

A Dynamic Study of Changes in Hepatic and Skeletal Muscle Lipid and Glycogen levels, due to 24h Starvation and Re-feeding: A ¹H and ¹³C MRS study

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Background: Short-term starvation (up to 36h) can induce skeletal muscle and hepatic insulin resistance (IR)¹, changes which are attenuated by administration of carbohydrate (CHO) drinks². The cellular mechanisms underlying this process remain unknown. This period of starvation has been shown to deplete liver glycogen stores³ and increase serum ketone and non-esterified fatty acid (NEFAs) levels, which are important as they determine the rate of NEFA flux into skeletal muscle⁴. The aim of this study was to measure changes in hepatic and skeletal muscle glycogen and lipid content in order to understand substrate metabolism leading to an insulin resistant state following short-term starvation and re-feeding using CHO drinks.

Method: 12 healthy males were studied following an overnight (12h) fast. Subjects abstained from alcohol and exercise for 3 days and caffeine intake for 24h prior to the study. At the start of the study (t=0h) they were given a standard meal which aimed to provide 40% of their daily energy requirements (calculated from a 3-day food diary) the macronutrient contribution of which was 50% from carbohydrate, 35% from fat and 15% from protein. MRS measurements (for observation of glycogen and lipid) were made 4h after the standard meal (to represent the baseline fed state), then at 12, 24, 28 and 32h. At 26h subjects were given an oral nutritional supplement (ONS) containing 100g of complex carbohydrates, antioxidants, and 30 g glutamine (Fresenius Kabi). Subjects were free to drink water throughout the study to avoid dehydration. MRS measurements were performed on a Philips Achieva 3T system using a transmit/receive body coil for ¹H MRS and a ¹³C surface probe with quadrature proton decouple coils for ¹³C MRS and localised ¹H imaging.

Liver Volume Measurement: Following an initial survey, to allow positioning of slices, a T₁-weighted 3D TFE scan was acquired to enable calculation of liver volume: resolution=2.08x2.08x7.00mm³, no. slices=36, matrix=180x182, TR=3.11ms with total scan time (equal to breath-hold time)=14.4s. Images were analyzed by drawing regions of interest in Analyze6 to calculate liver volume.

Lipid Measurements: Hepatic ¹H MR spectra were acquired, from a voxel positioned in the right lobe of the liver, using a respiratory triggered PRESS sequence and the following parameters: TE/TR = 40/5000ms, VOI = 30x30x30mm, N_{ave} = 16, BW = 2000Hz, 1024 samples. Two ¹H MR spectra were acquired in muscle; water-suppressed for measurement of intramyocellular lipid (IMCL)/water (N_{ave}=32) and non water-suppressed for total calf lipid/water measurement (N_{ave}=16). PRESS localization was used with the following parameters: TE/TR = 40/7000ms, VOI = 30x30x50mm, BW = 2000Hz, 1024 samples. Water un-suppressed spectra were post-processed using jMRUI and peak areas were calculated using in-house software built in Matlab. Water-suppressed spectra were post-processed using jMRUI and peak areas were calculated using the AMARES algorithm, fitting to Gaussian lineshapes. Lipid values are given as lipid/water ratios.

Glycogen Measurements: After acquisition of an initial survey to check positioning of the coil, ¹³C spectra were acquired using a proton-decoupled pulse acquire sequence with BW=7000Hz, samples=512, ¹³C adiabatic pulses and narrowband decoupling: Liver - Three pulse-acquire, proton decoupled ¹³C spectra with TR=2150ms, 288 averages, total acquisition time ~30 minutes; Calf - Two spectra at each time point. TR=1300ms, 336 averages, total acquisition time of ~15mins. ¹³C spectra were averaged and post-processed using jMRUI. Peak areas were determined using in-house software built in Matlab.

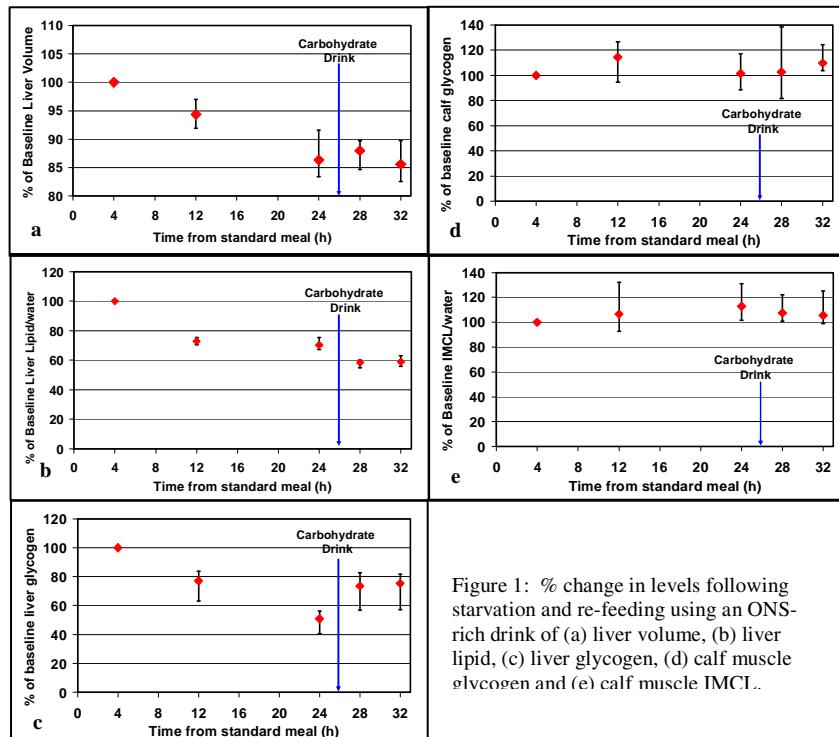


Figure 1: % change in levels following starvation and re-feeding using an ONS-rich drink of (a) liver volume, (b) liver lipid, (c) liver glycogen, (d) calf muscle glycogen and (e) calf muscle IMCL..

Results and Discussion: Liver: Liver volume (Fig. 1a) significantly decreased (p<0.03) at 12h to 94% of baseline, decreasing further (p<0.002) at 24h to 86%. Following ingestion of the drink, liver volumes were not significantly different from 24h levels. Decreases in liver volume are likely to result from loss of glycogen, water, and lipid. Liver lipid/water (Fig. 1b) decreased significantly (p<0.02) to 72% of baseline levels at 12h. Between 12 and 24 h, levels remained relatively constant (p=0.11). Following the drink, there was a tendency towards a decrease in liver lipid but this did not reach statistical significance. Since measurements of liver lipid are given as ratios to water, it is possible that decreases in lipid concentration could be due to an increase in water concentration. However, decreases in liver volumes measured imply decreases in liver water concentrations which are more likely to mask decreases in liver lipid. Liver glycogen (Fig. 1c) significantly decreased (p<0.01) after 12h of starvation to 77% of baseline, decreasing further to 50% of baseline following 24h of starvation (p<0.002). Following the ONS-rich drink, glycogen levels increased significantly (p<0.03) back to 74% of baseline, remaining at this level 6h after intake of the drink. Calf - As would be expected, 24h of starvation did not deplete skeletal muscle glycogen reserves (Fig. 1d). However, at 6h following the ONS-rich drink, levels increased to 110% of those seen following 24h of starvation (p<0.06). Unlike hepatic lipid levels, levels of IMCL (Fig. 1e) were significantly increased (p<0.02) following 24h of starvation and these paralleled changes seen in serum NEFA concentrations (data not shown). It is possible that the increase in muscle glycogen and lipid seen following 12h of starvation reflects continued energy storage following the standard meal (previous studies have shown levels of glycogen in calf muscle continue to increase 4-6h following mixed meal ingestion⁵).

Conclusion: 24 h of starvation in healthy volunteers resulted in a decrease in liver volume and decreases in liver glycogen and lipid reserves. The changes in liver volume and glycogen are attenuated following re-feeding using an ONS-rich drink, however liver lipid tended to decrease following the drink. This is unsurprising as no lipid was ingested during this time. During the starvation calf muscle glycogen was not depleted which is to be expected as muscle glycogen (unlike liver glycogen) does not directly contribute to the maintenance of glycaemia. Following the re-feeding calf muscle glycogen stores actually increased. Increases in muscle IMCL levels following 24 h of starvation parallels the increases in serum NEFA concentrations. This may be a mechanism by which muscle insulin resistance develops following starvation and is attenuated following re-feeding using ONS.

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