Assessment of the rate of Muscle Glycogen Synthesis using ^{13}C Magnetic Resonance Spectroscopy in Zucker fa/fa rats, Zucker lean rats and Sprague Dawley rats: Effect of treatement with Rosiglitazone

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Introduction

Non–Insulin Dependent Diabetes Mellitus (NIDDM) is characterized by defects in insulin secretion and insulin resistance in liver and muscle. By allowing repeated measurements of muscle glycogen concentration in rats, *in vivo* ^{13}C Magnetic Resonance Spectroscopy (MRS) has proven to be a valuable tool for accurately measuring the glycogen synthesis rate in the rat muscle and can be used as a measure of insulin resistance. By measuring the incorporation of ^{13}C glucose into muscle glycogen during a hyperinsulinemic–euglycemic clamp, it is possible to measure the effect of a therapeutic agent which alters the insulin sensitivity in skeletal muscle (Ref 1.)

Methods

MRS data from the gastrocnemius muscle group of rats was collected on a 4.7T/40 cm Varian Inova imaging console interfaced to a Magnex magnet and gradient set. The data were collected using a two–coil setup, a circular ^{13}C surface coil (~2 cm diameter) tuned to 50.3 MHz and a circular ^{1}H surface coil (~4 cm diameter) tuned to 199.9 MHz. The two coils are in the same plane with the smaller ^{13}C surface coil centered in the larger ^{1}H surface coil. The ^{1}H surface coil was used for decoupling (Waltz), shimming and collection of ^{1}H images of the gastrocnemius muscle group of the rat. The ^{1}H images were used to calculate the volume and position of the muscle group relative to the ^{13}C surface coil. For ^{13}C spectroscopy, a 90° hard pulse at the center of the ^{13}C surface coil was used with a repetition time of 200 ms. The linewidth of the water peak was routinely 30–40 Hz after localized shimming. Data were collected every five minutes for approximately an hour. Indwelling catheters for infusion of solutions and withdrawal of blood during the experimental procedure were implanted in the rats approximately one week prior to the clamp study. On the day of the study, the rats, which were fasted overnight, were anesthetized with Sodium Thiopental. An infusion of [1–^{13}C]glucose (45% glucose in water), insulin (10 mU/kg/min), and somatostatin (0.1µg/kg/min) was started to establish a hyperinsulinemic–euglycemic clamp (Ref 2) at a plasma glucose concentration of ~100 mg/dl. After the clamp was established, the rat was placed on a heating pad in the magnet with the muscle group positioned over the $^{13}C/^{1}H$ surface coils. The temperature of the heating pad was adjusted to maintain the body temperature of the rat at ~37° C. Blood (~20 µL) was withdrawn during the establishment and maintenance of the clamp. After the rat was positioned in the magnet, blood was withdrawn (~100 µL) to determine the percent enrichment of [1–^{13}C]glucose in the blood at the beginning (t=0 min), middle (t=30 min) and end (t=60 min) of the ^{13}C MRS data collection. Zucker fa/fa rats, their lean littermates and Sprague–Dawley rats were used in these studies. A group of the Zucker rats was treated with rosiglitazone twice a day (6 mg/kg/day) for one week. The other group of Zucker rats was treated with vehicle for one week.

Results

A representative time course of the peaks in the region between 90 and 100 ppm in the *in vivo* ^{13}C MRS data is shown in Fig 1. There are three resonances in this region: the glycogen peak at ~100 ppm and the β and α anomers of glucose at ~96 and ~92 ppm, respectively. The glycogen synthesis rate in the muscle can be determined by measuring the incremental increase of the glycogen resonance at ~100 ppm (Ref 3). The rate of increase in the glycogen resonance was determined by (1) measuring the entire integral of the three peaks in this region (the blood level of glucose is constant during this time) and (2) performing a line fit analysis on the three resonances. For comparing the glycogen synthesis rate in the different rats, both the integral and line fit data were normalized with respect to the amount of muscle and its position relative to the ^{13}C surface coil for each rat. The rate of glycogen synthesis for the different groups of rats and their treatment is given in Figure 2. The data show that the rate of glucose incorporation into muscle glycogen is significantly greater in Zucker fa/fa BRL treated and SD rats compared to vehicle treated Zucker fa/fa rats. Statistically there was no difference between the Zucker lean and vehicle treated Zucker fa/fa rats (p=0.07). The glucose infusion rate (GIR) was also determined for the different groups of rats during this experiment. There was no difference in any of the groups except for the vehicle–treated Zucker fa/fa rats in which suppressed global glucose utilization was observed (data not shown).

Discussion

The ^{13}C MRS data show that rosiglitazone significantly restores insulin sensitivity in the skeletal muscle when compared to a vehicle treated rat. The insulin sensitivity in the rosiglitazone treated rat is comparable to that in a normal Sprague Dawley rat and slightly increased, although not statistically significant, when compared to the lean Zucker rats.

References

Ref 1. G. Shulman, *et al.*, Quantitation of Muscle Glycogen Synthesis in Normal Subjects and Subjects with Non–Insulin Dependent Diabetes with ^{13}C Nuclear Magnetic Resonance spectroscopy, The New England Journal of Medicine, 1990; 322:223–228

Ref 2. B. Jucker, *et al.*, ^{13}C and ^{31}P NMR Studies on the Effects of Increased Plasma Free Fatty Acids on Intramuscular Glucose Metabolism in the Awake Rat, 1997, JBC; 272:10464–10473

Ref 3. G. Bloch, *et al.*, *In Vivo* Regulation of Rat Muscle Glycogen Resynthesis after Intense Exercise, AJP, 1994; 266:E85–E91

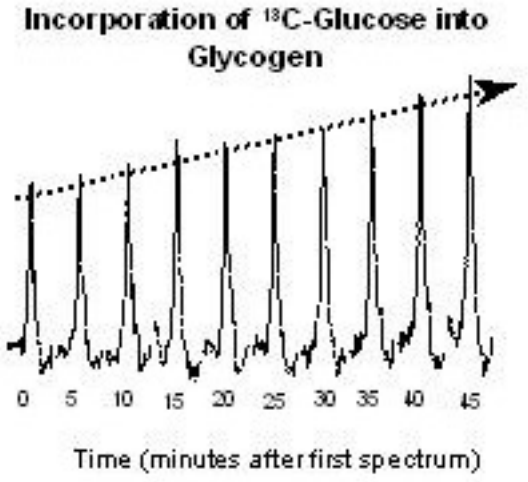

Figure 1

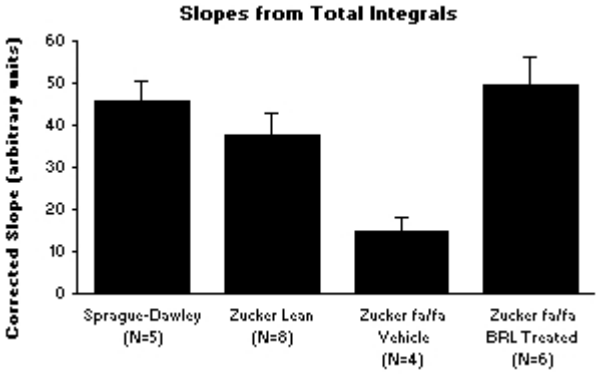

Figure 2