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^{13}C NMR Studies of Glucose Disposal in Normal and Non-Insulin-Dependent Diabetic Humans [and Discussion]

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^{13}C NMR studies of glucose disposal in normal and non-insulin-dependent diabetic humans

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To examine the extent to which the defect in insulin action in subjects with non-insulin-dependent diabetes mellitus (NIDDM) can be accounted for by impairment of muscle glycogen synthesis, we performed combined hyperglycemic–hyperinsulinemic clamp studies with [^{13}C]glucose in five subjects with NIDDM and in six age- and weight-matched healthy subjects. The rate of incorporation of intravenously infused [1-^{13}C]glucose into muscle glycogen was measured directly in the gastrocnemius muscle by means of a nuclear magnetic resonance (NMR) spectrometer with a 15.5 min time resolution and a ^{13}C surface coil.

The steady-state plasma concentrations of insulin (*ca.* 400 pmol l^{-1}) and glucose (*ca.* 10 mmol l^{-1}) were similar in both study groups. The mean ($\pm$SE) rate of glycogen synthesis, as determined by ^{13}C NMR, was 78 ± 28 and 183 ± 39 μmol-glucosyl units (kg muscle tissue (wet mass))$^{-1}$ min^{-1} in the diabetic and normal subjects, respectively ($p < 0.05$). The mean glucose uptake was markedly reduced in the diabetic (30 ± 4 μmol kg^{-1} min^{-1}) as compared with the normal subjects (51 ± 3 μmol kg^{-1} min^{-1}; $p < 0.005$). The mean rate of non-oxidative glucose metabolism was 22 ± 4 μmol kg^{-1} min^{-1} in the diabetic subjects and 42 ± 4 μmol kg^{-1} min^{-1} in the normal subjects ($p < 0.005$). When these rates are extrapolated to apply to the whole body, the synthesis of muscle glycogen would account for most of the total-body glucose uptake and all of the non-oxidative glucose metabolism in both normal and diabetic subjects.

We conclude that muscle glycogen synthesis is the principal pathway of glucose disposal in both normal and diabetic subjects and that defects in muscle glycogen synthesis have a dominant role in the insulin resistance that occurs in persons with NIDDM.

In 1983 we showed that glycogen, which is the storage polymer for glucose in mammals, gives a well-resolved ^{13}C NMR spectrum in which all of the carbons are nearly 100% visible (Sillerud & Shulman 1983). This has enabled us to measure liver and muscle glycogen concentrations in man (Jue *et al.* 1989*a*). The NMR technique has the advantage over existing biopsy techniques in that the measurements are made non-invasively with a time resolution of several minutes and are averaged over several cubic centimetres of muscle. Furthermore, the precision of the NMR measurements of glycogen concentration is better and can be enhanced several fold by infusing ^{13}C enriched glucose (Jue *et al.* 1989*b*). Recently we have applied these methods to examine the extent to which the defect in insulin action in subjects with non-insulin-dependent diabetes mellitus (NIDDM) can be accounted for by impairment of muscle glycogen synthesis (Shulman *et al.* 1990).

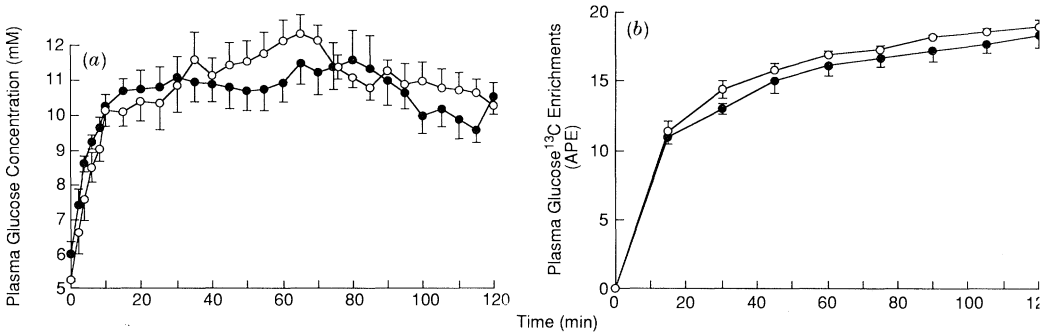

Figure 1. Plasma glucose concentrations and ^{13}C plasma glucose enrichment values in normal subjects (open circles) and subjects with NIDDM (solid circles) before and during the hyperglycemic–hyperinsulinemic clamp study. Values are means ±SE. APE denotes atom percent excess.

Non-insulin-dependent diabetes mellitus (NIDDM) is a hereditary disease characterized by defects in insulin secretion and in tissue sensitivity to insulin. Muscle is generally believed to represent the principle site of insulin resistance in NIDDM. Once taken up by the muscle glucose can be oxidized to carbon dioxide, converted to lactate, or stored as glycogen or fat. To examine whether the defect in insulin action can be accounted for by impaired muscle glycogen synthesis, we performed combined hyperglycemic-hyperinsulinemic clamp studies with ^{13}C glucose in NIDDM ($n = 5$) and age-mass-matched normal control subjects ($n = 6$). The rate of incorporation of intravenously infused [1-^{13}C]glucose into muscle glycogen was directly measured in the human gastrocnemius muscle with 15.5 min time resolution using ^{13}C NMR surface coil techniques in a 1 m bore 2.1 T Biospec NMR spectrometer. On the evening before the study, all the diabetic subjects were admitted to the clinical research centre and received an overnight infusion of insulin to induce normoglycemia. This allowed the diabetic and normal subjects to be studied under identical basal plasma glucose concentrations. Figure 1 shows the time course for both the plasma glucose concentrations (*a*) and ^{13}C plasma glucose enrichments (*b*) in both the normal and diabetic subjects. The mean starting plasma glucose (*a*) was 5.2±0.1 mM and 5.9±0.4 mM in the normal and NIDDM subjects respectively. After the start of the glucose infusion the plasma glucose concentration rapidly increased and reached a plateau of *ca.* 10.6 mM by *ca.* 15 min in both groups. The mean plasma glucose concentration from 15 to 120 min was 11.0±0.3 mM and 10.8±0.4 mM in the normal and NIDDM subjects respectively. The time course for the ^{13}C plasma glucose enrichment is shown in (*b*). After the start of the glucose infusion the enrichments increased rapidly and approached a plateau of *ca.* 18% atom percent excess (APE) by 80 min in both groups. To determine how much of the ^{13}C label was in the C1 position of plasma glucose, ^{1}H NMR was performed on plasma samples taken at 30, 60 and 90 min into the glucose infusion. The mean C1 plasma glucose enrichments (from all subjects) were: 13.4±0.4 APE, 15.9±0.5 APE, and 17.6±0.5 APE at 30, 60 and 90 min respectively. These values corresponded very well with the mean plasma glucose ^{13}C enrichments determined by GC–MS at the same time points in the same subjects: 13.7±0.5 APE, 16.3±0.5 APE and 17.4±0.5 APE at 30, 60 and 90 min respectively. This implied that all of the administered label remained in the C1 position of glucose and was not scrambled to other positions. The mean initial plasma insulin

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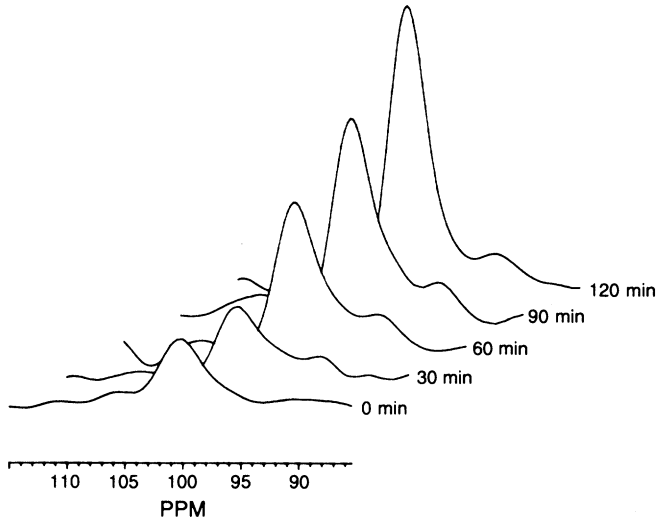

Figure 2. Typical course of ^{13}C NMR spectra of muscle glycogen in a representative normal subject during the hyperglycemic–hyperinsulinemic clamp study. The 1-C peak of glycogen appears at 100.4 p.p.m.

concentrations were 36 ± 6 pmol l^{-1} and 60 ± 12 pmol l^{-1} in the normal and diabetic subjects respectively. After the start of the combined infusion of glucose, insulin, and somatostatin, the plasma insulin concentration quickly rose and reached a plateau of 402 ± 54 pmol l^{-1} and 372 ± 30 pmol l^{-1} by 10 min in the normal and diabetic groups respectively. Figure 2 shows the time course of a typical decoupled ^{13}C NMR spectrum of leg glycogen acquired before the [1-^{13}C]glucose infusion (bottom spectrum) and the subsequent spectra obtained every 15.5 min. The natural abundance C1 peak of glycogen is clearly seen in the baseline spectrum and routinely yielded a minimal signal to noise ratio of *ca.* 25:1. This peak height increased over the next 120 min of [1-^{13}C]glucose infusion. The basal concentration of muscle glycogen was significantly lower in the NIDDM group (39 ± 6 mmol (l wet muscle)$^{-1}$) as compared with the normal subjects (73 ± 11 mmol l^{-1} $p < 0.01$). Figure 3 shows the time course for incremental changes in glycogen concentration over the baseline in both groups corrected for fractional labelling of the glucose pool. Despite almost identical concentrations of insulin and glucose the mean rate of glycogen synthesis in NIDDM subjects was *ca.* 60% less than the rates of glycogen synthesis in the normal subjects. The mean rate (40–120 min) of glycogen synthesis was calculated to be 183 ± 39 μmol-glucosyl units (kg muscle (wet mass))$^{-1}$ min^{-1} in the normals and 78 ± 28 μmol-glucosyl units (kg muscle (wet mass))$^{-1}$ min^{-1} ($p < 0.05$) in the diabetic subjects. This corresponded to rates of glucose uptake of 51 ± 3 μmol (kg (body mass))$^{-1}$ min^{-1} in the normal subjects and 30 ± 4 μmol (kg (body mass))$^{-1}$ min^{-1} ($p < 0.005$) in the NIDDM subjects. Rates of oxidative glucose metabolism determined by indirect calorimetry were 9 ± 1 μmol (kg (body mass))$^{-1}$ min^{-1} and 8 ± 2 μmol (kg (body mass))$^{-1}$ min^{-1} ($p < NS$) in the normal and NIDDM subjects respectively. Subtracting the rate of oxidative glucose metabolism from the rate of total body glucose uptake in both groups yielded rates of non-oxidative glucose metabolism of 42 ± 4 μmol kg^{-1} min^{-1} and 22 ± 4 μmol kg^{-1} min^{-1} ($p < 0.005$) in the normal and NIDDM subjects respectively.

A lag period before the onset of glycogen synthesis was observed in both groups

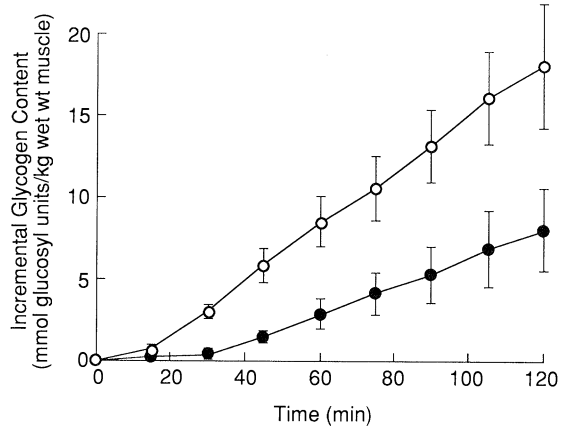

Figure 3. Incremental changes from base line in the muscle glycogen concentration in the normal (open circles) and diabetic subject (solid circles) during the hyperglycemic–hyperinsulinemic clamp study. Values are means ±SE, expressed in terms of the wet mass of muscle tissue.

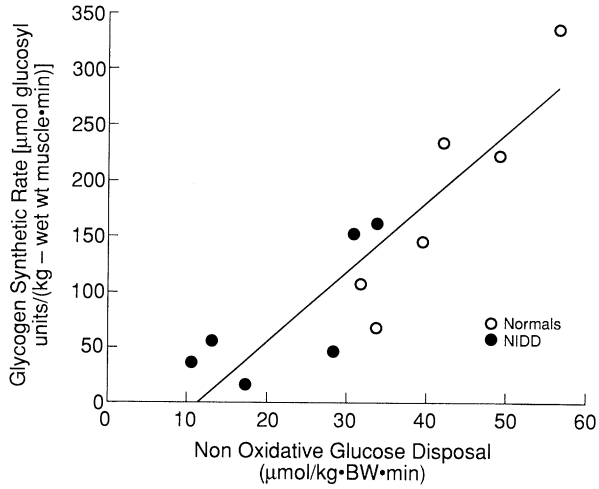

Figure 4. Muscle glycogen synthesis in relation to non-oxidative glucose metabolism during the hyperglycemic–hyperinsulinemic clamp study. Each normal subject is represented by an open circle, and each subject with NIDDM by a solid circle; the third and fourth solid circles along the abscissa represent the results of two studies in the same diabetic subject. $r = 0.89$ ($p < 0.001$).

(figure 3). By extrapolating the linear portion of the plots to the time axis (x-axis), the mean lag period for normal subjects was determined to be 12 ± 5 min while in NIDDM subjects it was 35 ± 6 min ($p < 0.01$). Figure 4 shows the relationship between rates of glycogen synthesis as determined by ^{13}C NMR in both diabetic (closed circles) and normal (open circles) subjects against the corresponding rates of non-oxidative glucose metabolism determined in those same subjects. As can be seen the rates of glycogen synthesis correlated very well with rates of non-oxidative glucose metabolism as determined by indirect calorimetry ($r = 0.89$, $p < 0.001$). When the rate of muscle glycogen synthesis was extrapolated to the whole body, the synthesis of muscle glycogen could account for (a) the majority of total body glucose uptake and (b) all of the non-oxidative glucose metabolism in both normal and diabetic man. From these studies we conclude that muscle glycogen synthesis is the major pathway

of glucose disposal in normal and diabetic subjects and that defects in muscle glycogen synthesis play a dominant role in the insulin resistance which is observed in non-insulin-dependent diabetic subjects.

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Discussion

K. Brindle (*Department of Biochemistry, University of Oxford, U.K.*). In the experiments where it was shown that the steady-state level of glycogen appears to correspond with the amount of 'work' being done by the muscle, has Professor Shulman measured, using ^{31}P NMR, the levels of P_i and phosphocreatine? It has been suggested that phosphocreatine represents a rapidly mobilizable store of inorganic phosphate which can be used for glycogenolysis. One might expect, therefore, that the steady-state levels of glycogen observed at different work loads would correlate with the steady-state levels of phosphocreatine and P_i.

R. G. Shulman. We have measured the ^{31}P NMR spectrum during the exercise described. If the glycogen phosphorylase is under substrate level control, as Dr Brindle suggests, the ^{31}P NMR spectrum could help to evaluate that possibility.

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