^{13}C MR spectroscopy of postprandial muscle glycogen metabolism to study the importance of the PPP1R3A gene

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Introduction

Activation of the glycogen-bound protein phosphatase-1 enzyme is a critical rate-determining step in muscle glycogen synthesis. The PPP1R3A gene encodes the regulatory subunit of protein phosphatase-1 in skeletal muscle. Disruption of this gene in mice results in a ten-fold decrease in muscle glycogen levels[1]. However, the precise role of PPP1R3A in muscle glycogen regulation in humans is unknown. Hence, we used ^{13}C MRS to observe the postprandial change in muscle glycogen levels in subjects carrying a genetic change in PPP1R3A gene.

Method

5 subjects with a mutation in PPP1R3A were studied. Two of these subjects had an additional unlinked mutation in the PPARG gene (target for glitazone drugs) and had severe insulin resistance[2]. The results from this group of subjects (n =5) were compared to healthy volunteers (n=9) and diet controlled type 2 diabetics (n=9) from a previous study[3]. Each subject fasted for 12 hours prior to the study. Baseline ^{13}C measurements for muscle glycogen were taken prior to the first standard meal (t =0). The meal consisted of 190.5g carbohydrate, 41.0g fat, 28.8g protein totalling 1253 kcal. Further measurements were taken at 60, 120 and 240 minutes, after which another standard meal of similar composition was given, followed by measurements at 300, 360 and 480 minutes.

All glycogen measurements were performed at 3.0T, using a circular ^{13}C surface coil and quadrature ^{1}H coils. A 100μs hard pulse was used to excite ^{13}C with CYCLOPS phase cycling, proton decoupling was achieved with a WALTZ-8 sequence at a power of 68 ± 2W. The repetition time for each acquisition was 360ms, keeping the power deposition within the SAR limits. For each time point 3000 acquisitions were made over 18mins.The C1-glycogen peak at 100.4 ppm was analysed with a MATLAB version of MRUI. Muscle glycogen concentration was quantified using a calf shaped phantom of known glycogen concentration. Blood samples were taken for each subject for measurements of glucose and insulin at the same times as the spectra.

Results

Baseline muscle glycogen concentration in the PPP1R3A group was 38.6±13.0 mmol/l. This was lower than the healthy volunteers (68.9±4.1 mmol/l) and diabetics (57.1±3.6 mmol/l). After the first meal, mean glycogen concentration in the healthy volunteers rose significantly from basal (97.1±7.0 mmol/l at 240 min; p = 0.005). After the second meal, the high level of muscle glycogen concentration in the control group was maintained, with a further rise to 108.0±11.6 mmol/l by 480 min (Fig 1a). In the PPP1R3A group, there was a blunted response after the first meal compared to the healthy volunteers (36.4±9.3 mmol/l at 240 min, p = 0.001) and glycogen levels remained significantly lower than the healthy volunteers after the second meal (50.8±18.0 mmol/l, p = 0.03) (Fig 1a). In comparison to the diabetes group, the PPP1R3A group had a significantly blunted muscle glycogen rise after the first meal (p = 0.033) but the mean glycogen levels were similar after the second meal (Fig 1a). The attenuated response in postprandial muscle glycogen synthesis in the PPP1R3A group was observed despite normal blood glucose concentrations (Fig. 1b)

Figure 1a.

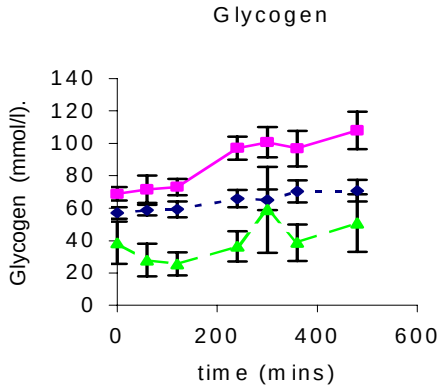

Figure 1b.

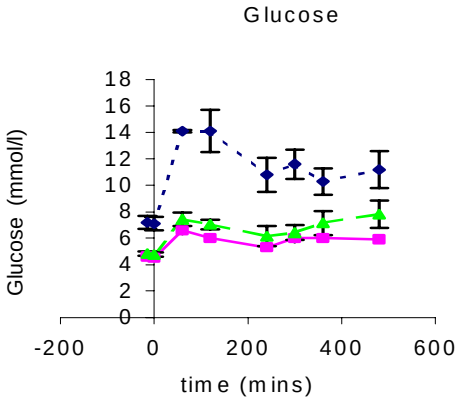

Fig.1: Glycogen (a) and blood glucose (b) concentrations as a function of time for controls (□), *diabetes* (◊) *and PPP1R3A subjects* (Δ).

Discussion

Baseline glycogen content in the PPP1R3A group was almost 2-fold lower than the healthy volunteers and 1.5 fold lower than the diabetes group. In sharp contrast to the healthy volunteers and diabetes subjects, the PPP1R3A group had a blunted muscle glycogen response following the first meal. Although the muscle glycogen concentration increased following the second meal, the mean glycogen concentration remained significantly lower in the PPP1R3A group compared to the healthy volunteers at the end of the study.

Overall, the results show that muscle glycogen regulation is markedly abnormal in subjects carrying the PPP1R3A genetic change. It is interesting to note that in healthy volunteers muscle glycogen concentration remains unchanged for two hours after the first meal because of the opposing effects of increased glucose oxidation from glycogen stores and increased glycogen synthesis. In the PPP1R3A group, the lack of rapid increase in glycogen synthesis led to a net decrease in glycogen concentration at 1 hour, which approached statistical significance (p = 0.052). Despite the defective muscle glycogen synthesis, the blood glucose levels of the PPP1R3A group were normal throughout the study suggesting that the muscle glucose oxidative capacity may be compensating for this defect.

References

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