

Direct observation of glycogen synthesis in human muscle with ^{13}C NMR

(glucose/insulin/diabetes)

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ABSTRACT On the basis of previous indirect measurements, skeletal muscle has been implicated as the major site of glucose uptake and it has been suggested that muscle glycogen formation is the dominant pathway. However, direct measurements of the rates of glycogen synthesis have not been possible by previous techniques. We have developed ^{13}C NMR methods to measure directly the rate of human muscle glycogen formation from infused, isotopically labeled $[1-^{13}\text{C}]\text{glucose}$. We show that under conditions of imposed hyperglycemia and hyperinsulinemia, a majority of the infused glucose was converted to muscle glycogen in a normal man. This directly shows that muscle is the major site of glucose disposal under these conditions, and provides quantitation of the glucose flux to muscle glycogen.

The maintenance of normal plasma glucose concentration after a glucose load depends upon three processes that occur simultaneously and in a coordinated fashion: (i) insulin secretion, (ii) suppression of hepatic glucose production, and (iii) glucose uptake by peripheral (muscle) and liver tissues (1). Arterial/venous difference measurements of liver and muscle have given evidence that the majority of the glucose load, whether given orally or intravenously, is disposed of by muscle (2-5). Once taken up by muscle, glucose can be either oxidized into CO_2 , stored in muscle as glycogen or fat, or released into the blood as lactate. Indirect measurements of O_2 consumption have shown that the majority of the glucose is not oxidized (1, 4, 6). Although it has been suggested that muscle glycogen is the primary storage compound, direct measurements of muscle glycogen formation in humans have yielded variable results (7-10). Even at whole-body glucose disposal rates above those likely to be encountered in everyday life, it has been difficult to demonstrate a significant increase in muscle glycogen concentration in response to insulin (9, 10). Until now quantitation of muscle glycogen synthesis has relied upon the ability to detect a small increment in the glycogen concentration in muscle biopsy samples obtained before and after an experimental maneuver. This has presented two problems. First, the expected increase in muscle glycogen concentration under physiologic conditions is less than the accuracy of currently available assays for the determination of unlabeled glycogen concentration. Second, the biopsy technique is invasive and repetitive sampling cannot be performed. This makes detailed kinetic studies impossible. In this report we show that ^{13}C nuclear magnetic resonance (NMR) spectroscopy can measure glycogen synthesis rates in a normal human with 15.5-min time resolution.

Although the glycogen molecule has a high molecular weight, it has been shown to be 100% ^{13}C NMR-visible (11,

12, 13). At the 1.1% ^{13}C natural abundance, ^{13}C NMR (14) has approximately the same or better accuracy for human muscle glycogen measurement as that claimed for the biopsy technique (7), but with the major advantages of being noninvasive, of being repeatable, and of averaging over a large volume of tissue. Previous ^{13}C NMR studies in animals have shown that ^{13}C -enriched precursors can be used to enhance the signal sensitivity of glycogen *in vivo* and thereby to improve the accuracy over unenriched ^{13}C spectra (15, 16). In the present experiment, $[1-^{13}\text{C}]\text{glucose}$ and ^{13}C NMR were used to measure the rate of synthesis of human muscle glycogen under hyperglycemic and hyperinsulinemic conditions.

^{13}C NMR spectra of human gastrocnemius muscle of a healthy 20-year-old man (height, 173 cm; weight, 70.5 kg) were collected on a 1-m-bore, 2.1-T Biospec NMR spectrometer. A $^1\text{H}/^{13}\text{C}$ concentric surface coil was used for both transmitting and receiving. The inner, ^{13}C coil was 8 cm in diameter; the outer, ^1H coil was 13 cm. Each spectrum required 15 min of signal accumulation and consisted of 11,250 scans.

The study began at 0800 following a 10-hr fast. Catheters were inserted into each antecubital vein for the infusion of test substances and blood withdrawal. After a 62-min baseline period during which four consecutive muscle glycogen signal accumulations were obtained from the natural-abundance ^{13}C nuclei, a hyperglycemic, hyperinsulinemic "clamp" was imposed (4, 17). At time zero a prime-continuous (1 milliunit per kg per min) infusion of insulin was administered to rapidly raise the plasma insulin concentration by approximately 70 microunits/ml above baseline for 180 min. At the same time a prime glucose infusion was given over 10 min to raise the plasma glucose concentration rapidly to 200 mg/dl. Thereafter, the plasma glucose concentration was measured every 5 min and a variable glucose infusion was adjusted to maintain the desired hyperglycemic level for 170 min. During the first 100 min all of the infused glucose was enriched to 8.7% with $[1-^{13}\text{C}]\text{glucose}$. At 100 min the enriched $[1-^{13}\text{C}]\text{glucose}$ was replaced with a glucose solution containing natural-abundance (1.1%) $[^{13}\text{C}]\text{glucose}$. Previous work (2, 3) showed that hepatic glucose production is suppressed under these hyperglycemic, hyperinsulinemic conditions.

Natural-abundance ^{13}C reference spectra were acquired before the infusion of glucose and insulin. A typical baseline spectrum is shown in Fig. 1, spectrum A. Within 15 min after the start of the insulin/glucose infusion, the C-1 glycogen signal began to increase. After 100 min, the glycogen signal had increased by a factor of 3 above the basal level (spectrum B). The spectral difference between the 100-min and baseline

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¶Hull, W. E., Zerfowski, M. & Bannasch, P., Sixth Annual Meeting of the Society of Magnetic Resonance in Medicine, 1987, New York, p. 488 (abstr.).

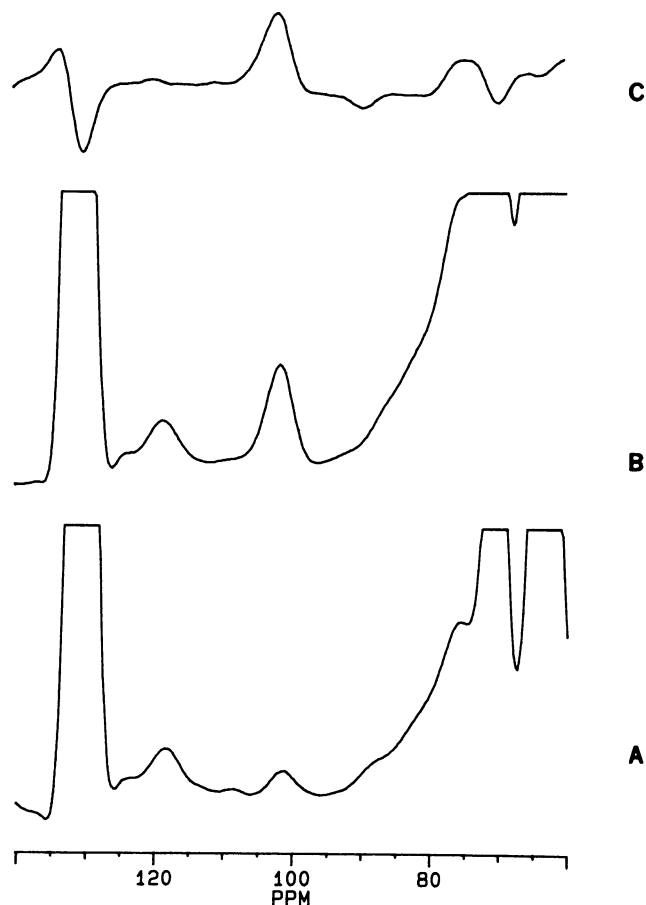


FIG. 1. Natural-abundance ^{13}C spectra were collected on a 1-m-bore, 2.1-T Biospec spectrometer. An 8-cm ^{13}C circular surface coil was used for both transmitting and receiving. A 13-cm concentric circular surface coil tuned to the ^1H frequency was used for decoupling. The coil was made from 99.9% pure copper wire (2.5 mm in diameter). At the coil center the loaded 90° pulse was $150\ \mu\text{sec}$ for ^1H and $120\ \text{sec}$ for ^{13}C . The rf transmitter power was 400 W. For single-frequency ^1H decoupling, 10 W was applied only during acquisition (20% duty cycle). The total heat deposition did not exceed the Food and Drug Administration guideline of 8 W/kg of tissue. The subject's right gastrocnemius muscle was placed atop the surface coil, separated by a 6-mm Lucite plate. Velcro straps anchored the leg in order to minimize motion during the experiment. The magnet was shimmed using the leg H_2O signal observed with the ^1H coil. ^{13}C spectra were acquired with a $1-t-1$ sequence, which was tailored to excite the 100-ppm region and to minimize the 30-ppm lipid region. Each pulse was $75\ \mu\text{sec}$. Interpulse delay was $320\ \mu\text{sec}$. The repetition time of 80 msec was optimized for the C-1 glycogen signal, which has relaxation times $T_1 = 240\ \text{msec}$ and $T_2 = 30\ \text{msec}$ (11). Each spectrum required 11,250 scans and 15 min of signal accumulation. The signal was then zero-filled to 4 K and apodized with a 40-Hz Gaussian-exponential function before Fourier transformation. Other spectral acquisition parameters were as previously reported (11). Spectrum A is a reference, natural-abundance ^{13}C spectrum of human gastrocnemius muscle. The C-1 glycogen signal appears prominently at 100.5 ppm. Spectrum B was obtained after 100 min of $[1-^{13}\text{C}]$ glucose/insulin infusion. The glycogen signal increased by a factor of 3.5. Spectrum C shows the difference between spectra A and B.

spectra is shown by spectrum C. Clearly the glycogen signal had increased substantially and the subtraction errors in the nearby spectral regions are close to noise, showing the high reproducibility of the signal. Fig. 2 shows a plot of the ^{13}C NMR signal amplitude of $[1-^{13}\text{C}]$ glycogen vs. time during the experimental period. A significant rise in muscle glycogen was first detected at 30 min, and thereafter the glycogen amplitude increased linearly until the end of the $[^{13}\text{C}]$ glucose

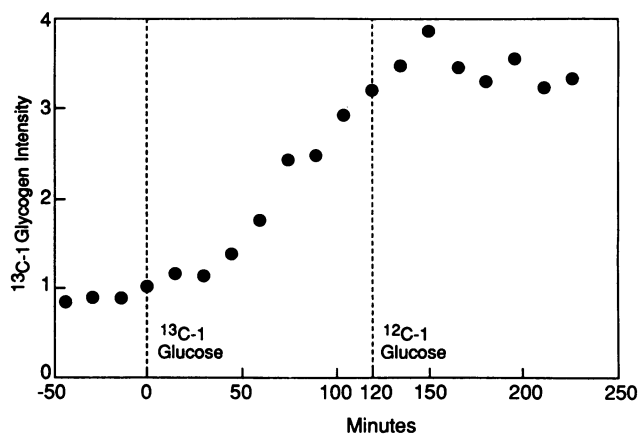


FIG. 2. Time course of change in the C-1 glycogen signal during combined glucose/insulin infusion. Three reference spectra were taken. Each spectrum required 15 min of accumulation. At time zero the $[1-^{13}\text{C}]$ glucose/insulin infusion was begun. At 30 min the C-1 glycogen signal was noticeably increased. Glycogen concentration continued to increase linearly until 100 min. After 100 min, the $[1-^{13}\text{C}]$ glucose infusion was switched to $[^{12}\text{C}]$ glucose (natural-abundance ^{13}C). No significant change in the glycogen signal intensity was observed from 120 min to the end of the ^{12}C infusion.

infusion at 100 min. After 100 min, when the infusate was switched to unenriched glucose, the $[^{13}\text{C}]$ glycogen peak continued to increase for approximately 20 min. From 120 to 170 min no significant change was observed in the C-1 glycogen signal intensity, indicating that no significant glycogen degradation occurred. This result demonstrates that the increase in the C-1 glycogen signal represents net synthesis rather than isotopic turnover. Additional studies should allow us to understand the control of phosphorylase activity under conditions of glycogen synthesis (18, 19).

From the NMR data, the rate of muscle glycogen synthesis during the time period between 45 and 90 min was calculated from the equation

$$R = K \times \frac{G_{90} - G_{45}}{G_0} \times \frac{1.1}{f} \times \frac{1}{45},$$

where G_{90} and G_{45} represent the signal intensities of glycogen at 90 and 45 min, times bracketing a period during which the rate of glycogen synthesis was constant; G_0 is the signal intensity of glycogen at time zero; and f is the percentage labeling of $[1-^{13}\text{C}]$ glucose in plasma from time 45 min to 90 min, which we measured to be 7.7%. This value of f was determined by using ^1H NMR at 360 MHz on four samples obtained at 45, 60, 75, and 90 min. The factor 1.1 is the percent natural abundance of ^{13}C , which was the degree of labeling in the basal glycogen, and K is the basal glycogen concentration.

The basal concentration (K) was calibrated by comparing the basal C-1 glycogen signal intensity against the signal obtained from a 4% (wt/vol) glycogen solution in 50 mM KCl in a cast shaped to match the leg. Any systematic error from this quantitation method will underestimate the actual glycogen concentration, since it assumes that 100% of the leg volume is muscle tissue. The reproducibility of the measurement has been established from repeated measurements on normal subjects to be within $\pm 10\%$ (14). For the subject in this experiment, the basal glycogen concentration, in glucosyl units, was determined to be $55\ \mu\text{mol/g}$ of wet weight, which is in the low range of values (mean $83\ \mu\text{mol/g}$, range $55\text{--}149\ \mu\text{mol/g}$) previously reported by Hultman (20) for biopsy samples obtained from normal subjects. By substituting $55\ \mu\text{mol/g}$ for K in the above equation, the muscle glycogen synthetic rate was calculated to be $0.21\ \mu\text{moles}$ of glucosyl units of glycogen per gram of muscle per minute.

During this period the glucose infusion rate needed to maintain the plasma glucose concentration at 200 mg/dl was constant at 89 μ moles per kilogram of body weight per minute. The fraction of infused glucose being stored as muscle glycogen can be calculated by multiplying the rate of glycogen synthesis per gram of muscle by the body muscle mass. Estimates of the fraction of body mass that is muscle in young men range from 38.8% by autopsy dissection (21) to 27.2% by neutron activation (12). Both measurements (12, 21) showed a wide range around these means. If one assumes that the first of these values (38.8%) is correct, it can be calculated that total body muscle stored 91% of the infused glucose as glycogen. A calculation using the second value for muscle mass (27.2%) indicates that 64% of the glucose is stored as muscle glycogen. Even with this large uncertainty in the body muscle mass, it is clear that under the present experimental conditions of hyperglycemia and hyperinsulinemia, most of the infused glucose is stored by the body as muscle glycogen. Hence, the basic physiological question about where the majority of an infused glucose load is stored is answered by the present measurements.

Looking ahead to the use of this method for studying pathological states, such as diabetes, it is clear that the ability to measure the rate of glycogen synthesis per gram of muscle tissue will provide an accurate method of distinguishing between normal and abnormal fluxes in this important insulin-regulated pathway. In addition to quantitating whole-body rates of glucose disposal, we can now focus upon quantitating the rate of glycogen formation in a clearly defined volume of muscle.

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