1 **TITLE**

2 **Investigating the effects of an Oral Fructose Challenge on Hepatic ATP Reserves in**
3 **Healthy Volunteers: A ^{31}P MRS Study**

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14 **DEPARTMENT AND INSTITUTION OF STUDY**

15 All work was conducted at the Sir Peter Mansfield Imaging Centre in the University of
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31 **LIST OF ABBREVIATIONS**

32 NAFLD – Non-Alcoholic Fatty Liver Disease

33 NASH – Non-Alcoholic Steatohepatitis

34 ATP – Adenosine Triphosphate

35 MRS – Magnetic Resonance Spectroscopy

36 Pi – Inorganic Phosphate

37 PDE – Phosphodiesters

38 PME – Phosphomonoesters

39 IV – Intravenous

40 ISIS – Image Selective *In vivo* Spectroscopy

41 NOE – Nuclear Overhouser Effect

42 SD – Standard Deviation

43 ADP – Adenosine Diphosphate

44 AMP – Adenosine Monophosphate

45 AMPK – AMP-activated protein kinase

46 UTP – Uridine Triphosphate

47

48 **KEYWORDS**

49 ATP; hepatic ATP; fructose; fructose infusion; oral challenge; NAFLD; 31P; MRS

50

51 **CONFLICT OF INTEREST**

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59 **AUTHORS CONTRIBUTIONS**

60 Study conception and design: SB; MS; LM; GA; PM; PG;

61 Acquisition of data: SB; MS

62 Analysis and interpretation of data: SB; MS; GA; IM; LM; PG

63 Drafting of manuscript: SB

64 Critical revision: SB; MS; EC; KH; LM; IM; GA; PM; PG

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ABSTRACT

Background: Impaired homeostasis of hepatic ATP has been associated with NAFLD. An intravenous fructose infusion has been shown to be an effective challenge to monitor the depletion and subsequent recovery of hepatic ATP reserves using ^{31}P MRS.

Aims: The purpose of this study was to evaluate the effects of an oral rather than intravenous fructose challenge on hepatic ATP reserves in healthy subjects.

Methods: Self-reported healthy males were recruited. Following an overnight fast, baseline liver glycogen and lipid levels were measured using Magnetic Resonance Spectroscopy (MRS). Immediately after consuming a 500ml 75g fructose drink (1275 kJ) subjects were scanned continuously for 90 minutes to acquire dynamic ^{31}P MRS measurements of liver ATP reserves.

Results: A significant effect on ATP reserves was observed across the time course ($P < 0.05$). Mean ATP levels reached a minimum at 50 minutes which was markedly lower than baseline (80 ± 17% baseline, $P < 0.05$). Subsequently, mean values tended to rise but did not reach statistical significance above minimum. The time to minimum ATP levels across subjects was negatively correlated with BMI (R^2=0.74, $P < 0.005$). Rates of ATP recovery were not significantly correlated with BMI or liver fat levels, but were negatively correlated with baseline glycogen levels (R^2=0.7, $P<0.05$).

Conclusions: Depletion of ATP reserves can be measured non-invasively following an oral fructose challenge using ^{31}P MRS. BMI is the best predictor of postprandial ATP homeostasis following fructose consumption.

INTRODUCTION

Both NAFLD and non-alcoholic steatohepatitis (NASH) have been associated with impaired homeostasis of hepatic adenosine triphosphate (ATP) levels [1] and baseline hepatic ATP reserves have been shown to be more depleted in obese subjects [2, 3]. It is widely accepted that the inhibition of AMP-activate protein kinase (AMPK) which stimulates ATP synthesis is an important part of liver lipid accumulation [4, 5] and it has also been suggested that an inability to maintain ATP levels may prime hepatocytes to become vulnerable to injury by reactive oxygen species.

Hepatic ATP reserves can be monitored noninvasively using ^{31}P magnetic resonance spectroscopy (MRS) [6]. Early animal studies used this method to monitor ATP following fructose injections and suggested its potential use as a diagnostic method for studying liver disease [7]. A number of more recent studies have used these techniques to measure ATP homeostasis following an intravenous (IV) fructose load [2, 8, 9]. Fructose infusion causes the depletion of hepatic ATP levels due to a lack of phosphorylation feedback which results in continued phosphorylation activating AMP deaminase and uric acid production (supplementary material) [10]. During these studies, subjects undergo continuous ^{31}P MRS immediately following a fructose bolus injection to measure minimum ATP levels and subsequent rates of replenishment.

The effects of fructose consumption on liver lipids [11] and NASH [12] have been considered in the literature, but little research has investigated the immediate ATP response to an oral fructose challenge. The present study investigated postprandial changes to hepatic ATP reserves following an oral fructose intake.

114 **MATERIAL AND METHODS**

115 **Subjects**

116 All subjects were self-reported healthy non-obese males with sedentary lifestyles and no
117 known metabolic disorders. All subjects consumed the oral challenge in the time required (5
118 minutes) and complied well with the lifestyle restrictions and scanning requirements. The
119 mean age for all subjects was 24 ± 4 years and BMI was 25 ± 3 kg/m^2.

120 **Study Design**

121 *Ethical Permission*

122 Ethical permission was obtained from the local Medical School Research Ethics Committee
123 and subjects provided written informed consent before participation.

124 *Subjects*

125 At the time of this investigation there were no published data on ^{31}P MRS ATP following an
126 oral fructose challenge which could be used to estimate the power of the study. We therefore
127 chose a sample size for this first exploratory study based on data reported in infusion studies
128 [13, n=8].

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129 Prior to study days subjects were asked to refrain from alcohol for 24hr. On the morning of
130 the study subjects arrived at the test centre between 7:30am and 8:00am having fasted
131 overnight.

132 On arrival, natural abundance ^{13}C MR spectra were acquired from the liver to determine
133 baseline hepatic glycogen levels, and localized ^{1}H MR spectra were acquired to determine
134 baseline hepatic lipid levels. Subjects were then asked to consume a 500ml drink of 75g
135 fructose solution (1275 kJ) within 5 minutes. Immediately following consumption, subjects

136 were placed in the scanner and ^{31}P MR spectra were acquired continuously for 90 minutes to
137 assess dynamic changes in ATP and related phosphate metabolites. During the 90 minutes of
138 scanning, subjects were asked to breathe regularly and remain as still as possible and were
139 allowed to listen to the radio or music.

140 **Data Acquisition**

141 All measurements were performed on a Philips Achieva 3T system (Philips, Best, The
142 Netherlands) using the built-in ^{1}H transmit / receive body coil for scout images and voxel
143 placement.

144 *ATP*

145 Dynamic changes in phosphate metabolites were measured using localized ^{31}P MRS. A ^{31}P
146 surface coil (Philips, Best, The Netherlands) was placed on the abdomen over the liver. Scout
147 ^{1}H images were obtained and used for voxel placement in the right lobe of the liver (60 x 60
148 x 60 mm^3 voxel size). ^{31}P spectra were obtained continuously for 90 minutes using a
149 respiratory triggered ISIS sequence with Nuclear Overhouser Effect (NOE) enhancement and
150 proton decoupling (3 kHz bandwidth, 2048 samples, 5000 ms repetition time) as described
151 previously [14, 15]. The voxel for β-ATP was positioned against the abdominal wall with the
152 chemical shift of all other metabolites directed away from the wall to minimize signal leakage
153 from the abdominal muscle (confirmed by a lack of spectral PCr peak) and maximise signal
154 for β-ATP.

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155 *Hepatic Lipids*

156 Baseline lipid levels were measured using the integrated ^{1}H body coil. Scout images were
157 obtained and used for voxel placement (30 x 30 x 30 mm^3 voxel size). ^{1}H spectra were
158 obtained using a respiratory triggered, water suppressed STEAM sequence (2 kHz

159 bandwidth, 1024 samples, 13 ms echo time, 5000 ms repetition time, 40 averages). Two
160 spectra were collected without water suppression for correction to absolute lipid fat fractions
161 as described previously [15].

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162 *Glycogen*

163 Baseline glycogen levels were measured using unlocalized ^{13}C MRS. A surface coil with
164 integrated quadrature proton decoupling (PulseTeq, Surrey, UK) was placed on the abdomen
165 over the liver. Scout ^{1}H images were used to determine correct placement. ^{13}C spectra were
166 obtained using a $\pi/2$ pulse-acquire sequence with an adiabatic half passage pulse shape to
167 minimise the effects of B_1 field inhomogeneity within the volume of interest, along with
168 narrow band proton decoupling (7 kHz bandwidth, 512 samples, 2150 ms repetition time, 576
169 averages, ~20 minutes total acquisition time) as previously described [16].

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170 **MRS analysis**

171 *ATP*

172 ^{31}P spectra were line broadening by 30 Hz and data were averaged over 15 minute windows
173 at 5 minutes intervals across the time-course. The β-ATP peak position was defined in the
174 spectra and peak area calculated across the time course (Figure1). The β-ATP peak provides a
175 way of measuring total ATP because the phosphate signal from ADP overlaps with the α-
176 ATP and γ-ATP peaks. The first time point was taken as a reference to measure changes in
177 ATP and recorded as % of baseline value.

178 Time to reach minimum ATP levels was calculated, and the rate of recovery of absolute ATP
179 was determined using the gradient across the first 4 time points of recovery using linear
180 fitting. For recovery rates, ratios of β-ATP to total phosphorous levels were taken as used in
181 previous studies [3].

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182 *Hepatic Lipids*

183 ^{1}H spectra were zero filled to 1024 datapoints and phase corrected before peak areas were
184 calculated using the AMARES algorithm in jMRUI (Universiteit Leuven, Belgium) [17]
185 (Lorentzian curve fitting of water peak at ~4.8ppm and -$[CH_2]_n$- at ~1.3 ppm). Water
186 suppression was applied during spectral acquisition for better resolution of the fat peak,
187 followed by unsuppressed spectra with identical parameters to determine the water peak area.
188 Peak areas were corrected for T_2 relaxation as determined from previous studies and
189 lipid/water ratios used to determine absolute fat fractions as described by Stephenson *et al*
190 [23].

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191 *Glycogen*

192 ^{13}C spectra were zero filled to 4096 datapoints and 100 Hz line broadening was applied
193 before Lorentzian curve fitting using in house software. Integrals of the C1-glycogen peak
194 (100.4 ppm) and of an external reference peak were measured and ratios used to account for
195 varying loading factors. Quantification was achieved by comparing glycogen/reference ratios
196 with a phantom [18].

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197 **Statistical Analysis**

198 All results are expressed as means (±SD). A repeated measures ANOVA F-test was used to
199 determine a significant effect across the timecourse, and a means difference T-tests were
200 subsequently used on individual time points to determine significant changes. Significances
201 in correlations were determined using linear regression analysis with Pearson correlation
202 coefficients quoted. In all cases significance was attributed to $P < 0.05$. The statistical
203 package used for analysis was SPSS version 21 for Windows (SPSS, Inc., Chicago, IL).

204

205 **RESULTS**

206 *Baseline Hepatic Lipid and Glycogen*

207 The mean baseline liver lipid fat fraction was 4 ± 3 % and correlated significantly with BMI
208 ($R^2 = 0.48$, $P \leq 0.05$) as expected.

209 The mean baseline hepatic glycogen concentration was 219 ± 81 mmol/l and there were no
210 correlations between individual values and age, BMI or baseline liver lipid levels.

211 *ATP Reserves following Oral Fructose Challenge*

212 Mean postprandial hepatic ATP levels began to decline from 15 minutes after the oral
213 fructose challenge (Figure 2). A statistically significant variation from baseline was found
214 across the time course (One way ANOVA F-test, $P < 0.05$). Mean values continued to decline
215 and were significantly below the first two points at t = 30 minutes (86 ± 14%, $P < 0.05$), t =
216 40 minutes (85 ± 16 %. $P < 0.05$) and t = 45 minutes (84 ± 14%, $P < 0.005$) until reaching
217 minimum at = 50 minutes (80 ± 17%, $P < 0.05$). There was a trend for values to recover after
218 50 minutes, but the increase was not statistical significance compared to nadir and levels
219 remained lower than baseline at the end of the study.

220 No subject showed any recovery of ATP levels during the first 6 time points (until t = 40
221 mins). The mean AUC across this period (t=0 to t=40 mins) was 232 ± 19 % h and showed a
222 strong negative correlation with BMI ($R^2 = 0.65$, $P < 0.01$).

223 *Time to minimum ATP*

224 For two subjects the minimum ATP time point was at the end of the scanning period, and as
225 such the final time point was taken as their time to minimum ATP (which may in fact have
226 been after the scan period). A significant negative correlation was found between time to

227 minimum ATP and BMI ($R^2 = 0.74$, $P < 0.005$) as shown in Figure 3. No such correlation
228 was observed with age ($R^2 = 0.01$, $P = 0.78$) or baseline glycogen ($R^2 = 0.003$, $P = 0.88$) but
229 the correlation approached significance with baseline liver fat ($R^2 = 0.39$, $P = 0.07$).

230 *Rate of recovery*

231 Figure 4 shows the relationship between rate of recovery and baseline glycogen reserves,
232 which had a strong negative correlation that was statistically significant ($R^2 = 0.71$, $P < 0.05$).
233 This correlation was not observed with BMI, liver fat, or any other baseline measures.

234

235 **DISCUSSION**

236 The underlying physiological hypothesis of this study is that ATP homeostasis, which
237 provides a measure of AMPK activity, acts as a biomarker for NAFLD and NASH. Rather
238 than fructose infusion, this study explored using ^{31}P MRS following an *oral* fructose
239 challenge, which is more physiological, more patient-acceptable and much simpler to
240 administer. The results showed that after oral consumption there is a measurable decline in
241 ATP reserves (β-ATP) followed by a partial recovery. This observation is characteristic of
242 fructose metabolism and can be explained as a result of the immediate rapid phosphorylation
243 of the monosaccharide. Under normal physiological conditions an increased cellular level of
244 adenosine monophosphate (AMP) activates AMPK resulting in the regeneration of ATP,
245 whereas under conditions where AMPK activity is lower (e.g. following fructose
246 consumption) the production of uric acid is favoured over ATP (supplementary material). In
247 addition to this, fructose has been shown to up-regulate Glut5 and Fructokinase [19], and
248 subjects with NAFLD and a higher intake of fructose have been shown to have a greater
249 hepatic mRNA expression of fructokinase [20].

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250 In a small study of 4 subjects Buemann *et al*. tested the effects of an oral dose of 30g D-
251 Fructose and D-Tagatose on hepatic ATP reserves at 1.5T [21] and reported no drop in ATP
252 following D-fructose consumption (however, they did find a drop following D-Tagatose
253 which reached a maximum at 51 minutes). The data from the present study suggests that a
254 greater concentration of fructose and high resolution spectra (3T scanner) may be required to
255 observe significant reductions.

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256 In the present study ATP levels took longer to recover compared to previous infusion studies.
257 This is probably due to the extra stages necessary to transfer fructose to the hepatic tissue,
258 namely gastric emptying and intestinal absorption. Gastric emptying has been shown to be

dependent on meal energy and volume [22], which becomes relevant to the techniques used here when considering the optimum energy content and volume of the fructose challenge to induce sufficient depletion of hepatic ATP. Another confounding factor is the variation in fructose intestinal absorption rates reported in the literature. A previous study showed a high variability in intestinal absorption of fructose in healthy subjects following an oral fructose drink [23]. The amount of fructose used in the present study was sufficient for intestinal absorption and delivery to the liver in all subjects, but this factor should be considered in future experiments, and it may be that lower doses and volumes of fructose will not have the same effect.

This study showed a negative correlation between BMI and time to minimum ATP levels. Given that the hepatic ATP response is a combination of depletion and recovery and fructose is known to deplete ATP reserves, these findings suggest that individuals with lower BMI have a more effective hepatic ATP recovery in response to a high fructose challenge. This result may be confounded by changes in gastrointestinal function, but also confirms previous studies that have shown that obese subjects have an impaired efficiency of ATP replenishment [2]. Surprisingly this correlation was not observed with liver fat levels as might be expected. Previous studies have shown that there is an impaired hepatic ATP homeostasis in Type2 diabetes [24] and it has been suggested that this may precede the development of steatosis in these patients [3]. Whether or not there is a causal link between rates of ATP synthesis and metabolic disorders remains to be established. Related to this, it has been suggested that regular consumption of fructose upregulates fructokinase and that this may be a factor in NAFLD development and the high incident rates observed currently [20]. In the present study we did not acquire a full dietary history, but future studies in this area should explore the effects of prior exposure and its relevance to ATP depletion and recovery rates and steatosis.

This experiment required 2 hours of scanning on a high field MRI scanner , which may be impractical and costly in a clinical setting. However, it is possible that other related measures may provide a more convenient marker. For example, there was a significant negative correlation between the AUC over the first 6 time points and BMI. These measures can be made over a shorter scan duration. Future studies should consider a wider range of liver fat, as well as NAFLD and NASH patients. In particular, studies that separate BMI from liver fat to determine which of these is a better predictor of ATP homeostasis, although admittedly it would be difficult to recruit for this given the correlation between BMI and liver fat.

Baseline glycogen measurements gave a wide range of values, which suggests variability in the timing and content of the previous evening meal across subjects [16]. Whilst this may reveal a potential limitation in the study design, the results showed for the first time a significant negative correlation between rates of ATP synthesis and baseline glycogen levels which may be relevant to patients with glycogen storage disease and other metabolic disorders. Previous studies have shown that a fructose load activates glycogen synthase resulting in increased glycogenesis, and also that fructose-1-phosphate produced during fructose metabolism is a competitive inhibitor of phosphorylase *a* [25] resulting in a slowed glycogenolysis. These factors result in an increase in glycogen synthesis following fructose consumption. The relationship between glycogen levels and ATP reserves has been explored in a number of publications and correlations between glycogen synthesis and ATP turnover in muscle [26] and between absolute hepatic glycogen levels and total hepatic ATP content during glycogen repletion [27] have been reported. This has been explained as the need for increased uridine triphosphate (UTP) during periods when unidirectional flux of glycogen synthesis is greater than glycogenolysis, which results in greater ATP synthesis. A possible explanation for the negative correlations between rates of ATP synthesis and baseline glycogen levels observed in the present study is that there is a greater demand from hepatic

309 glycogen in subjects with lower baseline glycogen levels, resulting in an increased rate of
310 glycogen synthesis and indirectly ATP synthesis. Whilst it is beyond the scope of this study
311 to determine this causal link, this study shows that baseline hepatic glycogen levels are an
312 important factor in the ATP response to fructose.

313 The present study has some limitations. Firstly, breath samples were not obtained to estimate
314 levels of intestinal fructose malabsorption [28]. Measuring changes in serum uric acid would
315 also be ideal as hyperuricemia has been associated with an impaired hepatic ATP
316 homeostasis in response to high fructose intake [3]. Future experiments should obtain blood
317 samples to measure this also. Secondly, histological comparisons were not made due to the
318 ethical considerations of liver biopsies in healthy subjects. As such, although all subjects in
319 this study had no known liver health problems this was not confirmed through histological
320 analysis. Other studies should investigate the postprandial ATP effects in patients with
321 NASH in comparison with healthy weight and obese people, as well as individuals with Type
322 2 diabetes. Similarly, all subjects in this study were healthy non-obese male volunteers and it
323 should be acknowledged that the response may be different in women or an obese cohort.
324 Subjects also found 500 ml fluid difficult to consume and the scan time was long and
325 potentially uncomfortable. Future studies should optimize the experimental protocol, in
326 particular the time duration, time resolution and volume or concentration of fructose
327 challenge used.

328 In summary, this study has shown that depletion in hepatic ATP reserves following an oral
329 fructose challenge is observable using ^{31}P MRS in healthy subjects, allowing for a completely
330 non-invasive assessment of ATP synthesis. BMI was negatively correlated with the time to
331 minimum ATP levels and with ATP levels immediately post consumption indicating an
332 impaired hepatic energy homeostasis in subjects with higher BMI.

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339 **REFERENCES**

340 1 Cortez-Pinto, H., J. Chatham, V. Chacko, C. Arnold, and A.M. Diehl. Impaired liver ATP
341 homeostais in human nonalcoholic steatohepatitis (NASH). *Gastroenterology*, 1999, **116**(4),
342 A1116-A1116;
343 2 Nair, S., V.P. Chacko, C. Arnold, and A.M. Diehl. Hepatic ATP reserve and efficiency of
344 replenishing: Comparison between obese and nonobese normal individuals. *American*
345 *Journal of Gastroenterology*, 2003, **98**(2), 466-470;
346 3 Abdelmalek, M.F., M. Lazo, S. Bonekamp, A.M. Diehl, and J.M. Clark. Increased Dietary
347 Fructose Impairs Hepatic Atp Homeostasis in Nafld. *Hepatology*, 2009, **50**(4), 777a-777a;
348 4 Ix, J.H. and K. Sharma. Mechanisms Linking Obesity, Chronic Kidney Disease, and Fatty Liver
349 Disease: The Roles of Fetuin-A, Adiponectin, and AMPK. *Journal of the American Society of*
350 *Nephrology*, 2010, **21**(3), 406-412;
351 5 Musso, G., R. Gambino, and M. Cassader. Emerging Molecular Targets for the Treatment of
352 Nonalcoholic Fatty Liver Disease. *Annual Review of Medicine*, 2010, **61**(375-392;
353 6 Oberhaensli, R.D., G.J. Galloway, D.J. Taylor, P.J. Bore, and G.K. Radda. Assessment of
354 Human-Liver Metabolism by P-31 Magnetic-Resonance Spectroscopy. *British Journal of*
355 *Radiology*, 1986, **59**(703), 695-699;
356 7 Roelsgaard, K., H. Stodkildejorgensen, S. Donstrup, and J.C. Djurhuus. Noninvasive
357 Investigation of Parenchymal Liver-Disease Using P-31 Nmr-Spectroscopy. *Nmr in*
358 *Biomedicine*, 1993, **6**(6), 383-388;
359 8 Nair, S., V.P. Chacko, C. Arnold, and A.M. Diehl. Basal hepatic ATP stores and recovery from
360 fructose induced depletion in obese and lean healthy individuals: Implications in the
361 pathogenesis of obesity associated liver diseases. *Hepatology*, 2001, **34**(4), 462a-462a;
362 9 Johnston, R.D., G.P. Aithal, S.D. Ryder, and I.A. MacDonald. Fast-food hyper-alimentation
363 and exercise restriction in healthy subjects. *Gut*, 2009, **58**(3), 469-470;
364 10 Johnson, R.J., L.G. Sanchez-Lozada, and T. Nakagawa. The Effect of Fructose on Renal Biology
365 and Disease. *Journal of the American Society of Nephrology*, 2010, **21**(12), 2036-2039;
366 11 Johnston, R.D., M.C. Stephenson, H. Crossland, S.M. Cordon, E. Palcidi, E.F. Cox, M.A. Taylor,
367 G.P. Aithal, and I.A. Macdonald. No Difference Between High-Fructose and High-Glucose
368 Diets on Liver Triacylglycerol or Biochemistry in Healthy Overweight Men. *Gastroenterology*,
369 2013, **145**(5), 1016-+;
370 12 Schultz, A., D. Neil, M.B. Aguila, and C.A. Mandarim-de-Lacerda. Hepatic Adverse Effects of
371 Fructose Consumption Independent of Overweight/Obesity. *International Journal of*
372 *Molecular Sciences*, 2013, **14**(11), 21873-21886;
373 13 Cortez-Pinto, H., J. Chatham, V.P. Chacko, C. Arnold, A. Rashid, and A.M. Diehl. Alterations in
374 liver ATP homeostasis in human nonalcoholic steatohepatitis - A pilot study. *Jama-Journal of*
375 *the American Medical Association*, 1999, **282**(17), 1659-1664;
376 14 Bawden, S.J., M.C. Stephenson, L. Marciani, G.P. Aithal, I.A. Macdonald, P.A. Gowland, and
377 P.G. Morris. Investigating Alterations in Hepatic ATP levels following Fructose and
378 Fructose+Glucose Ingestion: A Simple Non-invasive Technique to Assess Liver Function Using
379 31P MRS. *Proceedings 20th Scientific Meeting of the ISMRM, Melbourne*, 2012, **1369**(
380 15 Stephenson, M.C., E. Leverton, E.Y.H. Khoo, P.S. M., L. Johansson, J.A. Lockton, E.J. W., P.
381 Mansell, P.G. Morris, and I.A. Macdonald. Variability in fasting lipid and glycogen contents in
382 hepatic and skeletal muscle tissue in subjects with and without type 2 diabetes: a ^{1}H and ^{13}C
383 MRS study. *NMR in Biomedicine*, 2013, **26**(1518 - 1526;
384 16 Awad, S., M.C. Stephenson, E. Placidi, L. Marciani, D. Constantin-Teodosiu, P.A. Gowland,
385 R.C. Spiller, K.C.H. Fearon, P.G. Morris, I.A. Macdonald, and D.N. Lobo. The effects of fasting
386 and refeeding with a 'metabolic preconditioning' drink on substrate reserves and
387 mononuclear cell mitochondrial function. *Clinical Nutrition*, 2010, **29**(4), 538-544;

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388 17 Stefan, D., F. Di Cesare, A. Andrasescu, E. Popa, A. Lazariev, E. Vescovo, O. Strbak, S.
389 Williams, Z. Starcuk, M. Cabanas, D. van Ormondt, and D. Graveron-Demilly. Quantitation of
390 magnetic resonance spectroscopy signals: the jMRUI software package. *Measurement*
391 *Science & Technology*, 2009, **20**(10),
392 18 Jovanovic, A., E. Leverton, B. Solanky, B. Ravikumar, J.E.M. Snaar, P.G. Morris, and R. Taylor.
393 The second-meal phenomenon is associated with enhanced muscle glycogen storage in
394 humans. *Clinical Science*, 2009, **117**(3-4), 119-127;
395 19 Korieh, A. and G. Crouzoulon. Dietary-Regulation of Fructose Metabolism in the Intestine
396 and in the Liver of the Rat - Duration of the Effects of a High Fructose Diet after the Return
397 to the Standard Diet. *Archives Internationales De Physiologie De Biochimie Et De*
398 *Biophysique*, 1991, **99**(6), 455-460;
399 20 Ouyang, X., P. Cirillo, Y. Sautin, S. McCall, J.L. Bruchette, A.M. Diehl, R.J. Johnson, and M.F.
400 Abdelmalek. Fructose consumption as a risk factor for non-alcoholic fatty liver disease.
401 *Journal of Hepatology*, 2008, **48**(6), 993-999;
402 21 Buemann, B., H. Gesmar, A. Astrup, and B. Quistorff. Effects of oral D-tagatose, a
403 stereoisomer of D-fructose, on liver metabolism in man as examined by P-31-magnetic
404 resonance spectroscopy. *Metabolism-Clinical and Experimental*, 2000, **49**(10), 1335-1339;
405 22 Kwiatek, M.A., D. Menne, A. Steingoetter, O. Goetze, Z. Forras-Kaufman, E. Kaufman, H.
406 Fruehauf, P. Boesiger, M. Fried, W. Schwizer, and M.R. Fox. Effect of meal volume and
407 calorie load on postprandial gastric function and emptying: studies under physiological
408 conditions by combined fiber-optic pressure measurement and MRI. *American Journal of*
409 *Physiology-Gastrointestinal and Liver Physiology*, 2009, **297**(5), G894-G901;
410 23 Rumessen, J.J. and E. Gudmandhoyer. Absorption Capacity of Fructose in Healthy-Adults -
411 Comparison with Sucrose and Its Constituent Monosaccharides. *Gut*, 1986, **27**(10), 1161-
412 1168;
413 24 Szendroedi, J., M. Chmelik, A.I. Schmid, P. Nowotny, A. Brehm, M. Krssak, E. Moser, and M.
414 Roden. Abnormal Hepatic Energy Homeostasis in Type 2 Diabetes. *Hepatology*, 2009, **50**(4),
415 1079-1086;
416 25 Bollen, M., S. Keppens, and W. Stalmans. Specific features of glycogen metabolism in the
417 liver. *Biochemical Journal*, 1998, **336**(19-31;
418 26 Lim, E.L., K.G. Hollingsworth, F.E. Smith, P.E. Thelwall, and R. Taylor. Effects of raising muscle
419 glycogen synthesis rate on skeletal muscle ATP turnover rate in type 2 diabetes. *American*
420 *Journal of Physiology-Endocrinology and Metabolism*, 2011, **301**(6), E1155-E1162;
421 27 Gallis, J.L., H. Gin, H. Roumes, and M.C. Beauvieux. A metabolic link between mitochondrial
422 ATP synthesis and liver glycogen metabolism: NMR study in rats re-fed with butyrate and/or
423 glucose. *Nutrition & Metabolism*, 2011, **8**(
424 28 Choi, Y.K., F.C. Johlin, R.W. Summers, M. Jackson, and S.S.C. Rao. Fructose intolerance: An
425 under-recognized problem. *American Journal of Gastroenterology*, 2003, **98**(6), 1348-1353;

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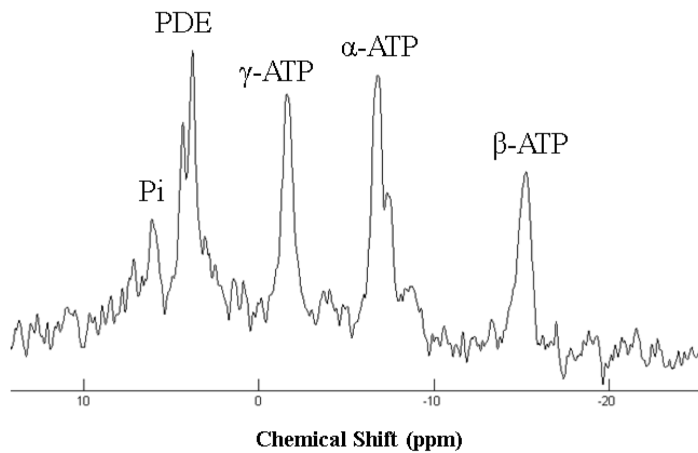

Figure 1. ^{31}P Magnetic Resonance Spectrum from one subjects showing signal peaks from ATP (β-ATP, α-ATP and γ-ATP), phosphodiesters (PDE) and inorganic phosphate (Pi).

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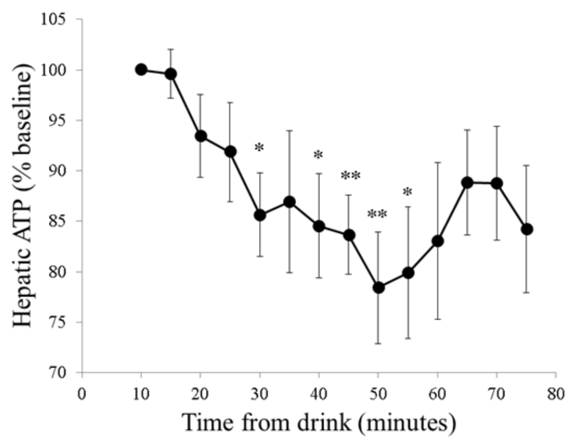

Figure 2. Changes in Hepatic ATP (β-ATP peak) from baseline in response to a 75g oral fructose challenge measured using ^{31}P MRS (n=9). * $P < 0.05$, ** $P < 0.01$

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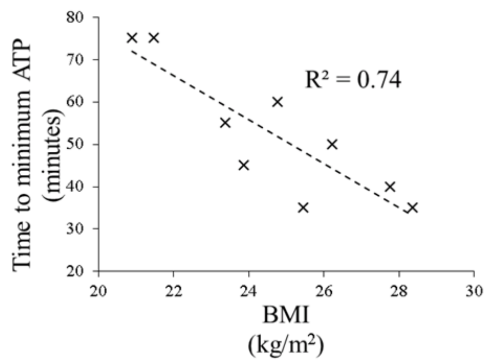

Figure 3. Correlation between time to minimum ATP (β-ATP peak) and BMI (P <0.005).

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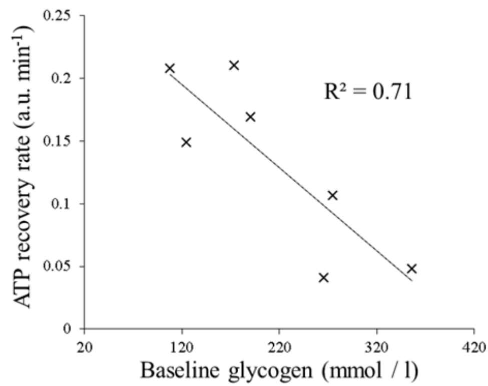

Figure 4. Correlation between rate of ATP recovery (β-ATP peak) and baseline glycogen levels measured using ^{31}P MRS ($P < 0.05$). Recovery rate is measured as the gradient of [β-ATP signal/total phosphorous signal] across the first four points of recovery

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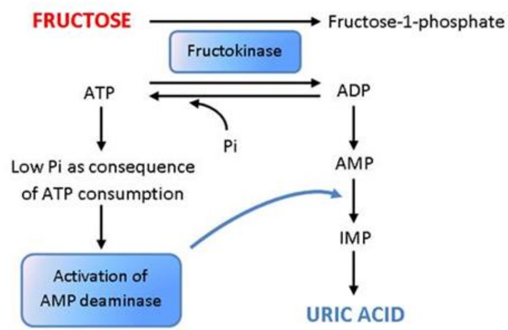

Supplementary Figure. Fructose metabolism showing ATP depletion and Uric Acid production

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