Glycogen Synthesis in Human Gastrocnemius Muscle Is Not Representative of Whole-Body Muscle Glycogen Synthesis

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The introduction of ^{13}C magnetic resonance spectroscopy (MRS) has enabled noninvasive measurement of muscle glycogen synthesis in humans. Conclusions based on measurements by the MRS technique assume that glucose metabolism in gastrocnemius muscle is representative for all skeletal muscles and thus can be extrapolated to whole-body muscle glucose metabolism. An alternative method to assess whole-body muscle glycogen synthesis is the use of [3-^{3}H]glucose. In the present study, we compared this method to the MRS technique, which is a well-validated technique for measuring muscle glycogen synthesis. Muscle glycogen synthesis was measured in the gastrocnemius muscle of six lean healthy subjects by MRS and by the isotope method during a hyperinsulinemic-euglycemic clamp. Mean muscle glycogen synthesis as measured by the isotope method was 115 ± 26 μmol · kg^{-1} muscle · min^{-1} vs. 178 ± 72 μmol · kg^{-1} muscle · min^{-1} ($P = 0.03$) measured by MRS. Glycogen synthesis rates measured by MRS exceeded 100% of glucose uptake in three of the six subjects. We conclude that glycogen synthesis rates measured in gastrocnemius muscle cannot be extrapolated to whole-body muscle glycogen synthesis. ***Diabetes*** **54:1277–1282, 2005**

Decreased insulin sensitivity is a key feature of type 2 diabetes. The exact pathophysiological mechanism underlying the defect in insulin action is still not fully understood. Under conditions of euglycemic hyperinsulinemia, glycogen synthesis accounts for 60–80% of glucose disposal in healthy subjects (1), whereas in insulin-resistant states, glycogen synthesis is decreased (2). Several mechanisms have been proposed to explain the decreased insulin-induced glycogen synthesis.

With the introduction of ^{13}C magnetic resonance spectroscopy (MRS) by Shulman and colleagues (2–4), it became possible to assess glycogen synthesis in humans in vivo. In 1999, Cline et al. (5) measured glycogen synthesis with ^{13}C MRS in the gastrocnemius muscle of patients with type 2 diabetes and healthy control subjects under hyperinsulinemic-hyperglycemic conditions. A decrease by 80% in glycogen synthesis was found in type 2 diabetic patients compared with control subjects. Because the intracellular glucose-6-phosphate concentration in muscle cells (as measured by ^{31}P MRS) was 1/25 of what would be expected if hexokinase was the rate-limiting step, it was concluded that the decrease in glycogen synthesis was caused by an impaired insulin-stimulated glucose transport. Up until now, this is the predominant view on insulin resistance. Conclusions drawn from ^{13}C MRS measurements are based on the assumption that glucose metabolism in gastrocnemius muscle is representative for muscle in general and thus can be extrapolated to whole-body muscle glucose metabolism.

In 1993, Rossetti et al. (6) introduced an alternative method to measure glycogen synthesis using [3-^{3}H]glucose. During glycolysis, [3-^{3}H]glucose loses its tritium atom completely to water. Another pathway of tritium loss may be during fructose-6-phosphate or pentose phosphate cycling. However, these cycles contribute for a minor part to glucose turnover (6). No significant label loss or recycling interferes with the results. The appearance of ^3H_2O in plasma therefore reflects the rate of whole-body glycolysis. Glycogen synthesis is then calculated by subtracting glycolysis from the rate of glucose disposal as measured during a hyperinsulinemic clamp. This method was validated by measuring the incorporation of [^{3}H]glucose in muscle glycogen in biopsies taken from the vastus lateralis muscle (6). To our knowledge, the indirect measurement of glycogen synthesis has never been validated by ^{13}C MRS, which is considered a well-validated technique for measuring human gastrocnemius muscle glycogen synthesis in vivo.

The aim of our study was to compare the results of whole-body muscle glycogen synthesis rates derived from the extrapolation of direct measured glycogen synthesis rates in gastrocnemius muscle by ^{13}C MRS with the results

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EGP, endogenous glucose production; GIR, glucose infusion rate; IMCL, intramyocellular lipid concentration; MRS, magnetic resonance spectroscopy; R_d, rate of glucose disposal.

of calculation of whole-body muscle glycogen synthesis rates measured by the isotope method introduced by Rossetti et al. (6).

RESEARCH DESIGN AND METHODS

We recruited six healthy nonobese young adults (three men and three women). They were not taking any medication except for oral contraceptives, had no family history of diabetes, and had a stable weight 3 months before the study. They did not perform any form of vigorous exercise.

All subjects were studied twice, serving as his or her own control. To rule out order effects, studies were done in balanced assignment. The ^{13}C MRS studies were performed in the University Medical Center Nijmegen, Nijmegen, the Netherlands, and the isotope studies were done in the Academic Medical Center in Amsterdam. The medical ethical committees of both hospitals approved the study protocol, and all participants gave written informed consent.

Measurement of glycogen synthesis by ^{13}C MRS (MRS study). The subjects attended the University Medical Centre Nijmegen for ^{13}C MRS after an overnight fast (14 h). Three days before the study, they consumed at least 250 g carbohydrates per day.

Insulin (100 kU Actrapid/l; Novo Nordisk, Alphen aan de Rijn, the Netherlands) infusion was started and continuously infused (60 mU · m^{-2} body surface area · min^{-1}) during the experimental protocol for at least 120 min. For the ^{13}C MRS study, glucose (20% wt/vol) in water was infused to maintain the plasma glucose concentration at 5 mmol/l. The glucose solution was 30% enriched at the C-1 position (Campro Scientific, Veenendaal, the Netherlands) to enrich plasma glucose with [1-^{13}C]glucose. Blood samples were obtained at 7.5-min intervals for measurement of plasma glucose concentration, at 15-min intervals for fractional plasma glucose ^{13}C enrichment or atom percentage excess, and at 60-min intervals for plasma insulin. Plasma glucose concentration was measured in duplicate by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman, Fullerton, CA).

During the measurements, the subjects were lying inside the magnet of the magnetic resonance spectrometer (1.5 T Magnetom Vision; Siemens, Erlangen, Germany) with the calf muscle of the right leg positioned on top of a custom-made radiofrequency coil. For ^{13}C magnetic resonance data acquisition, a concentric surface coil of 13 cm in diameter was used. For ^{1}H acquisition, decoupling, and shimming, a circularly polarized coil of 2 × 15 cm in diameter was used (7). ^{13}C magnetic resonance spectra were obtained in 7.5-min blocks consisting of 2,500 scans using an adiabatic pulse (2,560 μs length) and a repetition time of 180 ms. During the first 60 ms of the acquisition period, continuous wave decoupling at 26 W was applied, staying below the specific absorption rate safety limits (8). Increments in muscle glycogen concentration were calculated from the change in [1-^{13}C]glycogen integral and the plasma [1-^{13}C]glucose atom percentage excess. The rate of muscle glycogen synthesis was calculated from the slope of the least-squares linear fit to the glycogen concentration curve between t = 30–120 min and expressed in micromoles per kilogram muscle per minute.

Measurement of glycogen synthesis by radioisotopes and stable isotopes (isotope study). The subjects followed a diet with at least 250 g carbohydrates for 3 days before the study. They were admitted to the metabolic unit of the Academic Medical Centre of the University of Amsterdam and studied in the supine position. At 0900, after the subjects had fasted overnight (for 14 h), a catheter was inserted into an antecubital vein of each arm. One catheter was used to sample arterialized blood with use of a heated hand box (60°C). The other catheter was used to infuse [6,6-^{2}H$_2$]glucose, [3-^{3}H]glucose, a 20%-glucose solution, and insulin. After a blood sample was taken to measure the background enrichment and specific activity of plasma glucose, a primed continuous infusion of [6,6-^{2}H$_2$]glucose (>99% enriched; Cambridge Isotope Laboratories, Cambridge, MA) at a rate of 0.22 μmol · kg^{-1} · min^{-1} (prime: 17.6 μmol/kg) and a primed continuous infusion of [3-^{3}H]glucose (74 kBq/ml; Amersham Biosciences, Roosendaal, the Netherlands) at a rate of 0.0032 μCi · kg^{-1} · min^{-1} (prime: 0.4 μCi/kg) was started (T = 0). At the same time, insulin infusion (100 kU Actrapid/l; Novo Nordisk) was started at a rate of 60 mU · m^{-2} body surface area · min^{-1}. After 60 min, blood samples were drawn for 2 h at 10-min intervals for measurement of specific activity of plasma glucose and H_2O to calculate glycolysis. Between 160 and 200 min, a blood sample was drawn every 10 min for measurement of isotopic enrichment to calculate rate of glucose disposal. Plasma glucose concentrations were measured every 5 min with a Beckman Glucose Analyzer II, and the 20%-glucose solution was infused at a variable rate to maintain euglycemia at 5.0 mmol/l. [6,6-^{2}H$_2$]glucose and [3-^{3}H]glucose were added to the 20%-glucose infusate to approximate the values for enrichment and specific activity reached in plasma to prevent negative rate of glucose disposal (R_d) artifacts during the clamp. After 60 and 180 min, blood samples were drawn for measurement of insulin. During the study, subjects were allowed to drink water only.

Whole-body composition, appendicular fat, and lean body mass. Whole-body composition was measured with dual-energy X-ray absorptiometry using a total-body scanner (model QDR 4500 W; Hologic, Waltham, MA). This scanner produces two X-ray beams at 100 and 140 kVp. After passing through the body, the attenuated beams are detected by multiple detectors. Attenuation of the two beams depends on mass and type of tissue. Based on regional attenuation, bone mineral content, total fat mass, total body lean mass (i.e., fat-free mass), and lean mass of legs and arms were calculated (9). Appendicular lean mass (i.e., muscle mass in the extremities) was calculated from the sum of the lean mass of arms and legs (10) after subtraction of bone mass. Muscle mass in the extremities was assumed to represent total muscle mass.

Total body water was estimated as 60% of total body weight in men and 50% of total body weight in women (11).

Analytical procedures

Assessment of ^{13}C fractional enrichment. Blood plasma glucose enrichment levels were measured using high-resolution proton nuclear magnetic resonance (11.7 T). Preparation of plasma samples before nuclear magnetic resonance consisted of deproteinization by centrifugation for 1 h at 3,000*g* over a 10-kDa filter (Sartorius, Göttingen, Germany). From the filtrate, 500 μl was taken, and 20 μl D_2O with 2,2,3,3-tetradeuteropropionic acid as internal standard was added. Proton spectra were recorded on an AMX-500 spectrometer (Bruker, Karlsruhe, Germany). The number of averages was 128, and a repetition time of 10 s was used. Spectra were analyzed using WIN-MR software (Bruker). Fractional enrichment was calculated from the ratios of the Lorentzian fitted signals of the proton attached to [^{12}C/^{13}C-1]glucose.

Gas chromatography–mass spectrometry. Plasma samples for glucose enrichment of [6,6-^{2}H$_2$]glucose were deproteinized with methanol. The aldonitril pentaacetate derivative of glucose was injected into a gas chromatograph–mass spectrometer system (HP 6890 series II gas chromatograph equipped with a split-splitless injector and an HP 5973 model mass selective detector; Hewlett-Packard, Palo Alto, CA). Separation was achieved on a DB17 column (30 m × 0.25 mm, film thickness of 0.25 μm; J&W Scientific, Folsom, CA). Glucose was monitored at mass-to-charge ratios of 187, 188, and 189. Within each series, three control samples with known enrichments were measured for quality control. Glucose enrichments were calculated by dividing the area of the mass-to-charge 189 peak by that of the 187 peak (M2:M0) and correction for natural enrichments.

[3-^{3}H]glucose and $^{3}H_2O$. Titriated water and [3-^{3}H]glucose were measured as previously described by Rossetti et al. (6).

Insulin. The plasma insulin concentration was determined by radioimmunoassay (Insulin RIA 100; Pharmacia Diagnostic, Uppsala, Sweden) with an intra-assay coefficient of variation (CV) of 3–5%, an interassay CV of 6–9%, and a detection limit of 15 pmol/l.

Calculations and statistical analysis

Glycogen synthesis by MRS. The rate of glycogen synthesis was calculated from the slope of the increase of glycogen obtained by linear regression from increments in glycogen concentration as derived by Shulman et al. (2):

$$\Delta[\mathrm{Gly}, t] = [A_{\mathrm{gly},\,t} - A_{\mathrm{gly},\,T})/A_{\mathrm{gly},\,0}] \times \{1.1 \times [\mathrm{Gly}_0]/\mathrm{FE}(t)\}$$

with Δ[Gly, t] = glycogen concentration increment at time t. The increment is calculated from the data point at time T to the next data point at time t. $A_{\mathrm{gly},\,t}$ or $_T$ or $_0$ are the resonance areas of the glycogen C-1 signal at $t = t$, $t = T$, or $t = 0$. [Gly$_0$] = concentration of glycogen at $t = 0$, and FE (t) = fractional enrichment at time t.

For the quantification of glycogen ([Gly$_0$]), a phantom containing 100 mmol/l glycogen (rabbit liver glycogen; Sigma, St. Louis, MO), 50 mmol/l potassium chloride, 40 mmol/l creatinine, and 0.02% sodium azide was used according to a previously described method (12). Differences in volumes seen by the ^{13}C coil were determined by integration of the B1 profile of the ^{13}C coil over the segmented volume of both the entire phantom (V_{phantom}) and the skeletal muscle (V_{muscle}). Segmentation was performed on T1 weighted magnetic resonance images acquired with the ^{1}H coil (3D FLASH sequence [Tr/TE 8.1/4.0 ms], FoV 200 × 200 mm^2, slice thickness 5 mm). Corrections for differences in coil-loading were determined by using the acquired ^{13}C signal from a 5-ml reference phantom (10 mmol/l 100% ^{13}C-labeled aceton) at a fixed position inside the ^{13}C coil. The absolute glycogen content could be estimated from the following:

$$[\mathrm{Gly}_0] = \frac{A_{\mathrm{muscle,\,glycogen}} \times A_{\mathrm{aceton,\,phantom}} \times V_{\mathrm{phantom}} \times \mathrm{Gly}_{\mathrm{phantom}}}{A_{\mathrm{phantom,\,glycogen}} \times V_{\mathrm{muscle}} \times A_{\mathrm{aceton,\,muscle}}}$$

with A_{glycogen} being the glycogen resonance area at 100.5 ppm signal, A_{aceton} being the area of the aceton resonance at 200 ppm during muscle or phantom measurement, V_{muscle} being the segmented volume of the muscle area visible by the coil, and $\mathrm{Gly}_{\mathrm{phantom}}$ being the glycogen concentration in the phantom.

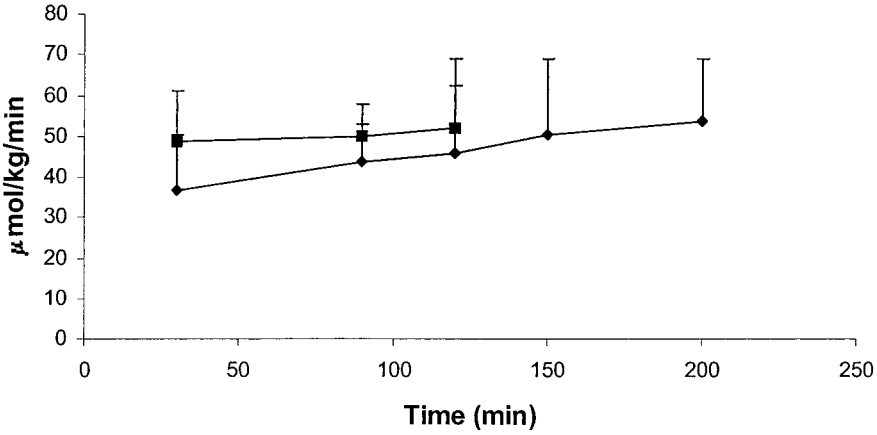

FIG. 1. Mean GIRs (*M* value) during the hyperinsulinemic-euglycemic clamp to maintain plasma glucose at 5 mmol/l. ◆, *M* value isotope study; ■, *M* value MRS study.

The glucose infusion rate (GIR) to maintain euglycemia during the clamp is expressed as the *M* value.

Glycogen synthesis by radioisotopes and stable isotopes. When R_d is calculated, the added source of labeled glucose entering the system and the exogenous glucose infusate should be taken into account. Thus, R_d was calculated using Steele equations for non–steady-state conditions adapted for stable isotopes (13). Reported R_d values represent the mean values from 60 to 120 min in the MRS study and from 160 to 200 min in the isotope study after the insulin infusion began. Glycolysis was calculated as described by Rossetti et al. (6). The slope of the linear regression line of plasma ^3H_2O concentration in time multiplied by the body water volume estimates the whole-body ^3H_2O production rate. Figure 3 shows a representative example of the regression line of plasma ^3H_2O concentration in time. The rate of glycolysis was obtained by dividing the ^3H_2O production rate by the specific activity of [3-^{3}H]glucose. Glycogen synthesis was then obtained by subtracting glycolysis from R_d and dividing glycogen synthesis by the muscle mass, measured by dual-energy X-ray absorptiometry and multiplying it by body weight.

We used two tracers for our calculations ([3-^{3}H]glucose to measure glycolysis and [6,6-$^2H_2$]glucose to measure R_d) because, in the literature, an underestimation of glucose turnover is reported because of [3-^{3}H] recycling (14), and the stable isotope tracer method is too insensitive to measure enrichment in body water (pilot study; data not shown).

Gas chromatography–mass spectrometry was used in our study to determine isotopic enrichment, and therefore recycled glucose (M+1) is excluded from the tracer-to-tracee ratio measurements, thereby not underestimating the true flux. All flux rates were expressed as micromoles per kilogram per minute, except for glycogen synthesis (which was expressed as micromoles per kilogram muscle per minute). All data are means ± SD.

Statistical analysis was assessed with Student's *t* tests, where $P < 0.05$ was defined as statistically significant.

RESULTS

We included six healthy young adults. Their characteristics are shown in Table 1. Mean muscle mass was 22.4 ± 5.7 kg or 32.8% (range 24–42) of body weight. The volunteers were all studied after an overnight fast of 14 h. On both occasions, they were admitted on the day of the study. Fasting insulin concentrations were all low, indicating a comparable fasting state on both occasions. The fasting insulin concentrations in the first and second study were not different (23 ± 8.8 pmol/l in the isotope study and 27 ± 13.8 pmol/l in the MRS study, $P = 0.63$). Furthermore, the GIRs in both studies were the same, indicating that there was no endogenous glucose absorption from the gut, because this would result in much more unpredictable and unstable GIRs.

TABLE 1
Characteristics of study participants

Men (*n*)	3
Women (*n*)	3
Age (years)	23.5 (19–31)
Weight (kg)	67.5 ± 7.7
BMI (kg/m^2)	22.3 ± 1.2
Fat-free mass (kg)	50 ± 9.8
Muscle mass (kg)	22.4 ± 5.7
Muscle mass (% of weight)	32.8 ± 5.8 (24–42)

Data are means ± SD except for age and muscle mass (range).

The R_d from 160 to 200 min in the isotope study (55 ± 17 μmol · kg^{-1} · min^{-1}) was equal to the GIR (53.8 ± 15 μmol · kg^{-1} · min^{-1}, $P = 0.84$), indicating that endogenous glucose production (EGP) was completely suppressed. The R_d in the MRS study from 60 to 120 min was 57 ± 18 μmol · kg^{-1} · min^{-1} and the *M* value was 52 ± 17 μmol · kg^{-1} · min^{-1} ($P = 0.04$), which indicates an underestimation of the R_d using the *M* value (from 30 to 60 min) by ~8%. However, the GIR (Fig. 1) to maintain euglycemia in the MRS study did not change from 30 min onward (8.76 ± 2.24 mg · kg^{-1} · min^{-1} from 30 to 60 min, 9 ± 0.5 mg · kg^{-1} · min^{-1} from 60 to 90 min [$P = 0.58$], and 9 ± 2.98 mg · kg^{-1} · min^{-1} from 90 to 120 min [$P = 0.2$ and $P = 0.78$, respectively]), indicating that also between 30 and 60 min after starting the insulin infusion, EGP contributed maximally 8% to the R_d. The rates of disposal of glucose in the isotope study and in the MRS study were not significantly different (55 ± 17 and 57 ± 18 μmol · kg^{-1} · min^{-1}, respectively; Table 2).

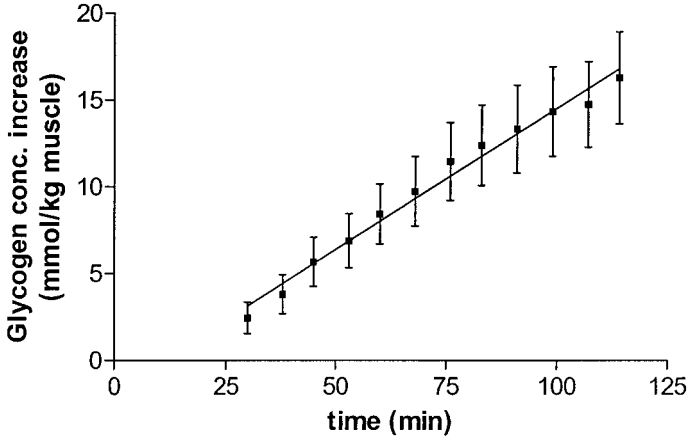

FIG. 2. Glycogen concentration increase (mmol/kg muscle), corrected for fractional enrichment, as a function of time during the euglycemic-hyperinsulinemic clamp. Each data point is an average of six individual data points grouped from measurement times that may vary in time within 3 min from the indicated time points. The error bars indicate SE. The straight line is the result of the least-squares fit through the averaged data points ($R^2 = 0.983$).

TABLE 2
Parameter values of euglycemic-hyperinsulinemic clamp conditions

	Isotope study	MRS	*P*
Plasma insulin (pmol/l)	588 ± 137	678 ± 124	0.07
Plasma glucose (mmol/l)	4.75 ± 0.16	5.0 ± 0.46	NS
R_d (μmol · kg^{-1} · min^{-1})	55 ± 17	57 ± 18	NS
M value (μmol · kg^{-1} · min^{-1})	53.8 ± 15	52 ± 17	NS
Glycolysis (μmol · kg^{-1} · min^{-1})	16 ± 6	—	—

Data are means ± SD. *M* value is the glucose infusion rate to maintain euglycemia.

Mean insulin-induced glycogen synthesis as measured by stable and radioactive isotopes was 115 ± 26 μmol · kg^{-1} muscle · min^{-1} (Table 3).

Glycogen synthesis rate was measured by assessing the glycogen C-1 signal by ^{13}C MRS between 30 and 120 min after the start of infusion of [^{13}C-1]glucose as described earlier (2,15). In all subjects, glycogen increased virtually linearly over this time period, as demonstrated in Fig. 2 for the average time-dependent glycogen concentration increases. The calculation of the individual glycogen synthesis rates was done from the slope of a linear regression line through the glycogen concentration data of each examination. Insulin-stimulated glycogen synthesis in gastrocnemius muscle measured in this way by ^{13}C MRS was 178 ± 72 μmol · kg^{-1} muscle · min^{-1} ($P = 0.03$ vs. isotope study) (Table 3). The glycogen synthesis rate, expressed as the percentage of R_d in the isotope study, was 69 ± 7% (range 58–78), and in the MRS study, it was 103 ± 39% (range 42–149) with three subjects exceeding 100% of glucose uptake ($P = 0.06$) (Table 3).

DISCUSSION

This study shows that measurements of insulin-induced glycogen synthesis rates in human skeletal muscle by isotope methods or by ^{13}C MRS yield different results. This conclusion is based on the finding that glycogen synthesis differed significantly despite comparable whole-body glucose disposal and plasma insulin concentration in the two studies. Whole-body glycogen synthesis expressed as the percentage of the rate of glucose disposal and extrapolated from ^{13}C MRS gastrocnemius muscle glycogen synthesis exceeded 100% in three of the six subjects.

TABLE 3
Comparison of glycogen synthesis rates measured by the isotope and the ^{13}C MRS approach

	Glycogen synthesis			
	Isotope study		MRS study	
	μmol · kg^{-1} muscle · min^{-1}	% of R_d	μmol · kg^{-1} muscle · min^{-1}	% of R_d
Subject				
1	127	78	253	137
2	127	75	214	115
3	74	58	46	42
4	140	64	191	90
5	132	73	204	149
6	92	68	158	83
Means ± SD	115 ± 26	69 ± 7	178 ± 72*	103 ± 39

*$P = 0.03$ vs. mean glycogen synthesis rate in the isotope study.

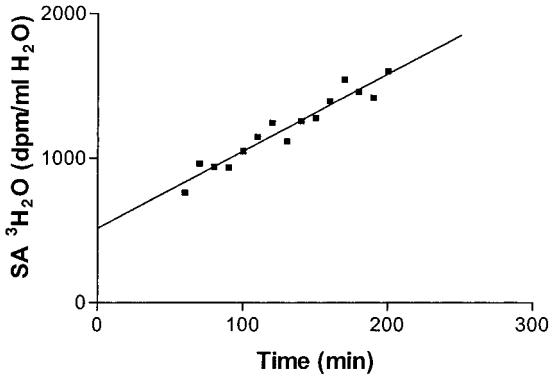

FIG. 3. Increase in ^3H_2O in plasma during infusion of tritiated glucose. SA, specific activity. $R^2 = 0.93$.

Glycogen synthesis rate assessed by radioisotopes and stable isotopes was 69% (range 58–78) of whole-body glucose uptake in all subjects.

Studies on the regulation of muscle glycogen synthesis measured by MRS showed independent influences by plasma insulin and plasma glucose. The glycogen synthesis rates measured by ^{13}C MRS are comparable with rates reported in the literature (2,5,15–19).

Glycogen synthesis rate assessed by radioisotope methods was validated by Rossetti et al. (6). Glycogen synthesis expressed as the percentage of R_d was 69% in our study and 51% in Rossetti's study.

This difference can be explained by differences in study design, with ~50% higher insulin concentrations in our study. After glucose is taken up by skeletal muscle, it can either be oxidized or converted to glycogen. Glycogen synthesis under hyperinsulinemic conditions accounts for 60–80% of disposed glucose (1). This corresponds well with our present findings of ~70% of R_d. The R_d in the MRS study was calculated from 60 min onward after achieving isotopic steady-state. The *M* value during that period was ~8% lower than the calculated R_d, meaning that EGP contributed by ~8% to the R_d. Glycogen synthesis rates were measured between 30 and 120 min. The GIRs to maintain euglycemia in the MRS study were stable after 30 min as described in RESULTS. Therefore, we concluded that, already after 30 min, EGP was almost completely suppressed and that the *M* value between 30 and 60 min can be used as a reliable representative of peripheral glucose uptake. This is in accordance with the literature on this subject (20). Glycogen synthesis rate during hyperinsulinemia is stable, at least from 30 min to over 120 min onward, as found in all studies that have applied measurements of glycogen synthesis rate using ^{13}C MRS (2,15,21).

Both fluxes (glycogen synthesis and glycolysis) depend on glucose uptake, represented in our study by the GIRs and R_d. The percentage of disposed glucose being oxidized or stored as glycogen is probably not changing within 2 h of stable hyperinsulinemic-euglycemic conditions, making a further increase in glycolysis after 60 min of insulin infusion unlikely. If there was a further insulin-stimulated increase in glycolysis after 60 min, the percentage of glycolysis from R_d would be greater and glycogen synthesis less than our reported 70% of R_d. This would make the difference between the two experiments even more obvious.

Although muscle mass is an important determinant in

calculation of glycogen synthesis rate, it is mostly estimated and not measured. Sometimes rather low percentages are chosen, resulting in a potential underestimation of the real flux (2). In our MRS study with glycogen synthesis expressed as a percentage of whole-body glucose uptake, it exceeded 100% in three of the six subjects studied. Shulman et al. (2) estimated the muscle mass in his subjects at 26%. Muscle mass, however, can be quite variable, even in lean nontrained subjects, as we obtained an average value of 33% with a range of 24–42%. Earlier studies on body composition showed a muscle mass in nonobese subjects ranging between 26 and 45% (22,23). Applying a percentage of muscle mass >26% of body weight, mean glycogen synthesis rates >100% of whole-body glucose uptake are found (2,16). Our assumption that extremity skeletal muscle mass represents total muscle mass may cause an underestimation of total muscle mass. This means that glycogen synthesis rate in the isotope study would be lower and in the MRS study would be higher if total muscle mass was used, leading to a greater difference between the two methods.

Apparently, depending on the design of the study and the conditions under which the clamp has been performed, glycogen synthesis rates can differ from 68% to >100% of whole-body glucose uptake. Murphy and Hellerstein (24) earlier addressed this issue while comparing methods of flux measurements and came to comparable conclusions. Possible explanations for this finding can be either related to the MRS technique itself or to the use of the gastrocnemius muscle as reference muscle. Although there is some discussion about the full visibility of glycogen using ^{13}C MRS (24), in particular for the liver, several studies have demonstrated that glycogen in skeletal muscle is fully visible by this method, and a close correlation was found between glycogen content in human gastrocnemius muscle measured by either MRS or biopsy (25,26). Assuming that the rates we found measured with ^{13}C MRS are accurate, the only explanation for the high glycogen synthesis rate (>100% of R_d) is that glycogen synthesis measured in gastrocnemius muscle is higher than in other skeletal muscles. If so, it follows that measurements in gastrocnemius muscle are not representative for glycogen synthesis rate of body muscle mass in general.

The question now rises by what physiological properties of the different skeletal muscles glycogen synthesis is influenced? Skeletal muscles consist of different types of muscle fibers. Type 1 fibers have a high oxidative capacity and a higher capillary density than type 2b fibers, which are characterized by a lower oxidative capacity but higher glycolytic capacity (27). Sensitivity for insulin also differs between the fiber types as many different studies indicate (28–33).

Therefore, the fact that different muscles differ in fiber type composition may explain differences in insulin-induced glycogen synthesis rate between different muscle compartments.

Another potential variable is the positive relationship between tonicity and GLUT4 protein levels in muscle (16,34). Gastrocnemius muscle is constantly active while standing (high tonicity). The difference between the glycogen synthesis rates found in the untrained (75% of glucose uptake, physiologically possible) and trained (107% of glucose uptake, physiologically impossible) gastrocnemius muscle (16) illustrates the impossibility of extrapolating the flux rates of an individual muscle group to whole-body muscle mass with a wide spectrum of muscle fiber types. Fiber type spectrum of whole-body muscle is not a constant characteristic but depends on physical activity and BMI.

A third issue that may affect glycogen synthesis rate is the intramyocellular lipid concentration (IMCL) in different skeletal muscles, which is thought to influence skeletal muscle insulin sensitivity. Indeed, an inverse correlation between IMCL in calf muscle measured with ^{1}H MRS and peripheral glucose uptake was observed (35). However, this correlation is probably not the same for all lower leg muscles.

Our conclusion that muscle groups are not representative for skeletal muscle in general is supported by a recent report on muscle-type specific lipid metabolism in rats (36). Neumann-Haefelin et al. (36) reported a muscle-type specific coping with starvation-induced elevated free fatty acid levels. IMCL in soleus muscle (i.e., oxidative muscle) remained constant but increased in tibialis anterior muscle (i.e., glycolytic muscle).

In conclusion, we found a significantly different glycogen synthesis rate when measuring with stable and radioactive isotopes compared with ^{13}C MRS. ^{13}C MRS calculated whole-body glycogen synthesis rates were >100% of glucose uptake in three of the six subjects. The most likely explanation for this finding is that glycogen synthesis rate in gastrocnemius muscle is higher than that in other skeletal muscles, and therefore measurements in gastrocnemius muscle cannot be extrapolated to whole-body muscle flux rates. The differences in flux rates between muscle groups may be determined by muscle fiber type, tonicity, and probably also by IMCL content. The effects of these latter factors on insulin sensitivity of different skeletal muscles are probably not of the same magnitude for each muscle. Glycogen synthesis rate in gastrocnemius muscle is thus not always representative of whole-body muscle insulin sensitivity, and one should be aware of this problem when evaluating potentially beneficial effects of different interventions on insulin sensitivity. The beneficial effect could only be true for the muscle under investigation. Future studies to measure glycogen synthesis in two different skeletal muscles simultaneously using ^{13}C MRS are needed.

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