Insulin-dependent glucose uptake in muscle has been shown to be reduced in non-insulin-dependent diabetes mellitus (NIDDM) under conditions of high plasma glucose and insulin. Although indirect measurements had suggested that the defect in glucose uptake was caused by impaired storage [1, 2], presumably as muscle glycogen, biopsy measurements of glycogen were not sensitive enough to detect the predicted small changes in concentration. Recently, we have used $^{13}$C n.m.r. spectroscopy in conjunction with [1-$^{13}$C]glucose infusion to measure glycogen synthesis in muscle in normal subjects and subjects with NIDDM under the same levels of plasma glucose ($\sim 10$ mmol/l) and insulin ($\sim 400$ pmol/l) [3]. These levels were chosen to simulate plasma conditions shortly after a meal.

Five non-obese men with NIDDM and six healthy age/weight-matched men were studied. On the evening before the study all the diabetic subjects received a variable infusion of insulin to achieve normoglycaemia ($\sim 5.5$ mmol/l), so that the normal and diabetic subjects had the same glucose concentration at the start of the study. At 08.00 h, after a 10–12 h fast, hyperglycaemic hyperinsulinaemia was induced with the insulin–glucose–clamp technique [4]. Insulin was administered in a priming and continuous infusion to raise and maintain plasma insulin concentration at approximately 400 pmol/l. Plasma glucose concentration was rapidly raised and maintained at 10 mmol/l, with a variable infusion of [1-$^{13}$C]glucose of 20% enrichment. Indirect calorimetry was performed at 40–60 min and 100–120 min during the infusion to determine the rate of whole-body glucose oxidation. The rate of non-oxidative glucose metabolism was determined by subtracting the rate of glucose oxidation from the rate at which glucose was infused. Hepatic glucose production was assumed to be suppressed by the high levels of plasma insulin and glucose.

During the infusion the concentration of the [1-$^{13}$C]glucosyl units of muscle glycogen was measured using $^{13}$C n.m.r. In previous studies we had shown that $^{13}$C n.m.r. can measure the $1.1\%$ $^{13}$C-labelled fraction of glycogen in human muscle with an accuracy similar to biopsy techniques [5]. The use of [1-$^{13}$C]glucose allowed the increment of muscle-glycogen concentration during the infusion to be measured with an accuracy of better than 1 mmol/l in 15 min. The n.m.r. measurements were performed on a 2.1 T, 1 m bore, Biospec spectrometer, that gave a resonant frequency for $^{13}$C n.m.r. of 22.49 mHz. The gastrocnemius muscle of the right leg was placed over a $^1$H–$^{13}$C concentric-surface coil. A gradient-refocused surface-coil image was obtained to confirm that the majority of the coil-sensitive volume was occupied by muscle. For $^{13}$C n.m.r. spectra a pulse-repetition time of 80 ms was used ($\sim 1t_I$ for glycogen) to optimize sensitivity without making the signal strength highly sensitive to $t_I$. Decoupling was applied during the 17 ms acquisition time at 10 W at the $^1$H frequency of 89.43 mHz. Tissue radio frequency (rf) power deposition owing to decoupling was estimated, using a magnetic-vector-potential model, to be less than 4 W/kg. The concentration of the [1-$^{13}$C]-glucosyl units of muscle glycogen in each subject was determined by comparing the n.m.r. signal intensity with the signal obtained from a orthoplast cast of the subject’s calf containing a 4% (w/v) solution of glycogen and 50 mM KCl. A 2 cm sphere containing [13C]formate was placed at the coil centre to act as an intensity standard and to allow pulse-angle calibration. The rate of glycogen synthesis was calculated by dividing the measured increment of [13C]glycogen concentration between spectra by the plasma [1-$^{13}$C]glucose fractional enrichment during the time of the measurement. The plasma glucose fractional enrichment was determined by $^1$H n.m.r. at 360 mHz and by g.c.–m.s. from plasma samples obtained every 20–30 min.

The basal level of glycogen was significantly lower in the diabetic (39 ± 6 mmol l$^{-1}$, mean ± s.e.m.) than the normal subjects (73 ± 11 mmol l$^{-1}$) in muscle. A similar reduction in basal-muscle-glycogen concentration in NIDDM has been found by Roch–Norland et al. [6]. During the infusion the mean rate of muscle glycogen synthesis was $0.18 \pm 0.04$ mmol l$^{-1}$ min$^{-1}$ muscle$^{-1}$ in the normal subjects and was reduced by 60% to $0.08 \pm 0.03$
mmol l⁻¹ min⁻¹ muscle⁻¹ in the diabetic subjects. The rate of non-oxidative glucose metabolism during the same period was 0.042 mmol (kg body wt)⁻¹ min⁻¹ in the normal subjects and 0.022 ± 0.004 mmol (kg body wt)⁻¹ min⁻¹ in the diabetic subjects. There was a close to linear correlation \((r=0.89, P<0.001)\) between the rate of glycogen synthesis and the rate of non-oxidative glucose metabolism for each subject.

The rate of synthesis of whole-body muscle glycogen in each subject was calculated by multiplying the rate of glycogen synthesis by the fraction of body weight which was muscle. Although estimates of whole-body muscle mass range from 26% to 38% of body weight [7, 8], even using the lowest reported value, the measured rates of glycogen synthesis accounted for effectively all of the non-oxidative glucose metabolism in both groups. Furthermore since in both groups non-oxidative glucose metabolism was the major pathway of glucose disposal, the reduced rate of muscle glycogen synthesis in the diabetic subjects was primarily responsible for their reduced insulin-stimulated glucose uptake rate. These results indicate that under conditions of high plasma insulin and glucose a defect in the pathway of muscle glycogen synthesis is the major source of reduced glucose disposal in NIDDM.

The defect in muscle glycogen synthesis in the NIDDM subjects may have been induced by long-term exposure to hyperglycaemia, which has been shown to reduce insulin-stimulated non-oxidative metabolism in rats [9] and insulin-dependent diabetic humans [10]. However, studies on populations and families with a high incidence of NIDDM have shown a strong correlation between reduced insulin-dependent non-oxidative glucose metabolism and the later development of NIDDM [11, 12]. This reduction in non-oxidative glucose metabolism occurs before the hyperglycaemia and alterations in post-prandial insulin production that accompany NIDDM, suggesting that impaired insulin-stimulated muscle glycogen synthesis is a primary defect in the pathogenesis of NIDDM.

There are several enzymic reactions which may be responsible for the reduced insulin-stimulated muscle-glycogen-synthesis rate in NIDDM. The muscle glucose transport and glycogen synthase enzymes are both insulin stimulated, and each has separately been suggested to be responsible for the reduced rate of insulin-stimulated glucose disposal in NIDDM. Under hyperinsulinaemic conditions a positive correlation has been found between the activity of semi-purified glycogen synthase enzyme and the rate of non-oxidative glucose metabolism in normal subjects and subjects with NIDDM [2, 13]. However, at physiological insulin concentrations similar to the \(^{13}C\) n.m.r. study the correlation is weaker than at maximally stimulating insulin concentrations, suggesting that additional factors limit the rate of muscle glycogen synthesis.

Glucose-transport activity has been difficult to assess in muscle. Evidence for defective glucose transport in NIDDM muscle comes primarily from studies of cultured adipocytes [14, 15] which express the insulin-sensitive Glut 4 transporter also found in muscle [16]. Studies of isolated adipocytes provide support for the defect being in transport, based on findings of reduced insulin-stimulated glucose transport and numbers of glucose transporter enzyme in adipocytes isolated from subjects with NIDDM. However, no reduction of Glut 4 or Glut 1 mRNA or protein has been found in the muscle of subjects with NIDDM [17].

As a result of its location between the transport and synthase enzymes in the pathway of glycogen synthesis, the concentration of glucose 6-phosphate (G6P) is sensitive to the relative activities of these enzymes. Measurements of human muscle G6P have been made by biopsy, under conditions of euglycaemic hyperinsulinaemia, where no change [18] or only a slight decrease [19] in G6P concentration was observed with increasing rates of non-oxidative glucose metabolism. A limitation of the biopsy technique is that the tissue must be excised before being frozen, during which time glycolgenolysis may substantially raise the G6P concentration. In a study by Rossetti & Giacarri [20] when rat-muscle freezing was delayed for several seconds, which is similar to the time between excision and freezing for human muscle biopsies, the G6P concentration increased by 3-4-fold.

An alternative method of measuring human muscle G6P that does not suffer from autolytic artefacts is \(^{31}P\) n.m.r. Under conditions of exercise, changes in the sugar-phosphate region of the \(^{31}P\) n.m.r. spectrum have been observed that are consistent with an elevation of G6P [21]. Based on these exercise studies and the sensitivity achieved in our 2.1 T whole-body spectrometer, it is possible to measure changes in G6P levels as low as 0.03 mmol/l. An additional advantage of the \(^{31}P\) n.m.r. method is that the other major effectors of the hexokinase reaction, which are \(P_e\), pH, ATP and ADP [22], can be measured or calculated.

An experiment which could potentially assess the relative roles of glucose transport and glycogen synthase in the reduced muscle-glycogen-synthesis...
rate observed in NIDDM would be to combine $^{31}$P n.m.r. measurements of G6P with $^{13}$C n.m.r. measurements of muscle glycogen synthesis in subjects with NIDDM and normal controls under the same conditions of hyperglycaemic hyperinsulinaemia. The relative concentration of G6P in normal and diabetic subjects found in this experiment can be interpreted using the crossover theorem to determine whether the defect in muscle glycogen synthesis is due to glycogen synthase or impaired glucose transport. The application of the crossover theorem is as follows. If the activity of the glucose transport and phosphorylation steps were the same in the diabetic as in the normal subjects, the rate of glucose phosphorylation to G6P by hexokinase would need to be reduced by allosteric inhibition to match the reduced rate of G6P outflow into muscle glycogen which would be measured by $^{13}$C n.m.r. The primary allosteric inhibitor of the hexokinase reaction is G6P$\,[ZZ]$, which would have a higher concentration in the diabetic subjects, indicating that glycogen synthase was the rate-limiting step in glycogen synthesis in NIDDM. In contrast, the finding of a lower steady-state G6P concentration in diabetic subjects would be inconsistent with normal activity of muscle glucose transport, indicating a reduced activity in this step in NIDDM. This proposed study is an example of how, by combining $^{13}$C n.m.r. measurements of rates of metabolic pathways with $^{31}$P n.m.r. measurements of metabolic intermediates, the regulation in vivo of glucose metabolism can be studied.


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