivity initially, it is important to note that the ^{31}P and ^{13}C coils used in these experiments were identical in size and shape, and care was taken to position the two coils identically with respect to the animal within a few millimeters. The coils have identical 90° pulse widths at their centers, and thus, we detect the signal from the same selected volume. It seems that while the lack of phosphocreatine on the ^{31}P spectrum monitors the absence of contributions from muscle to the spectrum, it does not indicate the lack of contributions from adipose tissue. This is reasonable since any ^{31}P signals from adipose tissue would come from the negligible amount of phosphates in the relatively small aqueous phase whereas ^{13}C signals from adipose tissue come from the highly concentrated triglycerides. Consequently, extrahepatic fat contributes to the in vivo ^{13}C NMR spectrum even after obtaining a ^{31}P spectrum lacking phosphocreatine. A quantitative analysis of the lipid content of the rat liver is then difficult to obtain on the basis of the in vivo spectrum. It is important to note that the rat liver is too small for the focusing available in our TMR-32/200 spectrometer. This is because the rat liver is, in at least one dimension, smaller than the smallest possible homogeneous volume. In larger animals with larger livers, it should be possible to focus more perfectly on the liver.

Although as mentioned above there are problems with maintaining hepatic glycogen levels during anesthesia, ^{13}C TMR is a quantitative method for studying carbohydrate metabolism in vivo. In a human, the liver is close enough to the skin and large enough so that it should be possible to focus more perfectly on the liver, while there is no reason to expect any trauma with its associated glycogenolysis. Therefore, we expect to have substantial improvement in tissue selectivity and in glycogen signal to noise. The exact improvement is difficult to predict because radio-frequency losses from larger coils will come into play (Hoult, 1978).

Analysis of adipose tissue in rats by the TMR method clearly indicates that there are no difficulties in observing the adipose tissue fat resonances of animals in vivo. In fact, as previously reported (Alger et al., 1981), the present instrumentation permits observation of the fat depot of the human arm. In this paper, we have shown how it is possible to resolve the polyunsaturated olefinic ^{13}C peak at 128.5 ppm from the peak at 130 ppm coming from all unsaturated fatty acids. Chronic restriction of essential (polyunsaturated) fat from the diet resulted in a decrease in the polyunsaturated fatty acid resonance at 128.5 ppm in adipose tissue spectra from the living rat (Figure 3B). Similarly, a chronic supplementation of the diet with essential fat resulted in an increase of the resonance at 128.5 ppm. Thus, the intensity of the peak at 128.5 ppm in the ^{13}C spectrum of adipose tissue reports the animal's history of polyunsaturated fatty acid intake. This kind of information obtained noninvasively in a short time should be of interest in the study of humans.

An analysis of the resolution available from natural abundance ^{13}C NMR spectroscopy is needed to evaluate the potential of the method for in vivo studies. The values of the line widths measured on liver and fat tissue spectra recorded at two magnetic fields (8.5 and 1.9 T) are listed in Table III. Line widths for carbon signals of fatty acyl chains and carbohydrates are not significantly different at 8.5 and 1.9 T. Hence, the resolution is expected to be reduced linearly with field, which from an examination of the spectra appears to be the case. The observed field independence of line widths agrees with a dipole–dipole relaxation mechanism for T_2. In contrast to ^{31}P NMR in vivo where the line widths are usually proportional to magnetic field and the resolution does not depend on the field, in the ^{13}C NMR the resolution is worse at low field because the line widths are approximately independent of field.

Within these limitations it is clear from the present results that natural abundance ^{13}C NMR can be very useful in following metabolic changes noninvasively in rats and larger animals. Both fatty acid peaks and hepatic glycogen peaks can be observed in natural abundance. Information about fatty acid composition can be obtained from the olefinic ^{13}C peaks near 130 ppm. Glycogen levels can be monitored by measuring the intensity of the glycogen C-1 peak at 100.5 ppm. The applications to human subjects should not present any insurmountable difficulties.

Registry No. Glycogen, 9005-79-2; α-glucose, 492-62-6; β-glucose, 492-61-5.

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Chemical Structure and Antigenic Aspects of Complexes Obtained from Epimastigotes of *Trypanosoma cruzi*†

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ABSTRACT: Cells of *Trypanosoma cruzi*, Y strain, were submitted to water extraction by successive freezing and thawing. Fractionation of soluble material on a P-10 column gave an antigenic glycoprotein (fraction I) whose carbohydrate portion (40%) contained galactose, mannose, glucose, and xylose in a molar ratio of 35:13:1:1. It was electrophoretically homogeneous (M_r ~25 000) and contained short chains of mannopyranosyl (23%) and galactopyranosyl (10%) nonreducing end units and 2-O-substituted mannopyranosyl units (19%). Extraction of the remaining cell fragments with phenol–water gave an antigenic $CHCl_3$–MeOH–H_2O-soluble fraction II (LPPG), which yielded a galactomannan (fraction III) (galactose and mannose in a molar ratio of 1:2.1) on degradation with hot aqueous NaOH–$NaBH_4$. It contained end units of galactofuranose (25%) and 2-O- (5%), 3-O- (32%), and 2,3-di-O- (19%) substituted mannopyranosyl units. Galactofuranose was directly linked (1→3) to mannopyranose and not via a phosphorodiester bridge. β-D-Gal*f*-(1→3)-Me-α-D-Man*p*, in contrast with Me-β-D-Gal*f*, was effective in inhibiting the precipitin reaction between LPPG and antiserum raised against LPPG. Fraction IV, insoluble in $CHCl_3$–MeOH–H_2O, contained galactose and mannose in a ratio of 1.4:1. After degradation with hot aqueous NaOH–$NaBH_4$, it gave a product (fraction V) containing galactose and mannose in a 1:2 ratio. Methylation analysis showed it to differ from fraction III since it contained a high proportion of nonreducing end units (41%) and 2-O-substituted units (16%) of mannopyranose.

Chagas' dissease is a complex clinical entity caused by the flagellate protoazoan *Trypanosoma cruzi*. This protozoan has a complex life cycle with developmental stages in different hosts (Brener, 1973). In the mammalian host, *T. cruzi* multiplies intracellularly as amastigotes and is subsequently released into the bloodstream as trypomastigotes. These are nondividing forms that can infect new host cells or be ingested by a triatomine bug. In the insect midgut lumen, trypomastigotes differentiate to epimastigotes, which are the invertebrate multiplying forms. In the insect rectum, epimastigotes differentiate to the nondividing metacyclic trypomastigotes, which are discharged in the feces and urine onto the vertebrate skin when the insect is feeding. Through skin discontinuities or through mucosal membranes, these forms can reach the blood circulation and thereafter penetrate cells, thus completing its biological cycle.

The clinical features of Chagas' disease are highly variable and depend on the interaction of several different host and/or parasite factors (Tafuri, 1979). Studies on the cellular membrane of *T. cruzi* have been performed with the aim of obtaining a better understanding of some of those complex interactions.

Surface glycoconjugates are the membrane components that are currently receiving special attention. Snary & Hudson (1979) isolated a surface glycoprotein of molecular weight (M_r) 90 000 that is present in epimastigote, trypomastigote, and amastigote forms and which was able to induce immunoprotection against experimental infections in mice (Scott & Snary, 1979). However, Nogueira et al. (1981) found that only bloodstream trypomastigotes contain a surface glycoprotein of M_r 90 000, whereas epimastigotes and trypomas-

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