Validation of ^{13}C NMR Measurements of Liver Glycogen *in Vivo*

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The natural abundance ^{13}C NMR intensity of the glycogen C1 resonance was measured in the surgically exposed liver of rabbits *in vivo* (n = 17) by *integration* from 98 to 104 ppm and compared double blindedly to the subsequent biochemical measurement. Coil loading was measured each time from a reference sphere at the coil center and the NMR intensity was normalized accordingly. For quantification, the normalized NMR intensity was calibrated using aqueous glycogen solutions ranging from 110 to 1100 μmol glucosyl units/g (n = 14). An *in vivo* range from 110 to 800 μmol glucosyl units/g wet weight was measured with a highly linear correlation with concentration (r = 0.85, P < 0.001). The *in vivo* NMR concentration was 0.95 ± 0.05 (mean ± standard error, n = 17) of the concomitant enzymatic measurement of glycogen content. We conclude that the ^{13}C NMR signal of liver glycogen C1 is essentially 100% visible *in vivo* and that natural abundance ^{13}C NMR spectroscopy can provide reliable noninvasive estimates of *in vivo* glycogen content over the physiological range of liver glycogen concentrations when using adequate localization and integration procedures.

Key words: glycogen; liver; biopsy; *in vivo* ^{13}C NMR.

INTRODUCTION

Glycogen is the major storage form of glucose in mammals. Glycogen metabolism is essential for glucose homeostasis in humans and may be deranged in a variety of diseases including diabetes mellitus. Liver glycogen can be measured *in vivo* using the biopsy technique, which is a difficult and invasive procedure to perform in humans and is associated with a high incidence of serious side effects. Noninvasive ^{13}C NMR spectroscopy of glycogen has potential to provide new insights into liver glycogen metabolism in humans (1–6). Despite the large molecular weight of this macromolecule, which is on the order of 10^7 Da, ^{13}C NMR studies of glycogen in aqueous solution have shown that approximately 100% of the ^{13}C atoms contribute to the C1 resonance at 100.5 ppm (7, 8). A study of relaxation times at different magnetic field strengths suggested that the presence of a short motional correlation time within the molecule ($\tau_c \sim 10^{-9}$ s) accounts for this high visibility (9, 10).

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In a recent study, we have shown in the rabbit that skeletal muscle glycogen is approximately 100% visible *in vivo* when the *in vivo* signal is compared with that from glycogen solutions (11). Such a comparison requires identical sensitive volumes *in vivo* and *in vitro* that were achieved by removing superficial tissue surgically and by controlling pulsing conditions with a reference sphere. We subsequently found that the NMR quantification correlated well with enzymatic assay of biopsied calf muscle of lean humans (12).

The earliest ^{13}C NMR experiments showing 100% visibility in solution also showed that the degradation of liver glycogen *in situ* gave a loss of signal proportional to the increase of glucose (7). From indirect *in vivo* evidence, it was suggested that the percentage of the NMR *visible* portion of the molecule does not vary in the liver under conditions of degradation or synthesis (13). However, the possibility remained that the C1 peak observed *in vivo* contained only a fraction of the carbon signal, because a direct comparison of the *in vivo* liver glycogen NMR signal with enzymatic assay has not been reported. This possibility has been supported by a recent study that implied a low visibility of 33% in fed liver and 100% in fasted liver (14), suggesting that glycogen visibility is concentration dependent. Such a concentration-dependent visibility has important implications for the measurement of net glycogen synthesis and breakdown rates by ^{13}C NMR (1).

In order to assess ^{13}C NMR visibility of the C1 glycogen resonance, we have compared the *in vivo* ^{13}C NMR quantification of glycogen with that by enzymatic determination over a wide range of glycogen concentrations in the rabbit liver.

MATERIALS AND METHODS

Animals

Seventeen New Zealand white rabbits (approximately 3.5 kg) were studied. Before the study, animals were fasted for approximately 40 h and then fed with a high carbohydrate diet (chow mixed with chocolate candy) for 18–24 h prior to the study in order to achieve a high liver glycogen content. On the day of the experiment, the animals were tranquilized with a subcutaneous injection of morphine (0.5 mg/kg) for transport from the animal care facilities to the MR Center. After exposure to a N_2O:O_2 plus isoflurane mixture applied via a face mask, an ear vein was cannulated and an α-blocker (phentolamine, 3 mg/kg) and a β-blocker (propranolol, 1 mg/kg) were quickly administered intravenously to suppress hepatic glycogenolysis. The animals were then tracheotomized and ventilated with a N_2O:O_2 plus isoflurane (0.5–2%)

mixture. Muscle movement was prevented by an intraperitoneal injection of curare (1 mg/kg) and pavulon (1 mg/kg).

The liver was exposed by an abdominal incision below the diaphragm and a sufficiently large area (approximately 5 cm diameter) was freed from any superficial muscle, fat, and skin. The rabbit was then positioned supine on a heating blanket (310 K) inside a semi-open Plexiglas® cylinder of 20 cm diameter. The subsequent fixation in this cylinder ensured that the liver surface made tight contact to the 2-mm thick Plexiglas® plate separating the sample from the coil. Movement during insertion into the magnet was thereby minimized. The transparent Plexiglas® plate separating the coil from the sample was screwed to the coil assembly which itself was tightly attached to the Plexiglas® cylinder.

NMR Measurements

NMR experiments were performed in a 4.7 T Bruker Biospec spectrometer with a 30-cm diameter bore. The RF probe was a 14-mm diameter ^{13}C double-turn coil with a concentric 40-mm diameter ^{1}H surface coil. A sealed external reference sphere of 8 mm diameter containing an aqueous solution of 99% enriched ^{13}C formic acid (Aldrich Chemical Co., Milwaukee, WI) was attached at the center of the inner carbon coil. The outer proton coil was used for shimming, proton decoupling, and imaging.

Characterization of the Sensitive Volume

The spatial extension of signal pickup (sensitive volume) of the carbon coil was identified using concentrated glycogen solutions (1100 m*M* glucosyl units) placed in suitable phantoms. The smaller coil was also tunable to the ^{23}Na resonance frequency, which was used to characterize the sensitive volume by gradient-echo recalled ^{23}Na imaging (echo time TE = 6 ms) of aqueous 50 m*M* NaCl solutions using the same pulse as for the glycogen measurements. Prior to tuning the coil to the sodium frequency, pulse power was adjusted at the carbon frequency on the formate sphere as for the ^{13}C NMR experiments (see below). The 5% increase in gyromagnetic ratio was compensated by a decrease in quality factor as measured on the bench with a spectrum analyzer. The ^{23}Na NMR NaCl imaging and ^{13}C NMR glycogen phantom solution experiments delineated the sensitive volume defined to contain more than 95% of total signal. Under the experimental conditions of this study, we found the signal to be within 9 mm depth, within ±10 mm along the magnet axis, and within ±14 mm laterally with the typical intensity falloff toward these boundaries.

In all studies presented in this paper, only liver tissue was present within this volume *in vivo*, as judged from multislice proton NMR images that were obtained in axial and saggital planes using heavily T_1-weighted gradient-echo imaging (15) with a repetition time (TR) of 60 ms and a TE of 6 ms. Further confirmation of the positioning was provided when the tissue was freeze clamped. Positioning was also verified by thorough visual inspections when the rabbit was inserted and removed from the magnet. These judgements were facilitated by a transparent Plexiglas® plate containing the carbon coil in a 28-mm diameter hole that contains thus the lateral dimensions of the sensitive volume.

In vitro ^{13}C NMR measurements were performed on several prewarmed aqueous solutions of oyster glycogen at 305–330 K (Sigma Chemical Co., St. Louis, MO) in 50 m*M* NaCl and 2 m*M* NaN_3. The preheated solutions were slowly poured into a 10-mm deep and 7-cm wide petri dish placed directly beneath the coil until the solution made contact with the separating Plexiglas® plate. This procedure ensured that the whole sensitive volume was filled with solution which was verified with proton imaging.

Natural Abundance ^{13}C NMR of Glycogen

All *in vivo* and *in vitro* ^{13}C NMR spectra of glycogen were collected as the sum of 3 × 3000 scans obtained with a 25-μs radio-frequency (RF) pulse (90°) applied 500 Hz downfield from glycogen C1 at a repetition time of 176 ms. RF power was adjusted to null the signal of the formate sphere with a 50 μs pulse applied on-resonance. Ten Watts WALTZ-16 proton decoupling (16) with a nominal 90° pulse width of 600 μs was used only during the acquisition time, which was set to 26 ms.

^{13}C NMR spectra were obtained by Fourier transformation of the free induction decays (FID) that were multiplied by a 20 Hz line-broadening exponential function and zero-filled to 216 ms. The C1 glycogen peak area was obtained by integrating from 98 to 104 ppm after correcting the baseline in the region from 80 to 120 ppm with a second-order polynomial. All spectral processing was performed with the spectrometer software DISNMR (Bruker, Karlsruhe, Germany). To eliminate effects of variable loading on the signal strength, integrated values of glycogen intensities were normalized to the total area of the undecoupled formate doublet obtained with one 25 μs (90°) pulse after a 60 s waiting time to ensure fully relaxed conditions. To minimize effects of minor spectrometer instability, these reference spectra (FA_i, FA_f) were obtained at the beginning and at the end of the ^{13}C NMR measurements of glycogen. The normalized NMR intensity was defined as I_{norm} according to Eq. [1],

$$I_{norm} = \frac{\int_{98}^{104} I_{decoupled}\, d\omega}{(FA_i + FA_f)/2}$$

Quantification of Glycogen C1

The above described determination of normalized glycogen C1 intensity I_{norm} (Eq. [1]) was calibrated using measurements of aqueous glycogen standard solutions obtained under experimental conditions identical with the *in vivo* measurements. The solution measurements were obtained on different days, which tested the stability of the method. The calibration curve was characterized by linear regression, with a resulting negligible y-intercept. The normalized *in vivo* intensity was converted to concentrations (μmol glucosyl units/ml liver vol) using the parameters of the linear regression. The resulting concentrations are equivalent to μmol glucosyl units/g wet weight, assuming a specific density of 1 g wet weight/ml liver volume.

Glucose peaks were observed in a number of *in vivo* spectra, consistent with the background tissue glucose. To reduce interference of the glycogen measurement from glucose signals *in vivo*, the integration bandwidth was from 98 to 104 ppm. In the solution measurements, a glycogen line width of 50–60 Hz was measured (including the 20 Hz line broadening). *In vivo*, the line width was slightly broader (70–80 Hz). We summed several spectra from six animals with low background glucose. Assuming that the integration from 94.5 to 106.5 ppm represented 100% intensity in both the *in vivo* and the *in vitro* measurements, integration of the *in vivo* C1 peak from 98 to 104 ppm was 83% of that intensity, and *in vitro* was 93%. The resulting correction factor of 1.1 was thus estimated from these values and *in vivo* NMR concentrations determined by integration over the limited region from 98 to 104 ppm were multiplied with this factor.

Enzymatic Determination of Liver Glycogen Content

Immediately after the NMR measurements, the tissue directly beneath the carbon coil (~4 g of tissue) was excised and quickly placed between two aluminium blocks precooled in liquid nitrogen and stored at −70°C. All tissue was analyzed within 4 weeks in a double-blinded fashion, i.e., by a different person that did not know the results of the previous NMR measurement. Liver glycogen content was determined in samples of 100–300 mg according to an established enzymatic assay procedure (17), as follows: immediately after being taken from the freezer and weighing, the tissue was homogenized at high speed in 5–15 parts/weight of ice-cold perchloric acid (0.6 mol/liter) using an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH). 0.1 ml $KHCO_3$ (1 mol/liter) was added to 0.2 ml homogenate in Eppendorf containers before 0.5 ml solution of amyloglucosidase (51 U/mg solid and 58 U/mg protein, 2 mg/ml) in acetate buffer (0.4 mol/liter, pH = 4.8) was added. The glycogen standard solutions were measured identically. Hydrolysis of glycogen was achieved by subsequent incubation at 313 K in a shaker bath for at least 2 h. After centrifugation, glucose concentration was determined in the supernatant of both the homogenate and the digested solution by the glucose oxidase technique using a Beckmann glucose analyzer (Beckmann, Fullerton, CA). All chemicals were purchased from Sigma Co., St. Louis, MO, unless otherwise stated.

Liver glycogen concentration was calculated from the glucose concentration of the digested solutions minus the concentration of the undigested tissue homogenates (background glucose). Intra-assay variability was 3% and inter-assay variability was 9.6%.

RESULTS

^{13}C NMR spectra were recorded in 17 rabbits and 14 aqueous glycogen solutions. An expansion of an *in vivo* spectrum of exposed rabbit liver is shown in Fig. 1. The C1 resonance of glycogen is at 100.5 ppm (resonance 2). The unfiltered line width of glycogen C1 was typically 60 Hz *in vivo*, corresponding to a T_2^* of 5 ms. The spectrum

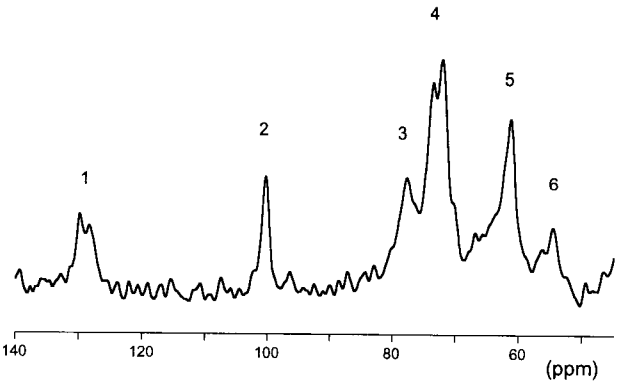

FIG. 1. Portion of an in vivo ^{13}C NMR spectrum of exposed rabbit liver. The spectrum (glycogen concentration was approximately 500 μmol glucosyl units/g wet weight) was obtained with a 90° pulse and a 14-mm diameter coil. Shown is the sum of 9000 scans obtained with WALTZ-16 proton decoupling (16) and a 176-ms repetition time. Processing included zero-filling, apodization with a mild gaussian and baseline correction with a fourth-order polynomial between 140 and 40 ppm. Peak assignments are according to those in refs. 7 and 18 (1) olefinic carbons of fatty acyl chains (128.4 and 130.0 ppm), (2) glycogen C1 (100.5 ppm), (3) glycogen C4 (78.0 ppm), (4) glycogen C3 (74.0 ppm), C2 and C5 (72.2 ppm) plus C2 of glycerol backbone (69.5 ppm), (5) glycogen C6 plus C1, C3 of glycerol backbone (61.4 ppm), (6) trimethylamine groups (e.g., choline, 54.6 ppm).

in Fig. 1 is the sum of three spectra that were acquired over a total time of ca. 30 min, during which the signals remained stable within the signal-to-noise for all *in vivo* and *in vitro* experiments. In one study, we added a measurement with an increased repetition time of 476 ms, corresponding to approximately $3T_1$ (9). The ratio of this spectrum to that obtained with the standard *TR* of 176 ms indicated that the saturation factor (which includes effects of T_1 and NOE when measured as here) *in vivo* was within a few percent of that measured in phantom solutions, i.e., indistinguishable with the current signal-to-noise.

To eliminate effects of variable loading on the signal strength, we normalized the area under the C1 peak to that of the formate sphere measured in separate reference spectra. Signal variations due to loading were approximately 15%. The normalized *in vivo* integral value I_{norm} (Eq. [1]) was calibrated versus the solution measurements shown in Fig. 2 (solid diamonds). The solid line was obtained by linear regression of all 14 measurements and has a *y*-intercept that is not different from zero (see legend of Fig. 2). The standard deviation of the NMR measurement was calculated from five measurements of the same glycogen solution (1055 m*M*) obtained on separate days and yielded an approximate standard deviation SD_{NMR} = 80 m*M*. The NMR error was increased by the low signal-to-noise resulting from the requirements to keep the signal entirely within the liver, as reflected by the choice of coil size and pulsing conditions. The standard deviation of the enzymatic determination was estimated similarly at SD_{assay} = 30 m*M*.

To account for differences in line width and possibly shape compared with the phantom solutions, *in vivo* NMR concentrations (determined by integration from 98

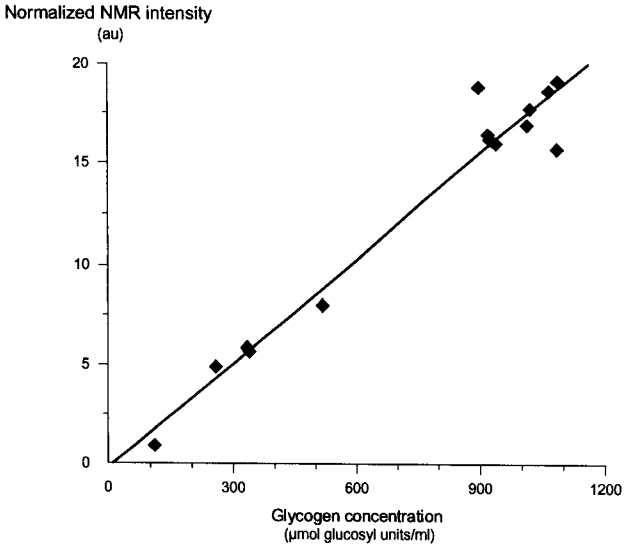

FIG. 2. Calibration of normalized glycogen C1 intensity (Eq. [1]) in aqueous glycogen solutions. NMR measurements consisting of 3 × 3000 scans were measured for each point. The solid line was obtained by fitting $y = ax + b$ to the 14 *in vitro* measurements yielding $a = 0.0174 \pm 0.0011$ and $b = -0.18 \pm 1.4$ ($r = 0.97$). The fit of $y = ax$ gave $a = 0.0172 \pm 0.0004$ ($r = 0.97$) which is not significantly different, as expected, because the NMR measurement as well as the chemical assay yield zero values in the absence of glycogen. Measurements were performed as described in the Materials and Methods section.

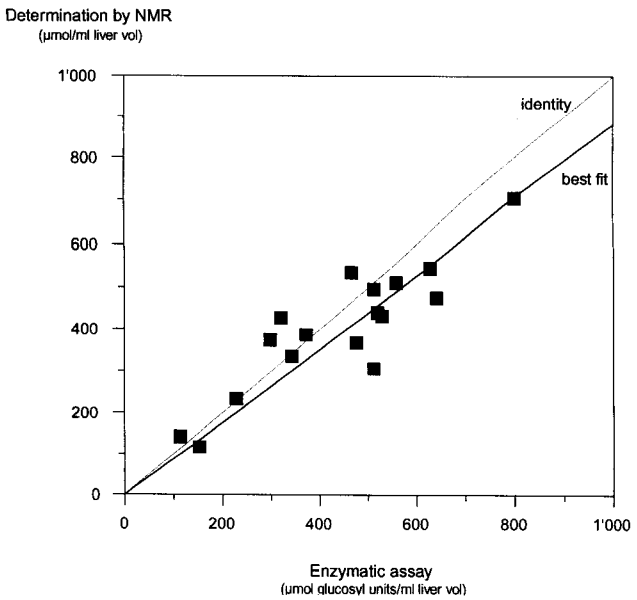

FIG. 3. Comparison of *in vivo* ^{13}C NMR determination of liver glycogen to that by enzymatic assay. The dotted line describes identity, i.e., the line where points fall in the case of perfect agreement of the two methods. Only two measurements were statistically significant different from the identity line (Fig. 4). The solid line describes the best fit ($y = ax$) which yielded a slope of 0.89 ± 0.04 ($r = 0.85$). The correlation was considered significant ($P < 0.001$) using Spearman's rank correlation test. The concentrations are expressed in μmol glucosyl units/ml liver vol.

to 104 ppm) were multiplied by the factor 1.1 derived as described in the Methods section to correct for the less complete integration bandwidth compared with the phantom spectra. The resulting *in vivo* NMR glycogen concentrations are plotted in Fig. 3 versus the enzymatically determined glycogen content (solid squares). The dotted line represents identity, i.e., perfect agreement between the two methods.

Table 1 lists the individual *in vivo* concentrations determined by NMR and by the chemical assay. The ratio is 0.95 ± 0.05 (mean standard error, $n = 17$), indicating close to 100% visibility. The difference of the concentrations derived by the two methods is plotted against the mean in Fig. 4 (solid squares). The differences do not exhibit a trend with glycogen concentration, as confirmed by linear regression ($r = 0.41$, $P > 0.05$ for correlation). The dashed line indicates the mean difference (37 μmol/g) and the dotted lines indicate mean ±2 SD of the data points (SD = 85 μmol/g). Note, that all points fall within these limits. The difference of 37 μmol/g is not statistically different from zero ($P > 0.05$). The shaded area indicates the 95% confidence interval assuming that the true difference is zero (solid line) and assuming an NMR measurement error of $SD_{NMR} = 80$ μmol/g assessed in solution as described above. Two points fall outside this range, indicated also in the Table (footnote b).

DISCUSSION

This study compared the ^{13}C NMR quantification of the *integrated* C1 resonance of liver glycogen *in vivo* to the enzymatic determination of glycogen content and found very good agreement between the two methods (Figs. 3 and 4, Table 1). The quantification procedure involved calibration of NMR intensity using external solutions whose glycogen concentrations were enzymatically measured as described in Materials and Methods. The excellent correlation of the two methods implies that the integrated *in vivo* signal equals the *in vitro* signal when measured with identical experimental parameters, such as flip angle distribution and repetition time, and when signal intensity variations due to loading were corrected in each study according to Eq. [1].

At the repetition time used, i.e., 176 ms, the signal of glycogen C1 is influenced by T_1 which is approximately 150 ms at 4.7 T (9). The signal-to-noise of the spectra (Fig. 1) generally did not allow long repetition times. However, on one occasion we measured the saturation factor *in vivo*, which equaled that measured in solution within a few percent. The similarity in saturation factors is supported by the observation that T_1 of hepatic glycogen measured *in vivo* was within 10% of the *in vitro* T_1 (9). In addition, relaxation studies suggested that T_1 of glycogen is determined mostly by fast internal motions of the molecule ($\tau_c \sim 10^{-9}$ s) and T_1 is therefore less likely to be affected by the overall motion (9). In fact, T_1 of liver glycogen in solution has recently been shown to be constant, when the particle size was increased (8).

Comparison of the *in vivo* signal with that from reference glycogen solutions depends strongly on identical sample volumes. Proton NMR imaging was used to verify that the sensitive volume of the carbon coil was filled

Table 1
Liver Glycogen Concentration Determined by *in Vivo* NMR and Enzymatic Assay[a]

Rabbit	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
NMR	702	433	117	307	477	428	441	369	336	536	546	496	512	375	233	142	387
Assay	800	529	152	512	641	320	520	476	343	466	628	512	558	298	228	113	372
Difference	−98	−96	−35	−205[b]	−164[b]	108	−79	−107	−7	70	−82	−16	−46	77	5	29	15

[a] All concentrations are in μmol/ml liver vol.
[b] Difference considered significant ($P < 0.05$) based on the estimated measurement errors (see caption of Fig. 4).

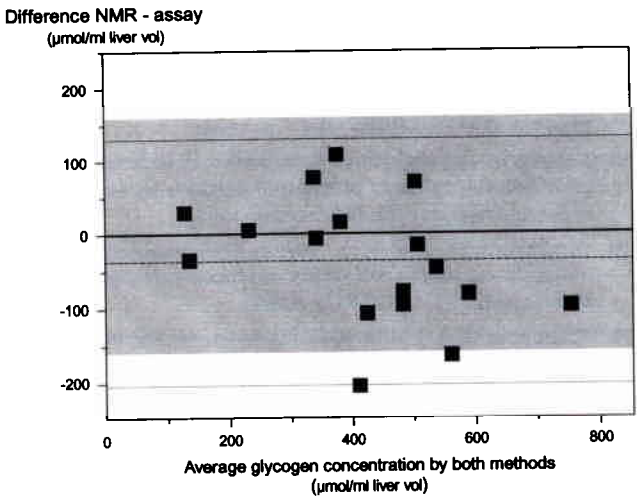

FIG. 4. Difference of the two methods against mean liver glycogen concentration. The ordinate shows the difference between NMR and biochemical measurement and the abscissa, the mean value. The dashed line indicates the average difference (37 μmol/ml liver vol), the dotted lines average ±2 SD (SD = 85 μmol/ml liver vol). The shaded area covers the 95% confidence interval of the measurements assuming perfect agreement of the methods, i.e., 0 ± 2 SD_{NMR} = 0 ± 160 μmol/ml liver vol. The units are equivalent to μmol glucosyl units/g wet weight liver assuming a specific density of 1 g/ml in liver.

entirely with liver tissue. This was possible because superficial nonliver tissue was surgically removed beforehand. We have used previously analogous procedures to determine *in vivo* glycogen visibility in rabbit muscle (11).

The error in NMR glycogen measurement was estimated from solution measurements on different days yielding SD_{NMR} = 80 m*M*, and only two differences fall outside ±2 SD_{NMR}, the rest being within ±1.35 SD_{NMR} (Table 1, Fig. 4). Most of the deviation from the identity line in Fig. 4 can thus be explained by the measurement accuracies involved.

Considering the size of the glycogen molecule (approximately 10^7 Da), it is conceivable that not all of the carbon atoms might contribute to the relatively narrow C1 resonance (T_2* approximately 5–10 ms). Several studies using natural abundance ^{13}C NMR have addressed the visibility question previously: An early study using liver glycogen solutions as well as excised liver from a rat fed ad libitum showed that the decrease in glycogen C1 intensity paralled the increase in glucose intensities when glycogenolysis was induced, suggesting that all of the atoms contribute to the narrow peak (7). A study of excised liver indicated that glycogen line widths narrow slightly with temperature *in situ*, yielding a fully visible signal at temperatures above 305 K (19). A recent *in vitro* study of liver glycogen excluded the presence of a very short T_2 component in solution (8). That study also reported a decrease in T_2 (approximately twofold) when the molecule size was increased, with T_1 remaining constant. These experiments together suggest that at *in vivo* temperature the liver glycogen line widths might vary slightly with molecular weight in a way that does not affect the measurement provided that sufficient integration limits are employed. The results of the present study support this.

Glycogen visibility has also been an issue in experiments using labeled glucose: An *in vivo* study in rat liver concluded that glycogen relaxation times of the labeled, visible peak do not change appreciably under conditions of net glycogen synthesis (14). A recent study of liver glycogen extracted from fed rats after [1,2-$^{13}C_2$] glucose administration noted a substantial change in isotopomer ratio when glycogen was hydrolyzed to glucose, suggesting substantial decreases in visibility when liver glycogen concentration was elevated (14). This change in isotopomer ratio was not observed in liver glycogen extracted from fasted rats.

The present study shows a very high visibility over a wide range of liver glycogen concentrations that is in agreement with one study using labeled glucose (16) and all of the aforementioned natural abundance studies (7, 8, 19). Observations of moderate changes in T_2 and line width of the C1 resonance have been reported (8, 19, 20) and are accommodated by our present integration limits. Simulations with Lorentzian line shapes showed that a twofold increase in line width results in less than 10% decrease in the integral over 300 Hz, used here. On the other hand, the integral over ±300 Hz is affected by less than 5%, which is within the given accuracy. It is conceivable that *in vivo* liver tissue contains glycogen molecules of different sizes with regions of different mobility. Hence, the *in vivo* peak shape may represent a distribution of moderately different line widths, which may be difficult to distinguish from a single line width considering the experimental signal-to-noise. With this possibility in mind, a careful choice in integration bandwidth must be made. In the present study, which was undertaken at 4.7 T, we made a 10% correction to extend the integration bandwidth to 600 Hz as described in Methods. Nevertheless, Fig. 3 indicates an excellent linear correlation ($P < 0.001$) between chemical glycogen measurement and the *in vivo* NMR determination, which clearly excludes the possibility of a drastic decrease in visibility with increasing concentration, as previously

suggested (14). At lower field strengths, such as 1.5 T, an integration bandwidth of less than 300 Hz may be necessary to avoid overlap with neighboring peaks. This reduced bandwidth may not accommodate all of the glycogen C1 intensity adequately.

When using labeled precursors, an incorporation of a few m*M* of label can produce a signal equivalent to the unlabeled bulk of the molecule, i.e., several 100 m*M*. If label is incorporated predominantly at a site in the glycogen molecule with different relaxation properties, the spectrum may not reflect the condition of the overall molecule. This may also explain some of the apparent discrepancy between this and the careful study by Künnecke and Seelig, who reported a concentration dependent visibility, with 33% visible in fed liver and approximately 100% in fasted liver (14).

The present range of concentrations studied (100–800 μmol glucosyl units/g wet weight) indicates a constant, very high visibility over the range of liver glycogen concentrations reported in humans, i.e., up to 620 μmol glucosyl units/g wet weight (1, 21). In the present study, interference of the measurement by superficial tissue was avoided by surgical retraction. Quantification of liver glycogen in humans and *intact* animals is more difficult because the superficial layer includes muscle tissue, which may contain variable amounts of glycogen. Experiments using a single pulse administered with the same surface coil used for detection will always detect surface signal. In humans, elimination of surface NMR signal is thus required, which has been done by using surface spoiling gradients (5) or by inversion of a slice on alternating scans (1).

CONCLUSIONS

We conclude that the C1 resonance of liver glycogen is close to 100% visible *in vivo*. We further conclude that ^{13}C NMR can provide noninvasive and reliable liver glycogen concentration measurements *in vivo* over the full range of physiological concentrations, when combined with suitable localization and integration schemes (1, 22).

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