Paired Studies of Glycogen Storage Using ^{13}C MRS: The Second Meal Effect

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Introduction

Studies have shown that the blood glucose rise following a meal is much less if a preceding meal has been ingested[1]. We aimed to study the dynamics of glycogen storage which could underlie this phenomenon by measuring glycogen levels in muscle and liver using ^{13}C-MRS following consecutive meals and conventional biochemical methods to measure blood glucose concentrations.

Method

6 healthy volunteers each attended on 2 separate days. The subjects fasted overnight before each study day. On the first day, an unlabelled breakfast (21.9g protein, 21.9g fat, 124.7g carbohydrate and 768kcal) was consumed at t=0hr followed 4hr later by lunch (35.6g protein, 47.3g fat, 102.8g carbohydrate and 967kcal) labelled with 3g U[^{13}C]-glucose. Baseline ^{13}C glycogen measurements were taken in the liver and quadriceps at t=0hr on both days. Further measurements were taken every 2 hrs. After two weeks, the subjects were studied again using the same protocol without the preceding breakfast.

Blood glucose measurements were taken at t=-0.25, 0, 1, 2, 4, 4.5, 5, 6 and 8hr using a Hemocue photometer analyser.

Glycogen measurements were recorded at 3T using a surface probe containing a ^{13}C coil and quadrature ^{1}H coils. The flip angle 2cm in from the centre of the ^{13}C coil was optimised to be 90º. A 100μs rectangular pulse with a peak power of 390±10W was used to excite ^{13}C and phase cycling performed using CYCLOPS. Decoupling was achieved using a WALTZ-8 pulse sequence with a peak power of 55±2W. For each scan 3000 acquisitions were made with a repetition time of 360ms, taking 18 minutes altogether. Spectra were analysed using a MATLAB version of MRUI. Quantification was performed by comparison with spectra acquired from an appropriately shaped phantom with a known glycogen concentration positioned appropriately for liver and quadriceps.

Results

Blood glucose concentrations were comparable in both groups at baseline (fig 1a). After breakfast at t=1hr the blood glucose concentration in the breakfast plus lunch group rose to 7.43±1.05mmol/l which was significantly higher than the lunch only group. This then decreased and by t=4hr blood glucose concentrations were comparable in both groups. After lunch the blood glucose concentrations rose in both groups and at t=5hr the blood glucose concentration in the lunch only group had risen to a higher level than the breakfast plus lunch group ($p<0.036$). This demonstrates the second meal effect. The concentrations then converged and were comparable by t=8hr.

Baseline liver glycogen concentrations in the breakfast plus lunch and lunch only groups were 126.6±42.8mmol/l and 158.3±27.3mmol/l respectively. At t=2hr the liver glycogen concentration in the breakfast plus lunch group had risen by 97.6±46.1% from baseline (fig 1b). Over this same interval the concentration in the lunch only group dropped by 44.4±11.9%. The concentrations did not change significantly in the second measurement. After lunch at t=5.5hr ^{13}C liver glycosyl units had increased in both groups from baseline levels. The increase in the breakfast plus lunch group (326.0±119.7%) was significantly higher ($p<0.042$) than in the lunch only group (38.8±36.2). The increases from baseline were maintained at t=9hr for both groups where the breakfast plus lunch group remained significantly higher than the lunch only group ($p<0.039$).

Baseline quadriceps glycogen concentrations in the breakfast plus lunch and lunch only groups were 70.0±21.1mmol/l and 71.5±18.1mmol/l respectively. At t=2hr the quadriceps glycogen concentration had not changed significantly from baseline in either of the groups (fig 1c). At t=4hr the quadriceps glycogen concentration was slightly lower than baseline in both groups. After lunch at t=6hr ^{13}C quadriceps glycosyl units had increased from baseline in both the breakfast and lunch group (80.3±87.6%) and the lunch only group (113.9±84.8%). At t=9hr this remained elevated from baseline in both groups. There was no significant difference in increase in ^{13}C quadriceps glycosyl units from baseline between both groups at any point in the day.

Discussion

At t=2hr the liver glycogen concentration had increased from baseline in the group that had eaten breakfast, whereas it decreased in the group that had not. This corresponds with a decrease in the blood glucose concentration in the breakfast plus lunch group and indicates that glucose from breakfast is stored as glycogen in the liver. Over the same time interval quadriceps glycogen concentration did not increase significantly from baseline in either of the groups. As previously reported, muscle glycogen levels do not begin to rise immediately after meals[2].

Before lunch ^{13}C labelled glycosyl units are at natural abundance and so absolute concentrations of glycosyl units can be determined. After lunch when 3g U[^{13}C]-glucose was supplied, the percentage enrichment of the glycosyl units is unknown, so absolute concentrations of glycosyl units can not be determined.

After lunch the rise in ^{13}C liver glycosyl units from baseline was much greater in the group that had eaten breakfast and this continued at t=9hr. The ^{13}C quadriceps glycosyl units also increased from baseline levels in both the groups after lunch but there was no significant difference in increases between the two groups.

In summary there was a greater accumulation of ^{13}C liver glycosyl units in the group that ate lunch after a preceding breakfast. These results suggest that the attenuation in blood glucose rise after a second meal is due to the priming of hepatic glycogen synthesis that occurs as a consequence of the first meal. Post-prandial suppression of plasma free fatty acids could explain this. As the second meal phenomenon occurs in type 2 diabetes, therapeutic manipulation of this mechanism can be applied to routine management of this condition. Use of ^{13}C spectroscopy has allowed non-invasive investigation of every day physiology.

References

1. Carey P.E et al, AJP 284: E688-694, 2003
2. Taylor R. et al, AJP 265:E224-229, 1993

Figure 1a

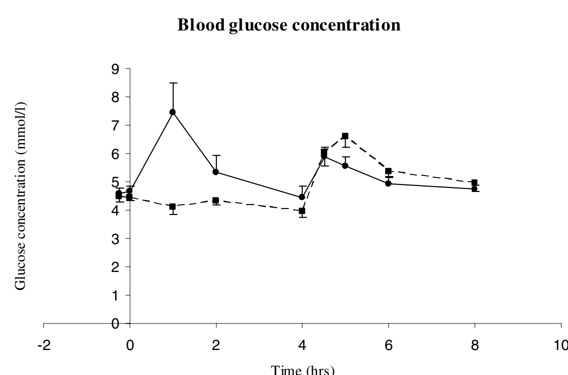

Figure 1b

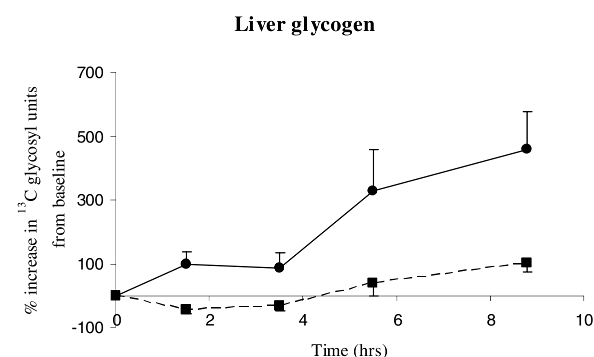

Figure 1c

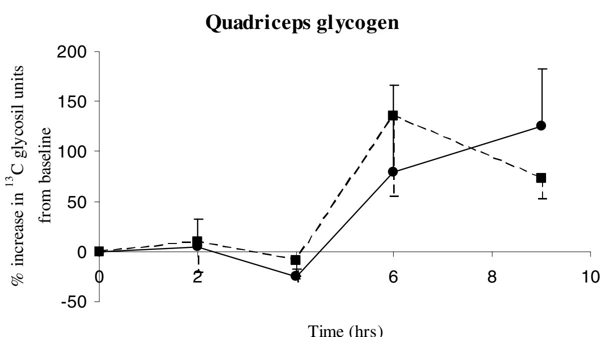

Fig 1: Blood glucose concentration (a), % increase in ^{13}C glycosyl units from baseline in the liver (b) and quadriceps (c), as a function of time for subjects having just lunch (▯) and breakfast plus lunch (▯)