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No Difference Between High-Fructose and High-Glucose Diets on Liver Triacylglycerol or Biochemistry in Healthy Overweight Men

Short title: Hepatic effects of fructose versus glucose

Authors

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Abbreviations used: ATP adenosine triphosphate, EGTA ethylene glycol tetraacetic acid, HFCS high fructose corn syrup, HOMA-IR Homeostasis model assessment of insulin resistance, HTGC hepatic triglyceride concentration, MRS magnetic resonance spectroscopy, NAFLD non-alcoholic fatty liver disease, NEFA non-esterified fatty acid, Pi inorganic phosphate, TAG triacylglycerol, TNF tumour necrosis factor, TR repetition time, TE echo time, VOI volume of interest.

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Author Contributions: RJ secured grant support and was the study designer, recruiter and organiser. RJ also was the principal data collator and analyser. MS designed the magnetic resonance protocol, supervised the scans, and analysed the ^{31}P and liver volume data. HC performed the gas chromatography. SC performed the radioimmunoassay, ELISA and spectrophotometry analyses. EP and EC performed MR scans and aided with analyses. MT supervised the dietetic design and planning. GA provided clinical supervision and advised on the study design and analysis. IM provided biomedical supervision and advised on the study design and analysis.

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Abstract:

Background & Aims: Diets high in fructose have been proposed to contribute to non-alcoholic fatty liver disease (NAFLD). We compared the effects of high-fructose and matched glucose intake on hepatic triacylglycerol (TAG) concentration and other liver parameters.

Design: In a double-blind study, we randomly assigned 32 healthy but centrally overweight men to groups that received either a high-fructose or high-glucose diet (25% energy). These diets were provided during an initial isocaloric period of 2 weeks, followed by a 6-week washout period and then again during a hypercaloric 2 week period. The primary outcome measure was hepatic level of TAG, with additional assessments of TAG levels in serum and soleus muscle, hepatic levels of ATP, and systemic and hepatic insulin resistance.

Results: During the isocaloric period of the study, both groups had stable body weights and concentrations of TAG in liver, serum, and soleus muscle. The high-fructose diet produced an increase of 22±52 μmol/L in serum level of uric acid, whereas the high-glucose diet led to a reduction of 23±25 μmol/L (P<.01). The high-fructose diet also produced an increase of 0.8±0.9 in the homeostasis model assessment of insulin resistance, whereas the high-glucose diet produced an increase of only 0.1±0.7 (P=.03). During the hypercaloric period, participants in the high-fructose and high-glucose groups had similar increases in weight (1.0±1.4 kg vs 0.6±1.0 kg; P=.29) and absolute concentration of TAG in liver (1.70±2.6% vs 2.05±2.9%; P=.73) and serum (0.36±0.75 mmol/L vs 0.33±0.38 mmol/L; P=.91), and similar results in biochemical assays of liver function. Body weight changes were associated with changes in liver biochemistry and concentration of TAGs.

Conclusions: In the isocaloric period, overweight men on neither a high-fructose nor a high-glucose diet developed any significant changes in hepatic concentration of TAGs or serum levels of liver enzymes. However, in the hypercaloric period both high-fructose and high-glucose diets produced significant increases in these parameters without any significant difference between the 2 groups. This indicates an energy-mediated, rather than specific macronutrient-mediated effect. Clinical trials.gov no: NCT01050140

Keywords

NAFLD, obesity, steatosis, insulin resistance

Introduction

Background

The current epidemic of non-alcoholic fatty liver disease (NAFLD) has a partial association with insulin resistance and obesity, though genetics and lifestyle have also been implicated. The specific impact of individual dietary and exercise factors on hepatic lipid metabolism remains largely unclear.[1]

Fructose has been proposed to have a greater hepatic steatogenic potential than glucose.[2] The initially separate pathways for hepatic catabolism of glucose and fructose converge and are identical following the formation of pyruvate. Pre pyruvate stages of glucose catabolism involve feedback inhibition via glucokinase and phosphofructokinase, which are absent for fructose. The full clinical impact of this lack of inhibition is unclear although increased serum uric acid is reported following a high fructose, compared to glucose, intake.[3]

The principal outcomes of pyruvate catabolism are energy release via anaerobic and aerobic pathways, or energy storage with glycogen or triacylglycerol (TAG) formation. A high intake of fructose, as compared to glucose, has been reported to result in a greater rate of de-novo lipogenesis[4] and hepatic venous lactate concentration.[5] As a result, some scientists have predicted greater whole body and hepatic TAG concentrations with intakes high in fructose as opposed to glucose. However, a greater lipogenic rate with fructose has been inconsistently shown,[6] and there is no difference in whole body TAG stores.[7]

Some clinical data have indirectly linked high fructose intakes with NAFLD. Insulin resistance and dyslipidaemia has been attributed to a high fructose, as opposed to glucose, intake.[4] Secondly, some groups have reported a higher fructose intake in NAFLD patients than controls.[8] It is important to note that the fructose and glucose content in most foodstuffs is practically identical. Caloric sweeteners contribute to 84% of dietary fructose intakes.[9] Sucrose, which contributes to more than 90% of caloric sweeteners globally, is a disaccharide containing equal amounts of fructose and glucose.[10] High fructose corn syrup (HFCS) is an additional sweetener in the United Sates. The principal HFCS product used in soft drinks differs little in its fructose and glucose contents (55% vs. 42%), and HFCS acts metabolically identical to sucrose.[10] Of the 16% of natural dietary fructose more than 80% comes from fruit and vegetables which rarely differ substantially in their fructose and glucose content.[6, 9] As a result, fructose and glucose intakes are similar and so much of the prior dietary analyses that have attributed outcomes to fructose will equally reflect intakes of glucose.

Objectives

The study aimed to compare the hepatic outcomes of a high fructose and a matched glucose intake. The study was performed in two novel scenarios: firstly, in centrally overweight healthy men with no evidence of liver disease; secondly, in both an energy neutral state (isocaloric or weight maintaining) and an energy overfeeding state (hypercaloric or weight gaining).

Methods

Trial design and setting

This double blind study took part at Nottingham University, UK. Healthy subjects were randomized to a high fructose or high glucose intake for 2 separate intervention periods of 2 weeks. The initial 2 weeks were energy balanced (isocaloric), followed by a 6 week washout, a repeat baseline assessment, and then a final 2 weeks of energy overfeeding (hypercaloric). This was a parallel study with a 1:1 ratio and no monosaccharide crossover.

Participants

Healthy men aged 18 to 50 years were recruited with a body mass index (BMI) between 25 to 32kg/m^2, and a waist circumference greater than 94cm. Subjects were excluded if they had a known active health problem, or an abnormal health screen which comprised: liver and renal biochemistry, serum ferritin, hepatitis B and C serology, full blood count, random blood glucose, electrocardiogram, and blood pressure. Exclusions for lifestyle factors included self-reported alcohol intake greater than 21 units per week, weight change greater than 3kg in prior 3 months, regular high intensity physical activity, vegetarianism, and habitual fructose intake from drinks greater than 25g per day (equivalent to that in 500ml of a standard fizzy drink). Subjects with contraindications for MRI scanning were excluded.

Interventions

During both intervention periods the monosaccharides provided 25% of the subjects' predicted total daily energy requirements and were consumed 4 times a day in divided amounts mixed with 500ml of water. Isocaloric status was ensured during the first period by the provision of daily foodstuffs containing 75% of individual energy requirements. The energy contributions from carbohydrate, protein and fat from the combined foodstuffs and monosaccharides provided were 55/15/30%, in line with prior work.[4, 11, 12] In addition, saturated fatty acid content was within recommended levels.[13] Hypercaloric status during the second assessment period resulted from *ad libitum* habitual foodstuff consumption with the addition of identical amounts of the monosaccharides to that during the isocaloric period. Additional sweetened drinks were forbidden. Continuation of habitual exercise was encouraged during the entire study period.

Outcomes

The primary outcome measure was hepatic steatosis, with secondary measures including hepatic ATP content, insulin resistance, and enzymes. In order to aid interpretation of these outcomes whole body metabolic data was collected to explain these findings including weight, satiety, insulin resistance, non-hepatic triglycerides, inflammatory cytokines and adipokines.

Pre study assessments

The Henry modified Schofield formulae[14] were used to predict resting energy expenditure (REE). Subjects were allocated activity factors of 1.5, 1.6 and 1.7 if the short form of the International Physical Activity Questionnaire (IPAQ)[15] classified them as leading low, medium or high levels of activity respectively. Subjects' habitual food intakes were assessed by self-completed 3 day unweighed food diaries, using household names. The nutrients and energy content was analyzed using 'Microdiet' software (Downlee Systems, Salford, UK). A Lunar Prodigy DEXA scanner (GE Medical, Bedford, UK) quantified whole body adiposity at baseline.

Assessment protocol

The assessment processes were identical pre and post each intervention period. An initial early morning fasted MRI and MR spectroscopy was followed by 20 minutes of rested indirect calorimetry. Finally venesection was performed and the subjects self-completed visual analogue scales responding to statements over their fullness, satisfaction by food, hunger and energy during the previous week. Following this, 20 out of the 32 subjects left the physiology laboratory. Twelve subjects remained fasted and underwent a hyperinsulinaemic euglycaemic clamp. One week into each intervention there was a brief fasted visit for venesection and monitoring of weight, compliance, side effects, and satiety.

MR measurements were made on a Philips Achieva 3T system using the Q-body coil for ^{1}H transmission and reception and a 14cm single loop coil for ^{31}P measurements. Liver volume was measured using a T_1-weighted Turbo Field Echo image (192x192 2.08x2.08mm^2 voxels in-plane, 36 7mm slices, Echo time/Repetition time (TE/TR) = 1.5/3.11ms, flip angle = 10°). All slices were collected within a single bre ath-hold. Liver volumes were assessed by region drawing in Analyze9 (AnalyzeDirect, Lenexa, TX, USA).

For measurement of hepatic TAG content (HTGC) a 27cm^3 cubic voxel was positioned within the right liver lobe. ^{1}H MR spectra were collected using a respiratory triggered Point RESolved Spectroscopy (PRESS) sequence (TR = 5000ms, 1024 points, bandwidth=2000Hz). 24 spectra were acquired with TE =40ms, and 8 spectra each with TE=50ms, 60ms and 70ms to allow individual correction for T_2 relaxation. Spectra were individually phase corrected, realigned and averaged across TE before peak areas from water and fatty acid methylene groups were calculated using an in-house built program. Absolute lipid content was calculated as described.[16]

For calf TAG assessments ^{1}H MR spectra were acquired from the soleus muscle using a STimulated Echo Acquisition Mode (STEAM) sequence (VOI=20x20x50mm^3, bandwidth=2000Hz, samples=1024, TE/mixing time (TM)/TR=13/17/7000ms). 16 averages were collected with water-suppressed and 2 without. Spectra were individually phase corrected and realigned before the peak areas of intra-myocellular lipid (IMCL) and extra-myocellular lipid

(EMCL) (CH_2 and CH_3) were fitted using AMARES in jMRUI.[17] Correction to absolute lipid content was performed using previously acquired T_2 values (T_2 water=31ms, T_2 IMCL CH_2=89ms, T_2 EMCL CH_2=78ms).

^{31}P MRS spectra were acquired using a respiratory triggered, Image Selected In vivo Spectroscopy (ISIS) sequence, with broadband ^{1}H decoupling (VOI = 60x60x60mm^3, TR=5000ms, 1024 samples, bandwidth=3000Hz, 96 averages). Spectra were zero-filled to 2048 points and 10Hz Lorentzian line broadening was added before phase correction, realignment and spectral averaging. Peaks were fitted using AMARES[17] with singlet peaks fitted for GPE, GPC, PE and PC; doublets for γ-ATP and α-ATP; and a triplet for β-ATP(J=20Hz). The inorganic phosphate peak was fitted to two peaks to account for intra and extracellular compartmentation. GPE and GPC results were summed to give phosphodiesters (PDE) and similarly PE and PC are given as phosphomonoesters (PME).

Indirect calorimetry was performed on the fasted, rested, awake and supine subjects. Air was collected via a ventilated canopy and analyzed by a Gas Exchange Monitor (Nutren Technologies, Manchester, UK). The hyperinsulinaemic euglycaemic clamp included an infused glucose tracer. A loading bolus dose of 4mg/kg of deuterated glucose [6, 6-$^2H_2$] (CK Gas Products, Hook, Hampshire, UK) was followed by a continuous infusion at a constant 40μg/kg/min for 5 hours. After 2 hours a peripheral insulin infusion was started at 30 mIU/min/m^2 in a 3 hour single stepped clamp according to standard protocol.[18] Arterialized blood glucose concentrations were measured every 5 minutes, and maintained at 4.5mmol/L by a variable rate infusion of 20% glucose (Baxter Healthcare, Norfolk, UK) spiked with 1g (1%) of deuterated glucose. Endogenous glucose production (EGP) was determined by subtracting the rate of exogenous infusion from its rate of appearance (Ra), and correcting for body weight.[19] Non-oxidative glucose disposal (NOGD) rates were the difference between the rates of systemic glucose disposal and carbohydrate oxidation during the final 30 minutes.

Fresh whole blood was analyzed for glucose (YSI 2300, Yellow Springs Incorporated, Ohio, USA). Serum and plasma samples were centrifuged for 10 minutes at 4000 rpm and at 4^{o}C. The supernatant was frozen at –80^{o}C prior to single batch analysis of each analyte. For analysis of non-esterified fatty acids (NEFAs) 75μl of EGTA-glutathione and 5μl of tetrahydrolipostatin were added to lithium-heparin tubes. Liver enzymes, uric acid, NEFAs, CRP, and TAGs were quantified by spectrophotometry by an ABX Pentra 400 (Horiba Medical, Montpelier, France). Radioimmunoassays quantified insulin with a ^{125}I-labeled Millipore HI-14K kit, and leptin with a Millipore HL-81K kit. TNFα was quantified by ELISA assays using Becton Dickinson antibodies. Plasma 6, 6-$^2H_2$ glucose isotopic enrichment was measured by gas chromatography mass spectrometry with a trace DSQ system (Thermofinnigan, Hemel Hempstead, UK).[20]

Sample size

The primary outcome was a change in hepatic TAG concentration (HTGC). No clinical data could directly guide power calculations. 35% energy overfeeding with fructose for 7 days increased HTGC by 79% with no control arm.[21] Insulin resistance and serum lipid profiles differ after 2 weeks of 25% energy *ad libitum* overfeeding with glucose and fructose.[4] As a result the same duration and amount of energy overfeeding was selected. We estimated a 75% increase in HTGC with fructose, and considering a type 1 error of 0.05 and a type 2 error of 0.20, 15 subjects were needed per arm.

Randomization process

Pharmacists dispensed identically labeled monosaccharide packets of either fructose or glucose according to a computer generated randomization list. Randomization was non-stratified with variable sized blocks. The study was designed to have 6 subjects in each group undergoing the assessments with a hyperinsulinaemic euglycaemic clamp, and 10 without. One subject in the fructose group was not able to tolerate the hyperinsulinaemic euglycaemic clamp and was transferred to the non-clamp group. This was inadequately adjusted for by the randomization package and hence there were 15 subjects in the fructose group and 17 in the glucose group.

Blinding

All participants and the investigators who obtained outcome measures were blinded to the allocation process. The pharmacy and randomization teams were not involved in data collection or analysis. Both fructose and glucose were provided as a sweet, fine powder.

Statistical methods

All data are tabulated as the mean ± standard deviation (SD). Comparisons between baseline values were done by the independent samples T test. The Student's T test was used for paired data. Analysis of the significance of change between the groups was made by an independent samples T test of the absolute change between the two paired assessments in both groups. Discrete data were analyzed by Chi squared analyses. Associations were assessed by 2-tailed Pearson correlations. All of the authors had access to study data and reviewed and approved the final manuscript.

Results

Participant recruitment and flow

The study started in April 2010 and completed in December 2010. All assessments were attended with no drop-outs. No participants were excluded from analyses.

Baseline data

The groups were well balanced at baseline, see table 1. All the subjects were centrally overweight: BMI 25.9 to 32.2kg/m^2, and waist circumferences 95.5 to 112 cm. The elevated total body fat percentage (range 26.8 to 45.0%) confirmed that the excess weight reflected adipose, as opposed to muscle tissue.

The 12 subjects who underwent the hyperinsulinaemic euglycaemic clamps matched the whole cohort in age, anthropometry and HOMA-IR (data not presented). The fructose and glucose clamp groups did not differ.

The groups reported similar habitual dietary energy and macronutrient intakes (supplementary table 1). The groups had equally similar predicted resting energy expenditure (2003±165 vs. 1974±158kcal/day, p=0.62) and activity factors (1.63±0.09 vs. 1.65±0.10, p=0.55). Consequently they did not differ in the amount of monosaccharide supplemented during both periods (217.6±16.1 vs. 215.5±13.7g/day, p=0.7) or the total energy provided during the isocaloric period (3276±260 vs. 3243±204kcal/day, p=0.7).

Study outcomes

Differences between the two baseline assessments

The findings at the baseline assessments undertaken before both assessment periods differed only for NEFAs, with a similar reduction in both groups (supplementary table 3). As a result only the first baseline assessment values of each parameter are presented in table 1. The changes from the first baseline assessment during the isocaloric period are presented in table 2, whereas the changes from the second baseline assessment during the hypercaloric period are presented in table 3.

Outcomes measured in the fasted state

At study entry both groups were similar in their fasted state metabolic parameters, see table 1. Despite being apparently healthy, serum leptin, TAG, uric acid and HTGC were elevated at study entry in 26, 9, 11 and 17 subjects respectively. There was sub-clinical fasting insulin resistance with normal glucose (4.50±0.20mmol/L) yet elevated HOMA-IR (3.58±1.04). Normal liver enzymes were a pre-requisite for study entry.

Impact of the isocaloric and hypercaloric periods on fasted state outcomes

The two differing energy periods were reflected by changes in the subjects' weights and leptin, see tables 2 and 3, and figure 1a. In contrast to this fullness from foods was reported as increased during the isocaloric period and unaltered during the hypercaloric period. Resting energy expenditure was unaltered, whilst carbohydrate oxidation rates increased during both periods, reflecting increased carbohydrate intakes.

During the isocaloric period there was a rise in insulinaemia (+1.8±3.6mIU/L) and a small reduction in liver enzymes in both groups combined, see table 2. The remaining variables were unaltered including TAG concentrations in the liver (+0.11±2.1%) (figure 1b), serum (+0.03±0.59mmol/L) (figure 1d) and soleus muscle (+0.3±3.8%) (figure 1c).

In contrast, during the hypercaloric period there was an increase in both groups for TAG concentrations in liver (1.88±2.72%, p<0.001), serum (0.35±0.57mmol/L, p=0.002), and soleus muscle (1.3±3.4%, p=0.04). Liver enzymes were elevated during this period along with inorganic phosphorus (Pi), though in the absence of changes in other ^{31}P parameters, CRP, IL6 or TNFα. Ultimately, this period resulted in a 0.8±1.2% gain in body weight and a 39±49% gain in HTGC. Energy status appeared to significantly impact on outcomes, as during the isocaloric period changes in weight were positively associated with changes in HTGC and liver enzymes, but not inflammatory markers (table 6). During the hypercaloric period there were positive associations with liver enzymes and IL6. Fructose and glucose did not differ in their pattern of associations.

Impact of the monosaccharide type on fasted state outcomes

The outcomes were less influenced by the type of monosaccharide than by energy status. During the isocaloric period, the monosaccharide groups only differed for their amount of change in uric acid (fructose +22±52µmol/L vs. glucose -23±25µmol/L, p=0.004) and HOMA-IR (fructose +0.76±0.9 vs. glucose +0.13±0.7, p=0.03). There were no differences between the monosaccharides in any parameter during the hypercaloric period. Fructose and glucose did not differ in any hepatic outcome measures.

Outcomes measured in the hyperinsulinaemic state

These analyses were performed on a subset of 12 subjects, and the study was not specifically powered for these assessments. Steady state insulinaemia was achieved during the final hour of the clamps at a concentration roughly 5 fold greater than at fasting (supplementary table 4). These steady state insulin concentrations did not differ between the four assessments or the two groups. At baseline there was impaired glucose disposal (table 4) which is in line with the baseline fasted HOMA-IR values. There was hepatic insulin resistance as evidenced by a failure to completely abolish endogenous glucose production (EGP) (table 4).

Hepatic insulin resistance and whole body glucose disposal were unaltered by the interventions (table 5). In contrast, NOGD (a proxy for glycogen synthesis) was reduced during the hypercaloric period, reflecting the overfed, and hence glycogen replete, state.

Side effects

The fructose group reported a trend for greater abdominal and diarrhoeal symptoms (8/15 versus 4/17, p=0.08), though this was only problematic in 1 subject. The macronutrient

profile of the food provided during the isocaloric period was similar to habitual intakes (supplementary table 2).

Discussion

Limitations

We acknowledge some limitations of our study. Firstly, a large amount of monosaccharide was supplied to provide the subjects with 25% of their energy requirements. Similar energy contributions are however feasible within habitual dietary patterns. According to the Low Income Diet and Nutrition Survey, non-milk extrinsic sugars provide low income UK men with 14.6±8.8% of their energy intakes, with an upper 2.5% percentile contribution of 34.1%.[22] A further issue was that the monosaccharides were provided as their constituent powders as opposed to either incorporated into the matrix of a foodstuff, or as a constituent of sucrose. This resulted in a nutrient, as opposed to dietary pattern, comparison. There is however no evidence that fructose differs in its metabolic outcomes when provided as a hexose or when bound to glucose in sucrose.[23-25] Other issues include the lack of cross-over between the groups which weakened analyses between the monosaccharides. The relatively short-term nature of the intervention means that an effect with a longer dietary alteration cannot be excluded. The hyperinsulinaemic euglycaemic clamp was only performed in a representative subset of the group, and the study was not specifically powered for this assessment. Hyperinsulinaemia was however stably reproduced at all four assessments with concentrations similar to those found in obese post-prandial subjects.[26] During the hypercaloric period foodstuffs were not provided, and hence nutrient intakes not precisely regulated. *Ad libitum* energy overfeeding is the standard dietary method employed in related studies.[4, 11, 27, 28] It improves the translatability of the findings into dietary intakes but fails to exclude for potential confounders. The magnitude of these would be predicted to be small in this study as habitual intakes of macronutrients, including fructose, were identical between the groups (supplementary table 1). Finally, it was not ethical to perform histological analyses via serial liver biopsies.

Gastrointestinal symptoms develop in a half of subjects who consume 50g of fructose alone on an empty stomach.[29] The current dose of 54±4g resulted in a similar proportion reporting mild symptoms, though not significantly greater than with glucose. Attempts to reduce malabsorption were made by concurrent foodstuff consumption.[30] The impact on outcomes of any malabsorption is unclear. There are no reliable biomarkers for fructose or glucose absorption. The presence of symptoms did not impact on weights.

Generalizability

The sharply defined cohort reduced variation in baseline metabolic state but it also limited the translatability of the findings. The cohort did however represent a substantial

proportion of the UK adult male population. Forty eight percent of UK males have both a waist greater than 94cm and a BMI between 25 and 35kg/m^2,[31] and 72% of UK males report drinking less than 21 units of alcohol a week.[32] Only men were recruited as gender appears to impact on fructose metabolism via hormonal or anthropometric mechanisms.[33]

Interpretation

We compared the effects of high intakes of glucose or fructose in isocaloric and hypercaloric conditions with predictable weight changes. The isocaloric period not only maintained weight, but also levels of leptin, rates of glycogen synthesis (non-oxidative glucose disposal), and serum and ectopic TAG stores. The hypercaloric period altered all of these parameters with increased HTGC, and liver volume, biochemistry and Pi content. Energy balance appeared to be the key determinant of outcomes. The lack of change in HTGC during the isocaloric period and increase in the hypercaloric period suggests an exquisite hepatic sensitivity to excess energy, as opposed to specific monosaccharide. Interestingly, satiety was unaltered in spite of the weight gain during the hypercaloric period. This reinforces the notion of 'hidden calories' in drinks.[34]

Fructose and glucose overfeeding resulted in equal outcomes in terms of: TAG in the serum, liver and muscle; hepatic volume, insulin resistance, and ^{31}P metabolites; and whole body substrate oxidation. This was more than just maintenance of the *status quo* as the hypercaloric period resulted in a clear hepatic challenge. The responses to the challenge were markedly similar in both groups (figure 1) with similar absolute elevations in key parameters including weight (+1.0±1.4% vs. +0.6±1.1%); and TAG in liver (+40±47% vs. +38±51%) and serum (+35±66% vs. +28±30%); and liver biochemistry. Hence the message appears clear that liver-related parameters do not differ with a 2 week period of glucose or fructose overfeeding, except for the impact from any energy excess. This lack of difference conflicts with the perception of fructose being highly lipogenic. There is however considerable debate as to whether any such effect exists.[6] High doses of fructose result in greater fasted serum TAG than matched intakes of starch,[35] but not in comparison to glucose[4, 36, 38] as has been shown again here. A greater postprandial triglyceride response following fructose than glucose was reported and attributed to increased DNL and reduced insulin excursion with fructose resulting in a lower activation of adipose tissue lipoprotein lipase (LPL).[4, 12] Only 1.5% of the energy from fructose overfeeding is used for DNL[4, 6] and the magnitude of any difference with glucose is minimal.[37]

The paper by Stanhope et al. generated a comprehensive metabolic assessment but only a limited assessment of adipose tissue storage and distribution.[4] There were significant increases in body weight, waist circumference and total body fat which did not differ between fructose and glucose. Visceral adiposity, as assessed by a single slice umbilical CT scan, was greater with fructose. Visceral adiposity has been linked to NASH via an increased portal NEFA concentration, a pro-inflammatory cytokine profile and hepatic insulin resistance.[38-41] Stanhope et al. did not present NEFA data; reported that fructose had a negative impact on whole body,

as opposed to hepatic, insulin sensitivity; whilst fructose appeared to result in a favorable adiponectin/TNFα gene expression ratio in gluteal subcutaneous adipose tissue and glucose resulted in a deleterious ratio . As a result we do not feel that hepatic outcomes can be inferred from this paper's visceral adiposity findings. Interestingly the current TAG changes were not associated with evidence of systemic inflammation as assessed by CRP, TNFα or IL6.

The lack of an alternative nutrient comparator in this study means that we cannot be certain that these changes are solely the effects of energy overfeeding, or specific to energy overfeeding with monosaccharides. To date the hepatic outcomes from carbohydrate overfeeding has not been compared with a matched amount of energy from an alternative macronutrient.[42] This current data does however highlight the impact that a short change in lifestyle can have on HTGC.

The two groups did differ in terms of their HOMA-IR and uric acid outcomes, findings which appear unrelated, isolated and hence whose interpretation is uncertain. Elevations in fasted glucose and insulin concentrations have previously been reported with both fructose and glucose overfeeding.[4, 27, 36] The current study found such changes only during the isocaloric period with fructose. There is no clear explanation for the difference with the glucose group during this period. The trend for a greater pre-existing HOMA-IR value in the fructose group may have impacted. We caution against over interpretation of this finding, especially in light of the current matched rates of glucose disposal and endogenous glucose production (EGP). The effects of glucose and fructose overfeeding on systemic insulin resistance and EGP have previously been compared twice by a Swiss group using the clamp method.[36, 43] A greater EGP with fructose has been the only difference reported; though this was in a sub-group analysis of a larger study with no baseline data to fully quantify the impact of both arms.[43]

Uric acid concentrations increased with fructose during both periods and reduced with glucose. This was only significant during the isocaloric period. The lack of associated hepatic ^{31}P MRS metabolite changes during this period may be because only fasted assessments were undertaken.[44] In accordance with the increase in hepatic Pi during the hypercaloric period with fructose, an increase in serum Pi has been reported with sucrose overfeeding,[45] with no prior controlled fructose data to our knowledge. The rise in uricaemia with fructose has been attributed to its lack of pre pyruvate feedback inhibition, though this outcome has not been consistently shown.[46] The mechanism behind the current reduction with glucose is unclear. It may reflect a lower purine content in the supplied than the habitual foods (analyses not possible).

The strengths of this study are the provision of all foodstuffs in the isocaloric phase, repeated baseline assessments, differing energy periods, ^{31}P MRS assessments, and the use of a hyperinsulinaemic euglycaemic clamp within a subset. The selection of glucose as the comparator maintained energy and macronutrient balance between the groups. Assessments of liver lipid, volume, biochemistry and inflammatory markers formed a global hepatic profile. A wider metabolic picture was formed with data on non-hepatic lipids, whole body metabolism

and insulin resistance. The repeat baseline confirmed that parameters had returned to baseline during the washout.

The cohort was recruited on the basis of being centrally overweight with no evidence of liver or metabolic disease. Central (visceral) obesity drives systemic and hepatic insulin resistance, which appears to result in an increased metabolic response to fructose.[21] The current baseline insulin resistance can be attributed to body composition. A cohort of 376 men with a similar BMI and body fat percentage to this cohort (28.8±3.9kg/m^2 and 33%) reported similar fat free mass glucose disposal rates of 8.2mg/kgFFM/min to the current 7.2mg/kgFFM/min.[47]

Two recent papers have also studied the hepatic effects of fructose versus glucose overfeeding.[27, 36] Both assessed a smaller number of slim subjects with a mean BMI less than 25kg/m^2, and had significant methodological limitations. The paper by Silbernagel *et al.* involved unsupervised overfeeding without weight monitoring or supplied foodstuffs. Significant weight gain occurred with glucose but not fructose. This disparity in weight, and hence presumably energy intakes, hampers all data interpretation. The paper by Ngo Sock *et al*. involved two periods of glucose or fructose overfeeding and a further control period that differed from the interventions by 800kcal/day. Data was only collected at the end of each period with no baseline assessments, and the washout periods were very short at 2-3 weeks. As a result it is impossible to ascertain how much can be truly ascribed to each intervention. This current study adds to these with similar outcomes in terms of triglyceride in the serum, liver and muscle; systemic, adipose and hepatic insulin resistance; hepatic volume and ^{31}P metabolites; and whole body substrate oxidation.

Conclusions

Features of NAFLD including steatosis, and elevated serum transaminases and triglycerides occurred during energy overfeeding. The present study reports no difference in these parameters between fructose and glucose. The greater uric acid concentration with fructose was evidence of a reduced pre pyruvate metabolic control, though it appears to have no hepatic impact in terms of hepatic volume, TAG storage, insulin resistance, glycogen synthesis, fasted ATP content, and biochemical assays of liver function. As such, any advice on low fructose diets in NAFLD remains unjustified. Further assessments are needed to assess if the energy overfeeding changes are monosaccharide specific, and to assess the outcomes of low monosaccharide intakes in NAFLD patients.

Study approval, registration and funding

The Nottingham University and NHS trust research ethics and research and development committees approved the study. The trial is registered at ClinicalTrials.Gov, number NCT01050140. Core charity, London, UK, provided a supporting grant but did not influence the study design, or the data collection, analysis and interpretation.

References

1. Thoma C, Day CP, Trenell MI. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. J Hepatol 2012;56:255-66.
2. Lim JS, Mietus-Snyder M, Valente A, et al. The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. Nat Rev Gastroenterol Hepatol 2010;7:251-64.
3. Cox CL, Stanhope KL, Schwarz JM, et al. Consumption of fructose- but not glucose-sweetened beverages for 10 weeks increases circulating concentrations of uric acid, retinol binding protein-4, and gamma-glutamyl transferase activity in overweight/obese humans. Nutr Metab (Lond) 2012;9:68.
4. Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 2009;119:1322-34.
5. Brundin T, Wahren J. Whole body and splanchnic oxygen consumption and blood flow after oral ingestion of fructose or glucose. Am J Physiol 1993;264:E504-13.
6. Livesey G. More on mice and men: fructose could put brakes on a vicious cycle leading to obesity in humans. J Am Diet Assoc 2011;111:986-90; author reply 990-3.
7. Sievenpiper JL, de Souza RJ, Mirrahimi A, et al. Effect of fructose on body weight in controlled feeding trials: a systematic review and meta-analysis. Ann Intern Med 2012;156:291-304.
8. Ouyang X, Cirillo P, Sautin Y, et al. Fructose consumption as a risk factor for non-alcoholic fatty liver disease. J Hepatol 2008;48:993-9.
9. Marriott BP, Cole N, Lee E. National estimates of dietary fructose intake increased from 1977 to 2004 in the United States. J Nutr 2009;139:1228S-1235S.
10. White JS. Straight talk about high-fructose corn syrup: what it is and what it ain't. Am J Clin Nutr 2008;88:1716S-1721S.
11. Le KA, Faeh D, Stettler R, et al. A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans. Am J Clin Nutr 2006;84:1374-9.
12. Bantle JP, Raatz SK, Thomas W, et al. Effects of dietary fructose on plasma lipids in healthy subjects. Am J Clin Nutr 2000;72:1128-34.
13. Saturated fat recommended level. Scientific Advisory Committee on Nutrition 2008.
14. Henry CJ. Basal metabolic rate studies in humans: measurement and development of new equations. Public Health Nutr 2005;8:1133-52.
15. Hagstromer M, Oja P, Sjostrom M. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. Public Health Nutr 2006;9:755-62.
16. Szczepaniak LS, Babcock EE, Schick F, et al. Measurement of intracellular triglyceride stores by H spectroscopy: validation in vivo. Am J Physiol 1999;276:E977-89.
17. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. J Magn Reson 1997;129:35-43.
18. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214-23.
19. Powrie JK, Smith GD, Hennessy TR, et al. Incomplete suppression of hepatic glucose production in non-insulin dependent diabetes mellitus measured with [6,6-2H2]glucose enriched glucose infusion during hyperinsulinaemic euglycaemic clamps. Eur J Clin Invest 1992;22:244-53.
20. Kury D, Keller U. Trimethylsilyl-O-methyloxime derivatives for the measurement of [6,6-2H2]-D-glucose-enriched plasma samples by gas chromatography-mass spectrometry. J Chromatogr 1991;572:302-6.
21. Le KA, Ith M, Kreis R, et al. Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. Am J Clin Nutr 2009.

22. Nelson M EB, Bates B, Church S, et al. Low income diet and nutrition survey: Foods Standards Agency, 2009.
23. McDevitt RM, Poppitt SD, Murgatroyd PR, et al. Macronutrient disposal during controlled overfeeding with glucose, fructose, sucrose, or fat in lean and obese women. Am J Clin Nutr 2000;72:369-77.
24. Stanhope KL, Griffen SC, Bair BR, et al. Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup-, sucrose-, fructose-, and glucose-sweetened beverages with meals. Am J Clin Nutr 2008;87:1194-203.
25. Tappy L, Le KA, Tran C, et al. Fructose and metabolic diseases: new findings, new questions. Nutrition 2010;26:1044-9.
26. Parra MD, Martinez de Morentin BE, Martinez JA. Postprandial insulin response and mitochondrial oxidation in obese men nutritionally treated to lose weight. Eur J Clin Nutr 2005;59:334-40.
27. Silbernagel G, Machann J, Unmuth S, et al. Effects of 4-week very-high-fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. Br J Nutr 2011:1-8.
28. Perez-Pozo SE, Schold J, Nakagawa T, et al. Excessive fructose intake induces the features of metabolic syndrome in healthy adult men: role of uric acid in the hypertensive response. Int J Obes (Lond) 2010;34:454-61.
29. Rao SS, Attaluri A, Anderson L, et al. Ability of the normal human small intestine to absorb fructose: evaluation by breath testing. Clin Gastroenterol Hepatol 2007;5:959-63.
30. Rumessen JJ, Gudmand-Hoyer E. Absorption capacity of fructose in healthy adults. Comparison with sucrose and its constituent monosaccharides. Gut 1986;27:1161-8.
31. Health Survey for England by National Centre for Social Research, University College of London, 2006.
32. Statistics on Alcohol: England 2010 by NHS Information Centre. 2010.
33. Tran C, Jacot-Descombes D, Lecoultre V, et al. Sex differences in lipid and glucose kinetics after ingestion of an acute oral fructose load. Br J Nutr 2010;104:1139-47.
34. Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. Am J Clin Nutr 2006;84:274-88.
35. Sievenpiper JL, Carleton AJ, Chatha S, et al. Heterogeneous effects of fructose on blood lipids in individuals with type 2 diabetes: systematic review and meta-analysis of experimental trials in humans. Diabetes Care 2009;32:1930-7.
36. Ngo Sock ET, Le KA, Ith M, et al. Effects of a short-term overfeeding with fructose or glucose in healthy young males. Br J Nutr 2010;103:939-43.
37. Chong MF, Fielding BA, Frayn KN. Mechanisms for the acute effect of fructose on postprandial lipemia. Am J Clin Nutr 2007;85:1511-20.
38. Tilg H, Hotamisligil GS. Nonalcoholic fatty liver disease: Cytokine-adipokine interplay and regulation of insulin resistance. Gastroenterology 2006;131:934-45.
39. Schaffler A, Scholmerich J, Buchler C. Mechanisms of disease: adipocytokines and visceral adipose tissue--emerging role in nonalcoholic fatty liver disease. Nat Clin Pract Gastroenterol Hepatol 2005;2:273-80.
40. Nielsen S, Guo Z, Johnson CM, et al. Splanchnic lipolysis in human obesity. J Clin Invest 2004;113:1582-8.
41. Musso G, Cassader M, De Michieli F, et al. Nonalcoholic steatohepatitis versus steatosis: adipose tissue insulin resistance and dysfunctional response to fat ingestion predict liver injury and altered glucose and lipoprotein metabolism. Hepatology 2012;56:933-42.
42. Sobrecases H, Le KA, Bortolotti M, et al. Effects of short-term overfeeding with fructose, fat and fructose plus fat on plasma and hepatic lipids in healthy men. Diabetes Metab 2010;36:244-6.
43. Aeberli I, Hochuli M, Gerber PA,et al. Moderate amounts of fructose consumption impair insulin sensitivity in healthy young men: a randomized controlled trial. Diabetes Care 2013;36:150-6.
44. Abdelmalek MF, Lazo M, Horska A, et al. Higher dietary fructose is associated with impaired hepatic adenosine triphosphate homeostasis in obese individuals with type 2 diabetes. Hepatology 2012.

45. Israel KD, Michaelis OEt, Reiser S, et al. Serum uric acid, inorganic phosphorus, and glutamic-oxalacetic transaminase and blood pressure in carbohydrate-sensitive adults consuming three different levels of sucrose. Ann Nutr Metab 1983;27:425-35.
46. Wang DD, Sievenpiper JL, de Souza RJ, et al. The effects of fructose intake on serum uric acid vary among controlled dietary trials. J Nutr 2012;142:916-23.
47. Ferrannini E, Natali A, Bell P, et al. Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). J Clin Invest 1997;100:1166-73.

Figures 1a-d. 1a. Weight of subjects (kg); 1b Hepatic triglyceride concentration (HTGC%); 1c Intra-myocellular lipid content (IMCL%); 1d Fasted serum triglyceride (mmol/L), at the beginning and end of the isocaloric and hypercaloric periods (fructose pre■, fructose post▨, glucose pre□, glucose post⬚, mean±SEM, *=p<0.05, **=p<0.01).

Supplementary data

	Energy (kcal/day)	Fat	Carbo hydrate	Protein	Alcohol	Saturated fat	Fructose
All, n=32	2835±375	38.4±4.1	43.9±4.4	16.1±2.4	2.0±2.1	14.3±2.0	5.4±2.1
Fructose, n=15	2724±282	37.3±3.9	44.2±4.3	17.0±2.2	1.7±1.7	14.2±1.6	6.1±2.2
Glucose, n=17	2932±427	39.3±4.3	43.7±4.6	15.3±2.4	2.4±2.4	14.5±2.4	4.8±1.9

Table 1. Energy intakes and its percentage macronutrient origin in the subjects' habitual intakes, mean±SD.

	Energy (kcal/ day)	Fat	Carbohy drate	Protein	Alcohol	Saturated fat	Fructose in fructose group	Fructose in glucose group
Food alone	2444	40.0	42.2	17.9	0	15.5	3.1	3.1
Food and drink	3259	32.0	52.8	14.3	0	12.4	27.5	2.3

Table 2. Energy intakes and its percentage macronutrient origin in the food supplied with or without the monosaccharide drinks during the first period, mean±SD.

	All, n=32	Fructose, n=15	Glucose, n=17	Significance between groups (p)
Weight (kg)	0.33±1.37	0.59±1.7	0.09±1.0	0.32
Resting energy expenditure (kcal/day)	-39.6±114.1	-72.0±130.8	-11.0±91.7	0.13
Respiratory quotient (RQ)	0.05±0.038	0.00±0.31	0.01±0.04	0.50
Lipid oxidation (mg/kg/min)	-0.16±0.31	-0.20±0.35	-0.13±0.29	0.54
Carbohydrate oxidation (mg/kg/min)	0.48±0.51	0.44±0.54	0.51±0.50	0.78
Hepatic triglyceride (%)	0.15±2.09	0.25±1.88	0.07±2.30	0.81
Intra-myocellular lipid (%)	0.01±2.26	0.23±1.96	-0.18±2.55	0.62
Extra-myocellular lipid (%)	-0.54±3.13	0.42±3.37	-1.39±2.72	0.10
Triglycerides (mmol/L)	-0.14±0.56	-0.18±0.63	-0.10±0.50	0.68
HOMA-IR	0.32±1.22	0.25±1.52	0.38±0.91	0.76
Glucose disposal (mg/kg/min)	-0.14±1.11	-0.23±0.86	-0.05±1.40	0.80
Noradrenaline (nmol/L)	-0.07±0.62	-0.01±0.69	-0.13±0.57	0.60
Adrenaline (nmol/L)	-0.04±0.17	-0.04±0.19	-0.04±0.17	0.97
Non-esterified free fatty acids (mmol/L)	-59±161*	-34±149	-80±171	0.44
Creatinine (µmol/L)	-1.8±5.6	-0.9±6.8	-2.5±4.5	0.43
Uric acid (µmol/L)	-20.6±57.6	0.2±64.2	-38.9±45.4	0.054

Alkaline phosphatase (IU/L)	-1.6±6.2	-1.1±7.4	-2.1±5.0	0.66
Alanine transaminase (U/L)	-1.7±9.0	-4.3±9.3	0.7±8.3	0.12
Aspartate transaminase (U/L)	-1.4±5.2	-2.2±5.6	-0.8±4.9	0.45
Gamma glutamyl transpeptidase (U/L)	-1.9±6.5	-2.2±4.2	-1.7±8.2	0.82

Table 3. The absolute change within the entire cohort and each group at the second baseline assessment compared to the first baseline assessment, mean±SD. (*=p<0.05)

			Arterialised glucose (mmol/L)	**Difference between groups (p)**	**Arterialised insulin (mIU/L)**	**Difference between groups (p)**
Isocaloric period	**Baseline**	Fructose	4.48±0.09	0.76	89.1±11.6	0.65
		Glucose	4.47±0.06		84.9±14.3	
	Week 2	Fructose	4.43±0.04	0.21	82.2±9.2	0.15
		Glucose	4.47±0.04		73.6±8.0	
Hypercaloric period	**Baseline**	Fructose	4.45±0.06	0.23	85.5±13.9	0.42
		Glucose	4.48±0.02		79.3±10.6	
	Week 2	Fructose	4.45±0.03	0.61	80.8±8.1	0.58
		Glucose	4.46±0.04		86.8±22.0	

Table 4. Whole arterialised blood glucose concentrations and arterialised serum insulin concentrations during the final hour of a three hour hyperinsulinaemic euglycaemic clamp, mean ± SD.

	Fructose, n=15	Glucose, n=17	p
Age	35±11	33±9	0.60
Weight (kg)	96.8±7.4	93.9±8.7	0.32
BMI (kg/m^2)	30.0±1.4	28.9±1.7	0.07
Body fat (%)	34.5±4.6	33.9±4.2	0.70
Waist (cm)	103.8±4.9	103.3±5.2	0.77
Carbohydrate oxidation (mg/kg/min)	0.353±0.27	0.356±0.21	0.97
Fullness from habitual food	66±17	59±20	0.30
Leptin (µg/L)	11.5±6.0	8.8±4.0	0.16
HTGC (%)	7.20±5.6	7.98±5.2	0.69
Liver volume (l)	2.09±0.4	2.02±0.3	0.53
IMCL (%)	8.8±4.1	8.3±2.1	0.64
Triglyceride (mmol/L)	1.45±0.67	1.44±0.58	0.97
ALT (IU/L)	31±15	27±10	0.31
AST (IU/L)	24±8	24±5	0.74
GGT (IU/L)	39±17	32±12	0.14
Glucose (mmol/L)	4.50±0.20	4.65±0.42	0.20
Insulin (mIU/L)	17.9±5.1	14.6±5.6	0.09
HOMA-IR	3.58±1.04	3.02±1.24	0.19
NEFA (µmol/L)	415±80	405±110	0.78
Uric acid (µmol/L)	406±60	410±77	0.87
Hepatic ATP	239±63	270±99	0.34
Hepatic Pi	223±72	228±72	0.86
CRP (mg/L)	1.01±1.08	1.40±1.46	0.41
IL6 (pg/ml)	3.56±4.84	4.99±13.86	0.71
TNFα (pg/ml)	1.92±0.5	2.00±0.3	0.61

Table 1. Clinical characteristics and fasting data from the first baseline assessment (mean±SD).

	All, n=32	Fructose, n=15	Glucose, n=17	p
Carbohydrate oxidation (mg/kg/min)	0.38±0.32**	0.37±0.21	0.39±0.37*	0.65
Fullness from supplied food and supplements	7±19*	6±14	8±23	0.69
Weight (kg)	-0.2±0.8	-0.3±0.9	-0.1±0.7	0.62
Leptin (µg/L)	-0.1±2.4	-0.4±3.2	0.2±1.3	0.51
HTGC (%)	0.11±2.1	0.29±2.2	-0.05±2.1	0.65
Liver volume (l)	0.02±0.13	0.02±0.14	0.02±0.13	0.96
IMCL (%)	0.3±3.8	-0.4±4.7	0.9±2.9	0.34
Triglyceride (mmol/L)	0.03±0.59	-0.07±3.5	0.13±0.74	0.35
ALT (IU/L)	-3.4±7.1*	-4.0±7.9	-2.9±6.5	0.67
AST (IU/L)	-1.9±4.4*	-3.2±5.2	-0.7±3.3	0.11
GGT (IU/L)	-4.0±7.4**	-1.6±7.0	-6.2±7.3	0.08
Glucose (mmol/L)	0.07±0.4	0.21±0.3*	0.00±0.4	0.12
Insulin (mIU/L)	1.8±3.6**	2.9±4.3*	0.8±2.7	0.11
HOMA-IR	0.43±0.8**	0.76±0.9**	0.13±0.7	0.03^{+}
NEFA (µmol/L)	-34±168	-80±120*	4±195	0.17
Uric acid (µmol/L)	-2±45	22±52	-23±25	0.004^{++}
Hepatic ATP	5±96	6±60	4±122	0.96
Hepatic Pi	11±86	19±94	0±79	0.59
CRP (mg/L)	-0.02±1.3	-0.22±1.2	0.16±1.4	0.43
IL6 (pg/ml)	-0.08±2.76	0.15±0.54	-0.29±3.80	0.66
TNFα (pg/ml)	-0.02±0.2	0.01±0.2	-0.05±0.2	0.36

Table 2. Absolute changes during the isocaloric period ((mean±SD); significance of change within the group *=p<0.05, **=p<0.01, ***=p<0.001; significance of change between the groups $^{+}$=p<0.05, $^{++}$=p<0.01).

	All, n=32	Fructose, n=15	Glucose, n=17	P
Carbohydrate oxidation (mg/kg/min)	0.47±0.78**	0.50±0.61	0.46±0.90*	0.77
Fullness from food habitual food and supplements	1±13	3±13	0±12	0.60
Weight (kg)	0.8±1.2**	1.0±1.4*	0.6±1.0*	0.29
Leptin (µg/L)	1.2±3.2*	1.4±3.4	1.0±3.1	0.70
HTGC (%)	1.88±2.72***	1.70±2.6*	2.05±2.9*	0.73
Liver volume (l)	0.10±0.20	0.16±0.25	0.05±0.13	0.09
IMCL (%)	1.3±3.4*	0.9±3.2	1.7±3.5	0.51
Triglyceride (mmol/L)	0.35±0.57*	0.36±0.75	0.33±0.38**	0.91
ALT (IU/L)	4.9±9.2**	5.8±8.7	4.1±9.8	0.62
AST (IU/L)	1.4±5.0	0.9±4.4	1.8±5.7	0.64
GGT (IU/L)	4.7±9.7*	7.2±12.1	2.5±6.5	0.17
Glucose (mmol/L)	0.01±0.4	0.14±0.4	-0.10±0.4	0.10
Insulin (mIU/L)	-0.8±4.2	-1.0±4.6	-0.6±4.0	0.82
HOMA-IR	-0.13±0.9	0.00±1.1	-0.24±0.7	0.47
NEFA (µmol/L)	-51±145	-50±166	-43±129	0.90
Uric acid (µmol/L)	5±55	22±64	-10±41	0.10
Hepatic ATP	4±85	12±82	-4±91	0.65
Hepatic Pi	32±75*	28±78	36±74	0.81
CRP (mg/L)	0.26±2.0	-0.9±1.0	0.56±2.6	0.37
IL6 (pg/ml)	-0.70±6.41	0.85±2.01	-1.97±8.37	0.23
TNFα (pg/ml)	-0.04±0.1	-0.09±0.2	0.01±0.1	0.07

Table 3. Absolute changes during the hypercaloric period ((mean±SD); significance of change within the group *=p<0.05, **=p<0.01, ***=p<0.001; significance of change between the groups $^{+}$=p<0.05, $^{++}$=p<0.01).

	Fructose, n=6	Glucose, n=6	p
Glucose disposal (mg/kg/min)	4.50±1.84	5.01±1.59	0.62
Fasting EGP (mg/kg/min)	2.07±1.31	1.93±1.49	0.87
End of clamp EGP (mg/kg/min)	1.02±0.68	1.17±0.73	0.73
NOGD (mg/kg/min)	2.3±1.6	2.2±1.2	0.97

Table 4. Data derived from the first baseline hyperinsulinaemic euglycaemic clamp (mean±SD).

	isocaloric				hypercaloric			
	All, n=12	**Fructose, n=6**	**Glucose, n=6**	**p**	**All, n=12**	**Fructose, n=6**	**Glucose, n=6**	**p**
Glucose disposal (mg/kg/min)	-0.49±1.2	-0.29±1.5	-0.68±0.83	0.58	-0.51±1.2	-0.29±0.84	-0.73±1.51	0.55
Fasting EGP (mg/kg/min)	-0.04±1.82	-0.35±1.73	0.27±2.01	0.58	0.24±1.62	0.32±2.27	0.17±0.81	0.89
End of clamp EGP (mg/kg/min)	0.19±1.38	-0.11±1.36	0.50±1.47	0.47	0.11±1.03	-0.20±1.01	0.41±1.05	0.33
NOGD (mg/kg/min)	0.2±1.7	0.5±2.1	-0.2±1.2	0.48	-1.1±1.5*	-1.2±1.4	-1.0±1.8	0.87

Table 5. Change in hyperinsulinaemic euglycaemic clamp data during the isocaloric and hypercaloric periods (mean±SD).

		Whole cohort, n=32	Fructose, n=15	Glucose, n=17
HTGC (%)	Period 1	0.54**	0.56*	0.54*
	Period 2	0.18	0.41	-0.03
ALT (IU/L)	Period 1	0.64**	0.54*	0.66**
	Period 2	0.33	0.20	0.58*
AST (IU/L)	Period 1	0.52**	0.45	0.62**
	Period 2	0.31	0.19	0.52*
GGT (IU/L)	Period 1	0.25	0.22	0.57*
	Period 2	0.40*	0.67**	0.32
CRP (mg/L)	Period 1	0.05	0.02	0.09
	Period 2	-0.3	-0.49	0.01
TNFα (pg/ml)	Period 1	0.07	-0.24	0.13
	Period 2	0.14	0.46	-0.10
IL6 (pg/ml)	Period 1	0.08	-0.46	0.18
	Period 2	0.36*	0.35	0.43

Table 6. The associations between changes in weight and changes in hepatic and inflammatory parameters (2-tailed Pearson correlations). (*=p<0.05, **=p<0.01).