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Impact of liver fat on the differential partitioning of hepatic triacylglycerol into VLDL subclasses on high and low sugar diets

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Abstract

Dietary sugars are linked to the development of non-alcoholic fatty liver disease (NAFLD) and dyslipidaemia, but it is unknown if NAFLD itself influences the effects of sugars on plasma lipoproteins. To study this further, men with NAFLD (n=11) and low liver fat 'controls' (n= 14) were fed two iso-energetic diets, high or low in sugars (26% or 6% total energy) for 12 weeks, in a randomised, cross-over design. Fasting plasma lipid and lipoprotein kinetics were measured after each diet by stable isotope trace-labelling.
There were significant differences in the production and catabolic rates of VLDL subclasses between men with NAFLD and controls, in response to the high and low sugar diets. Men with NAFLD had higher plasma concentrations of $VLDL_1$-triacylglycerol (TAG) after the high ($P<0.02$) and low sugar ($P<0.0002$) diets, a lower $VLDL_1$-TAG fractional catabolic rate after the high sugar diet ($P<0.01$), and a higher $VLDL_1$-TAG production rate after the low sugar diet ($P<0.01$), relative to controls. An effect of the high sugar diet, was to channel hepatic TAG into a higher production of $VLDL_1$-TAG ($P<0.02$) in the controls, but in contrast, a higher production of $VLDL_2$-TAG ($P<0.05$) in NAFLD. These dietary effects on VLDL subclass kinetics could be explained, in part, by differences in the contribution of fatty acids from intra-hepatic stores, and *de novo* lipogenesis. This study provides new evidence that liver fat accumulation leads to a differential partitioning of hepatic TAG into large and small VLDL subclasses, in response to high and low intakes of sugars.

Summary Statement

This study shows that raised liver fat can influence the effects of a high intake of sugars on lipid metabolism, and provides new evidence for a mechanism by which sugars could contribute to the development of non-alcoholic fatty liver disease (NAFLD).

Clinical Trial Registration: NCT01790984

Key words: Sugar, Triacylglycerol, NAFLD, De novo lipogenesis, Stable isotopes, VLDL, LDL, NEFA, hepatic lipase, kinetics

Abbreviations: apolipoprotein (apo), atherogenic lipoprotein phenotype (ALP), de novo lipogenesis (DNL), fractional catabolic rate (FCR), gas chromatography mass spectrometry (GCMS), hepatic lipase (HL), Intermediate density lipoprotein (IDL), intra-hepatocellular lipid (IHCL), lipoprotein lipase (LPL), magnetic resonance imaging (MRI) and spectroscopy (MRS), non-alcoholic fatty liver disease (NAFLD), National Diet & Nutrition Survey (NDNS), non-milk extrinsic sugars (NMES), tracer/tracee ratio (TTR), production rate (PR), small dense low density lipoprotein (sdLDL), steady state (SS),

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common condition, defined histologically, as an excess of macro-vesicular steatosis (>5%) in the absence of a high intake of alcohol [1]. In addition to being a progenitor of end-terminal liver diseases, NAFLD has been linked to the metabolic syndrome, and is a potential source of elevated plasma TAG and abnormalities in plasma lipoproteins, known as an atherogenic lipoprotein phenotype (ALP) [2-4].

Elevated plasma TAG promotes the development of an ALP through the extra-cellular remodelling of plasma low and high density lipoproteins (LDL and HDL) into small, dense particles with increased potential to promote atherosclerosis [5]. Plasma TAG may be raised by an overproduction of its principal transporter VLDL in the liver, and/or impaired clearance of VLDL from the plasma [6]. VLDL is secreted from the liver as a spectrum of particles that vary in size, composition and metabolic properties, which can be subdivided on the basis of hydrated density into two discrete subclasses of large, TAG-rich $VLDL_1$ and smaller $VLDL_2$ (Svedberg flotation units (Sf) of 60-400 and 20-60, respectively) [7]. The particle size of VLDL in the liver in the fasted, post-absorptive state is largely determined by the availability of lipid in the form of non-esterifed fatty acids (NEFA) from peripheral adipose tissue (systemic source) or splanchnic sources, the latter of which includes visceral adipose tissue, intra-hepatic stores, and synthesis of fatty acids by *de novo* lipogenesis (DNL) in the liver [8].

Free sugars in food and sugar-sweetened beverages have been implicated in the development of dyslipidaemia and NAFLD, either through the direct lipogenic effects of sugars on liver fat and VLDL metabolism, and/or via the indirect effects of the energy from sugars on body weight [9]. While DNL makes a relatively small contribution to VLDL-TAG production, this has been shown to increase substantially when a high proportion of dietary energy is supplied as sugars, especially sucrose and fructose (>20% total energy)[10]. However, the extent to which liver fat affects the handling of hepatic fatty acids and alters VLDL metabolism in response to intakes of sugars representative of a Western diet, is currently unknown. This study tested the hypothesis that liver fat influences the metabolic effects of a high relative to a low intake of sugars (representative of the upper and lower 2.5^{th} percentiles of intake in the UK), on plasma lipoproteins, by altering the kinetics and source of fatty acids for the production of VLDL subclasses.

MATERIAL AND METHODS

Participants

Participants were men (aged 40-65y, BMI 25-30) at increased cardio-metabolic risk, as determined by a 1 to 10 risk score used previously in the *'RISCK'* study [11]. Men with a cardio-metabolic score of ≥4 and *APO ε3/ε3* genotype (to exclude possible confounding effects of different apo E isoforms on lipid metabolism), underwent an assessment of intra-hepatocellular lipid (IHCL) by magnetic resonance spectroscopy (MRS) for assignment to a group with NAFLD (>5.56% IHCL, n=11) or low liver fat (Controls) (<5.56% IHCL, n=14) [12]. Exclusion criteria included diabetes and any medical condition other than NAFLD, lipid-lowering medication, unstable weight in the preceding 3 months, and an intake of alcohol exceeding 20g/day. All participants provided written informed consent before taking part in the study, which received favourable ethical opinions from Surrey Research Ethics Committee (Ref. 08/H1109/227), and the University of Surrey's Ethics Committee (Ref. EC/2009/29). The trial was registered on Clinical Trials.gov (Ref. NCT01790984).

Study design

The study had a randomised, two-way crossover design, with two 12 week dietary interventions. After an initial 4 week run-in period on their habitual diet, participants were randomised to either a high or low sugar diet, using a computer-generated sequence of treatments in sealed envelopes. The two diets were iso-energetic and contained the same macronutrient composition. Participants returned to their habitual diet for 4 weeks washout, before crossing-over to the alternative diet for a further 12 weeks. During the dietary interventions, participants were instructed to maintain their habitual level of physical activity. Certain outcome measures were determined before and after each diet (body weight, percentage body fat, plasma lipids, glucose and serum insulin), while others were measured at the end of each diet (stable-isotope tracer kinetics, lipoprotein composition, IHCL and body fat distribution by MRS).

Dietary interventions

Intakes of total carbohydrates and sugars were based on mean intakes for men aged 40-65 years in the UK's National Diet & Nutrition Survey (NDNS), with target intakes for non-milk extrinsic sugars (NMES) on the high and low sugar diets corresponding to the upper and lower 2.5th percentile of intake in the UK population, respectively [13] . The term NMES, as originally defined by the UK's Department of Health [14], included free sugars added to food (including 50% of sugars in tinned and dried fruit), but excluded sugars in whole fruit, and lactose, primarily from cows' milk [15]. The content of sugars in the two diets was achieved by a dietary exchange of sugars for starch using a range of commercially available foods, as described in **Supplementary Material**. Dietary intakes were assessed by the completion of 3-day diet diaries during the final week of each dietary intervention (2 weekdays and 1 weekend day). Diaries were analysed by a single operator using *DietPlan 6* (version 6.50, Forestfield Software Ltd, UK).

Metabolic study (Post-diets)

The study protocol is shown in **Supplementary Figure 1**. The evening before the metabolic study, participants consumed a set volume of deuterated water (^2H_2O 3g/kg body water; 50% after a standardised low fat, low fibre meal (1900h) and 50% 3h later at 2200h). They then fasted and drank only water enriched with ^2H_2O (4.5g ^2H_2O/litre drinking water). The following morning, a blood sample was taken to measure deuterium enrichment of palmitate in $VLDL_1$ and $VLDL_2$-TAG, and plasma water to measure DNL (For calculation see **Supplementary Material**). A primed, 10h constant iv [1-^{13}C]leucine infusion (1mg/kg; 1mg/kg/h) (99%, Cambridge Isotopes) was administered to measure $VLDL_1$, $VLDL_2$, IDL, LDL_2 and LDL_3-apoprotein B (apoB) kinetics. An 8h constant iv infusion of [U-^{13}C]palmitate (99%, Cambridge Isotopes) bound to human albumin (5%, 0.01 µmol.kg^{-1}.min^{-1}), was administered to measure palmitate production rate (PR, assumed to be mainly from systemic adipose tissue lipolysis), and the percentage contribution of systemic NEFA to the export of TAG in $VLDL_1$ and $VLDL_2$. An intravenous bolus of [1,1,2,3,3-$^2H_5$]glycerol (75µmol/kg) (99%, Cambridge Isotopes) was administered to measure $VLDL_1$ and $VLDL_2$-TAG PR and fractional catabolic rate (FCR). Blood samples were taken at sequential time intervals to measure the isotopic enrichment and concentrations of plasma palmitate, αketoisocaproate (αKIC) and glycerol, and the enrichment and concentrations of apoB, TAG-palmitate and TAG-glycerol in the lipoprotein fractions, as reported previously [16, 17]. At the end of each dietary intervention period, the activity of lipoprotein lipase (LPL) and hepatic lipase (HL) in plasma was measured before and 15 minutes after an intravenous injection of 50U/kg heparin, as previously described [18]

Magnetic resonance imaging (MRI) and spectroscopy (MRS)

Whole body MRI scans were obtained on a 1.5T Phillips Achieva system (Philips Medical Systems, Best, The Netherlands). Volumes of intra-abdominal and subcutaneous abdominal adipose tissue were calculated from the abdominal region between the slices containing the bottom of the lungs/top of the liver, and femoral heads. Spectra were analysed by a single trained observer (ELT) using AMARES. Liver fat (IHCL) was measured relative to liver water content, as described previously [19]. Seventeen of the 25 participants who completed both diets, underwent a post-dietary analysis of IHCL and body fat distribution by MRS.

Laboratory methods

$VLDL_1$, $VLDL_2$, IDL, LDL_2 and LDL_3 were separated by sequential ultracentrifugation [7]. Plasma TAG, $VLDL_1$ and $VLDL_2$-TAG were extracted, and the isotopic enrichment of glycerol and TAG-palmitate in these extracts was measured by gas chromatography mass spectrometry (GCMS), as described previously [16]. The isotopic enrichment of leucine in

$VLDL_1$, $VLDL_2$ and IDL-apoB, and plasma αKIC enrichment, was measured by GCMS [17]. Leucine enrichment in LDL_2 and LDL_3-apoB was measured as the *N*-acetyl, n-propyl-ester derivative and analyzed by GC-combustion isotope ratio MS (Delta plus XP isotope ratio MS, Thermo Scientific). Plasma ^2H_2O enrichment was measured with a Gasbench II inlet system and isotope ratio MS using platinum catalyst rods to liberate hydrogen gas. Isotopic enrichment was measured relative to laboratory standards, which had been previously calibrated against international standards; Vienna Standard Mean Ocean Water and Standard Light Arctic Precipitation (International Atomic Energy Agency, Vienna, Austria). LPL and HL were measured in post-heparin plasma by the Confluolip Lipase test (Progen Biotechnik, Heidelberg). Plasma NEFA, total cholesterol, TAG, lipoprotein fraction TAG and cholesterol were measured by enzymatic assays using a Cobas MIRA (Roche, Welwyn Garden City, UK). Apolipoprotein B (apo B) in lipoprotein fractions was measured by an in-house ELISA. Plasma apolipoproteins CII, CIII and E were measured by commercially available ELISAs (Biomedica, GmbH &Co Wien, Austria), and small dense (sd) LDL-cholesterol by a precipitation method (Randox Laboratories Ltd) on an ILab 650 (Werfen). *APO E* genotype was determined by quantitative polymerase chain reaction and Southern blotting.

Data analysis

Tracer enrichment of αKIC, leucine, palmitate and glycerol was expressed as tracer/tracee ratio (TTR) corrected for baseline enrichment. Lipoprotein kinetics were analysed by compartmental modelling, as described previously [16, 17]. These models and the calculation of the fatty acid contribution to VLDL-TAG PR, together with further details of the methods, are described in **Supplementary Material**.

Statistical methods

Data are expressed as means (± SEM) for normally distributed variables, and $\log_{10}$ transformed geometric means for non-normally distributed variables.

Statistical modelling. Data are expressed as estimates of contrasts of least squares means for normally distributed variables, and as ratios of geometric means for logarithmically transformed variables. For outcome measures for which there were four samples from each participant (pre and post-diets, for each period), the post-diet measurements (NAFLD and controls for the 2-period cross-over, logarithmically transformed or not, as appropriate), were analysed as dependent variables in a general linear mixed model, with the following fixed categorical, non-random, explanatory effects: period, treatment (low and high sugar diet), period by treatment interaction (to detect carry-over effects), liver fat level (NAFLD and control) and treatment by liver fat level interaction. The pre-diet measurements (logarithmically transformed or not, as appropriate) for each period, and body weights (pre and post-diets) were included as covariates in the model, with participant as a model random effect. For outcome measures for which there were two samples for each participant (post-diets; end of each dietary intervention period only), each measurement for the combined groups (NAFLD and Controls), for the 2-period cross-over, were analysed in a general linear mixed model with the same fixed categorical effects as above, and body weights (pre and post-diets) as co-variates. Variables for which there was no significant carry-over effect, were modelled as above, omitting the period-by-treatment interaction from the model. There was only evidence of significant treatment by period interactions (carry-over effect) at the 5% level for $VLDL_1$-TAG fractional catabolic rate, which was modelled using data from the first period only. Modelling was performed using procedure MIXED of SAS Version 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

Twenty five men completed the study. The baseline characteristics of the NAFLD and control groups, including age, body weight, BMI, waist circumference and biochemical measures, were similar, except for plasma TAG, which was 42% higher in men with NAFLD than controls (P<0.05, **Table 1).**

Dietary intake and changes in body weight

Self-recorded dietary intakes were monitored by regular visits to the homes of participants, and indicated that dietary compliance was maintained. There was no difference in reported energy intake between diets, or differences in energy intake, macronutrients or alcohol between NAFLD and controls on either diets **(Supplementary Table 1)**. The high sugar diet resulted in a higher intake of total sugars and NMES (26% total energy) in comparison to the baseline and low sugar diet (6% total energy) in both men with NAFLD and controls (P<0.01 for all comparisons). The high sugar diet was also lower in starch (P<0.01) than the low sugar diet in both groups. Percent energy intake from dietary fat was significantly lower on the high sugar diet in controls (P<0.001).

Body weight was higher after the high versus low sugar diet in NAFLD (P<0.001) and controls (P<0.01), with both groups gaining and losing approximately 2kg on the high and low sugar diets, respectively (**Table 2**). All variables were adjusted for these differences in body weight in the statistical analysis (see Statistical Methods). There was no significant difference in body weight between groups after either diet, or differences in the change of body weight between groups on either diet, over time.

Plasma lipids and lipoprotein kinetics

Summary of model interactions: There was an overall difference in the response to the two diets between the NAFLD and control groups, as evidenced by significant **Group**

(NAFLD vs control) x Diet (high vs low sugars) interactions for our primary outcome variables. These interactive variables included: **1)** the plasma concentration and production rate of large, TAG-rich $VLDL_1$-TAG (*P* = 0.026, *P* = 0.015), which were higher in NAFLD compared to controls, but which increased in the controls in response to the high sugar diet; **2)** the rate of $VLDL_2$-TAG production (*P* = 0.04), which was higher in NAFLD than controls after the high sugar diet; **3)** the rate of removal of plasma small, dense LDL_3-apo B (*P* = 0.02), which was lower in NAFLD than controls after the low sugar diet; **4)** plasma NEFA (*P*=0.004) which was higher in NAFLD than controls after the high sugar diet; and **5)** the contribution of DNL to $VLDL_1$TAG production (*P*=0.02), which tended to be greater in controls after the high versus the low sugar diet, and higher in NAFLD relative to controls after the low sugar diet.

Post-hoc differences between groups: Men with NAFLD had higher plasma concentration of total VLDL-TAG and $VLDL_1$-TAG than controls, after the high (*P*<0.02 for both comparisons), and low (*P*<0.001, for both comparisons) sugar diets **(Table 3, Figure 1a)**, and a higher $VLDL_1$-TAG production rate and lower $VLDL_1$-TAG FCR than controls, after the low and high sugar diets, respectively (**Figure 1c**, and **Table 4**, *P*=0.01 for both comparisons). Men with NAFLD also had a higher concentration of plasma small, dense LDL cholesterol (sdLDL), and lower FCR for small, dense LDL_3-apo B than controls, after the low sugar diet (*P*<0.05 for both comparisons) **(Tables 3** and **4)**.

Post-hoc differences between diets: Men with NAFLD had a higher production rate of $VLDL_2$-TAG (*P*=0.036, **Table 4, Figure 1d**) after the high versus the low sugar diet. In contrast, controls had a significantly higher production rate of $VLDL_1$-TAG (*P*=0.02), and trend towards a higher plasma concentration of $VLDL_1$-TAG (*P*=0.058), after the high versus low sugar diet **(Figures 1c and 1a)**. Men with NAFLD had a higher plasma concentration of IDL-apo B (*P*=0.025, **Table 3**), IDL-apo B pool size (*P*=0.025, data not shown), and trend for a higher IDL-apo B production rate (p=0.06, **Table 4**), after the high versus the low sugar diet.

Sources of fatty acids for VLDL production

Post-hoc differences between groups: Men with NAFLD had a greater contribution of fatty acids from splanchnic fat for the production of $VLDL_1$ and $VLDL_2$-TAG relative to controls, after the high sugars diet (**Figures 1c & 1d**, $P<0.05$ for both comparisons). This group also expressed a greater contribution of fatty acids from splanchnc fat, and DNL for the production of $VLDL_1$-TAG after the low sugars diet (**Figure 1c,** $P=0.006$, $P=0.003$, respectively), and a markedly higher plasma concentration of NEFA after the high sugars diet, relative to controls **(**$P=0.0007$, **Table 5**).

Post-hoc differences between diets: There were no significant effects of diet on the source of fatty acids for VLDL production, other than a trend for a greater contribution from DNL to the production of $VLDL_1$ and $VLDL_2$-TAG in controls, after the high versus the low sugars diet ($P=0.08$ for both VLDL subclasses). The production and metabolic clearance rates of palmitate were higher in men with NAFLD ($P=0.025$, $P=0.006$, respectively), after the high versus low sugars diet **(Table 5)**.

Plasma apoproteins and post-heparin lipase activities

Post-hoc differences between groups: Men with NAFLD had a higher plasma apoprotein C-III than controls, after the high and low sugars diets ($P=0.042$, p=0.002, respectively), and a higher plasma apoprotein C-II than controls after the low sugars diet ($P=0.033$). The activity of hepatic lipase was higher in men with NAFLD versus controls after the high sugars diet ($P<0.05$) **(Table 5)**.

Liver fat, intra-abdominal and subcutaneous adipose tissue (subgroup n=17, post-diet)

Liver fat was higher after the high sugars diet in men with NAFLD and controls, relative to the low sugars diet (*P*=0.01 for both comparisons, **Table 2).** However, the significance of these differences was not maintatined after adjustment for body weight. There were no differences in the masses of visceral and subcutaneous adipose tissue between groups after each diet. (**Supplementary Table 3**). There were also no associations between post-dietary liver fat, body weight, visceral fat, plasma TAG, or changes in these variables.

DISCUSSION

This study provides new evidence that liver fat can influence the weight-adjusted partitioning of hepatic TAG into different plasma VLDL subclasses, in response to a high intake of sugars that is common to the UK diet [13]. Men with NAFLD were distinct from controls in having a higher plasma and production rate of large, TAG-rich $VLDL_1$, after both diets. This finding is consistent with the previous observation that $VLDL_1$ overproduction is driven by increased liver fat [20]. In the present study, this effect originated, in part, from a greater contribution of fatty acids from splanchnic fat (hepatic TAG storage pools, visceral fat, and to a lesser extent DNL in the liver). A highly original finding in this study, was that these metabolic characteristics in men with NAFLD were shown to develop in response to the high sugars diet in low liver fat controls. In contrast, the high sugars diet upregulated the production of $VLDL_2$ in NAFLD relative to controls, a difference that was also ascribed to a greater contribution of splanchnic fatty acids for the production of this smaller VLDL subclass **(Figure 2)**.

Large TAG-rich $VLDL_1$ has been associated with increased liver fat and dyslipidaemia in the metabolic syndrome [20, 21], but there is no previous evidence to link its plasma concentration or kinetics directly with a high intake of sugars in humans. There have also been no studies to date on the effect of dietary free sugars on VLDL kinetics in NAFLD. In healthy subjects, the production rate of VLDL-TAG has been shown to be higher after a 6-day hyper-energetic diet enriched with fructose as a liquid supplement (25% total energy) versus a 6-day, low-fructose diet [22]. VLDL-TAG production rate was also higher after a two-week high carbohydrate, low fat diet, compared to a two-week iso-energetic, low carbohydrate, high fat diet in healthy subjects [23]. In the present study, the production rate and plasma concentration of large, TAG-rich $VLDL_1$ were higher in the low liver fat controls on the high sugars diet compared to the low sugars diet. Moreover, the difference in production rate of large TAG-rich $VLDL_1$ between groups was removed on the high sugars diet, as the values in controls approached that of men with NAFLD, possibly because the controls also gained liver fat. In contrast, the production rate of smaller $VLDL_2$-TAG was significantly higher in NAFLD after the high relative to the low sugar diet. Since $VLDL_2$ is known to be the main precursor of IDL and LDL [24], this finding is consistent with an increase in IDL apoB production rate and the pool size of IDL apoB and plasma concentration of apoB in IDL, and sdLDL, both of which are components of an atherogenic lipoprotein phenotype [25]. Interestingly, there was no evidence in our study of any group or dietary effects on the production and secretion of new VLDL particles, as indicated by a lack of significant effects on plasma VLDL apoB or changes in the kinetics of VLDL-apoB.

Men with NAFLD had a higher DNL relative to controls after the low sugars diet, in accord with previous reports of increased contribution of DNL to hepatic fat and dyslipidaemia in men with NAFLD [8, 26]. However, this finding was only significant on the low sugars diet, possibly because the contribution of DNL to both $VLDL_1$ and $VLDL_2$-TAG increased to a greater extent in controls than in men with NAFLD after the high sugars diet. DNL made relatively minor contributions (between 4-8%) to $VLDL_1$ and $VLDL_2$-TAG production in both groups, after both diets, as reported previously in healthy subjects [27]. DNL has been shown to contribute approximately 12% of palmitate to VLDL-TAG in a previous study in NAFLD, when measured over a comparable time period to the present study [8]. In a previous study, an 8 week diet with fructose-sweetened beverages, providing 25% of total energy, increased DNL, whereas glucose-sweetened beverages had no effect in the healthy overweight participants [28]. Similarly, a 6-day high-fructose diet (25% total energy) was shown to increase DNL from 1.6 to 9.4% in VLDL-palmitate in healthy, normal weight men [29].

In the present study, there was no significant difference in the systemic contribution of fatty acids to $VLDL_1$-TAG or $VLDL_2$-TAG production between the diets in either groups. This is perhap surprising, given the marked increase in plasma NEFA and higher production and clearance rates of palmitate after the high sugars diet in the NAFLD group, which might be expected to increase the delivery of NEFA to the liver. There was, however, a greater contribution of splanchnic fat to $VLDL_1$-TAG and $VLDL_2$-TAG production in NAFLD relative to controls, which might help to explain how liver fat influences the differential partitioning of hepatic TAG in these groups in response to dietary sugars.

Splanchnic fat includes hepatic TAG storage pools and visceral adipose tissue, the NEFA from which drains directly into the liver via the portal vein. Hepatic TAG storage pools will expand in the fed, postprandial state, with an estimated 22% of dietary TAG being taken-up by the liver in chylomicron remnants [30], some of which will be stored and contribute to VLDL synthesis in the post-absorptive state [31].

The flux of NEFA from visceral adipose tissue has been estimated to be 20% of total NEFA delivery to the liver in obese subjects, but only 5% in lean subjects [32, 33] based on a model partially validated in dogs [34]. Visceral adipose NEFA flux was also shown to correlate with visceral fat measured by computer tomography [32].

Since the men in our study were generally overweight, but not obese, visceral adipose tissue is likely to have made a small contribution (5-20%) towards the delivery of total NEFA to the liver [32, 33]. However, since visceral fat was not different between groups and unaffected by the diets in the present study, this suggests that the relatively greater contribution of splanchnic-derived NEFAs to $VLDL_1$ and $VLDL_2$-TAG production on the high sugars diet in NAFLD relative to controls, came from hepatic TAG storage pools. This possibility introduces the established effect of dietary sugars in augmenting post-prandial lipaemia [35], and highlights the importance of postprandial TAG as a potential source of lipid for the accumulation of liver fat [36]. While postprandial responses were not measured in our study, the high sugar diet increased $VLDL_1$ in controls, and serum apo C-III in NAFLD, an apoprotein with roles in the assembly of $VLDL_1$ in the liver and inhibition of LPL [37]. These effects are consistent with dietary free sugars impairing the clearance of plasma TAG in the postprandial phase [35, 36].

The intake of sugars on the low sugars diet was close to the current recommendation for the intake of free sugars, of no more than 5% total energy (NMES 6 ± 2% total energy or 586 kJ (140kcal) /day) [38, 39]. In contrast, the intake of sugars on the high sugars diet (NMES 26 ± 7% total energy) was five-fold greater than this recommendation (2,721kJ (650kcals) /day), but still within the upper 2.5th percentile of intake in a typical UK diet [13]. Although we cannot exclude the possibility that the small differences in the intake of dietary fat between the iso-energetic diets contributed to the metabolic effects (5% and 8% energy in NAFLD and controls, respectively), the overall, weight-adjusted response of outcome variables is consistent with the marked differences in intake of dietary sugars between the two diets (19% and 20% energy, in NAFLD and controls, respectively).

It is well documented that hyper-energetic diets, high in sugars, increase liver fat in healthy men [40], but there is less evidence that iso-energetic diets, high in sugars, exert the same effect. A weight-maintaining high fructose diet (25% total energy) has been reported to increase liver fat by 137% in healthy men [41]. Similarly, an iso-energetic diet containing sucrose-sweetened regular cola increased liver fat by 132% in overweight subjects [42]. In the present study, the high sugars diet increased liver fat to a relatively greater extent in subgroups of men with NAFLD, compared to controls. While this might suggest greater sensitivity to dietary sugars in NAFLD, the statistical significance of this difference in liver fat was lost after adjustment for the small gain in body weight. This finding reaffirms that liver fat is very sensitive to increased body weight in response to dietary sugars [43].

Strengths of our study include the dietary exchange, which achieved its targets for sugar intake in a free-living setting, and stable isotope trace-labelling methodology to simultaneously track the metabolism of plasma lipoproteins, fatty acids and DNL. Limitations of our study include its sample size and the dependence of our main outcomes measures on the assumptions inherent in mathematical modelling. In addition, results derived from the infusion of stable isotope labelled palmitate are dependent on the validity of assumptions regarding fatty acid fluxes to the liver. While we adjusted all data for the small and consistent changes in body weight in response to differences in energy intake between diets, we cannot exclude the possibility of acute metabolic effects arising from these differences. Nevertheless, the overall pattern of metabolic responses to the diets, and significance of weight-adjusted differences in our outcome variables, including $VLDL_1$-TAG production rate, on which the sample size was originally powered, provide confidence that the data is robust. It also suggests that the effects of a high and low intake of sugars on lipoprotein metabolism were independent of the relationship between changes in body weight and liver fat.

This study provides new evidence that liver fat influences the effects of dietary free sugars in partitioning plasma TAG into different VLDL subclasses. This finding has major implications for the potential mechanism by which dietary free sugars could contribute to the development of NAFLD, and dyslipidaemia.

CLINCAL PERSPECTIVES

- A high intake of dietary sugars consumed in foods and sugar sweetened beverages, has been implicated in the development of fatty liver disease, possibly through adverse effects on lipid metabolism. This study was undertaken to determine if liver fat influences the plasma lipid and lipoprotein response to sugars, and the mechanism by which sugars contribute to the accumulation of liver fat.

- High and low sugar diets produced differential effects on the metabolism of plasma VLDL subclasses in men with raised liver fat (NAFLD) and low liver fat controls. A high intake of sugars produced changes in the lipoprotein metabolism of controls that were characteristic of men with NAFLD.

- These findings indicate that the accumulation of liver fat can influence the plasma lipid and lipoprotein response to dietary sugars, and provide new evidence for a mechanism to explain how sugars may contribute to NAFLD and dyslipidaemia.

AUTHOR CONTRIBUTION

AMU, BG, JL and GF designed the study, FSM, JW, AM and XL performed the clinical studies, BG, JL, GF, CI and AA were involved with the dietary design and supervision, FSM, NJ, AM, XL,NA, MS and BF the laboratory work, supervised by AMU. RH and MW did the modelling, JB and ELT performed the MRI and MRS measurements, SJ performed the statistical analysis. BG and AMU were the lead writers. All authors were involved in drafting the article or revising it critically for important intellectual content and approved the final version. AMU is the guarantor of this work and, as such, had full access to all the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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COMPETING INTERESTS

JL has previously received financial support for other research studies from Sugar Nutrition UK and PepsiCo. All authors, including JL, declared there to be no duality of interest associated with this manuscript.

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Table 1. Group characteristics at baseline

	NAFLD (n=11)	Controls (n=14)
Age y (range)	59 (49-64)	54 (41-65)
Body weight kg	90.0±2.2 (75.6-102.4)	89.7±2.4 (78.3-107.9)
BMI kg/m^2	28.9±0.3 (26.9-30.8)	28.4±0.5 (26.0-31.0)
Waist circumference cm	104±2 (93-113)	104±1 (100-114)
Liver fat %	17.2±2.7^b (7.9-36.8)	2.5±0.3 (0.5-4.6)
Triacylglycerol mmol/l	1.89±0.27^a (1.10-4.01)	1.33±0.23 (0.60-3.80)
Cholesterol mmol/l	5.91±0.25 (4.60-7.20)	5.51±0.28 (4.30-7.20)
HDL cholesterol mmol/l	1.22±0.08 (1.00-2.00)	1.24±0.08 (0.90-2.10)
Glucose mmol/l	5.73±0.11 (4.90-6.10)	5.46±0.12 (4.90-6.40)
Systolic BP mmHg	131±7 (113-177)	134±3 (110-156)
Diastolic BP mmHg	86±4.5 (67-113)	84±2.7 (62-93)

Values are means ± SEM (Ranges). Significant difference between groups: ^aP <0.05, ^bP<0.001.

Table 2. Effects of high and low sugars diets on anthropometrics and plasma lipids

	NAFLD (n=11)		Controls (n=14)	
	High sugars	Low sugars	High sugars	Low sugars
Body weight (kg)	89.8±2.5	87.7±2.4[c]	88.9±2.8	86.7±2.9[b]
BMI (kg/m^2)	28.8±0.4	28.2±0.5	28.1±0.6	27.4±0.6
Liver fat %[1]	24.2±6.8	14.2±3.2	3.6±1.3	1.5±0.3
Body fat %[2]	27.3±0.8	26.5±0.9	24.8±0.7	23.8±0.9
Plasma TAG[3] mmol/l	2.05±0.24[a]	1.77±0.22	1.33±0.15	1.13±0.08
Plasma cholesterol mmol/l	5.59±0.33	5.24±0.30	5.10±0.25	4.82±0.26
Plasma LDL-C mmol/l	3.40±0.26	3.23±0.28	3.27±0.19	3.13±0.21
Plasma HDL-C mmol/l	1.21±0.09	1.15±0.07	1.19±0.07	1.16±0.08
Plasma glucose mmol/l	5.35±0.09	5.39±0.09	5.08±0.11	5.11±0.08
Plasma insulin mU/l	21.2±2.6	21.4±1.0	17.9±1.4	17.7±2.4
HOMA2-IR	2.72±0.33	2.76±0.12	2.28±0.17	2.26±0.29

Values are arithmetic means ± SEMs unless stated otherwise. [1]Measured by MRS on subgroup n=17. [2]Measured by bio-electric impedance. [3]Geometric mean ± SEM. Significant difference between groups (within diet) [a]*P* <0.02. Significant difference between diets (within group) [b]*P* <0.01, [c]*P* <0.001. All differences adjusted for body weight.

Table 3: Effects of high and low sugars diets on plasma lipoprotein fraction concentrations

	NAFLD (n=11)		Controls (n=14)	
	High sugars	Low Sugars	High sugars	Low Sugars
Total VLDL-TAG µmol/l[1]	996±142[b]	872±117[d]	651±72	490±51
$VLDL_1$-TAG µmol/l	849±109[b]	761±97[d]	547±67	386±39
$VLDL_2$-TAG µmol/l	147±21	110±10	104±11	104±14
IDL-TAG µmol/l	61±5	52±5	54±5	65±11
VLDL-Chol µmol/l	509±121[a]	381±70[d]	290±30	206±23
$VLDL_1$-Chol µmol/l	345±76	283±49	207±26	127±14
$VLDL_2$-Chol µmol/l	163±45	97±13	82±10	80±13
IDL-chol µmol/l	167±53	88±13	88±11	99±16
$VLDL_1$-apoB mg/l	15.6±2.5	17.4±3.0	15.1±2.6	11.5±1.9
$VLDL_2$-apoB mg/l	12.5±2.1	11.6±1.5	13.1±3.4	11.0±2.7
IDL-apoB mg/l	21.9±4.6	14.1±1.8[e]	20.2±5.0	20.9±5.8
LDL-TAG µmol/l	1231±165[b]	1088±130[c]	843±71	705±57
LDL_2-TAG µmol/l	99±10	93±13	75±12	71±8
LDL_3-TAG µmol/l	79±12	72±8	60±7	65±6
LDL_2-chol µmol/l	1019±87	931±106	781±95	881±82
LDL_3-chol µmol/l	1222±68	1252±60	1141±94	1172±45
LDL_2-apoB mg/l	306±53	255±40	258±33	249±32
LDL_3-apoB mg/l	567±92	574±98	570±48	459±53
Small dense LDL µmol/l	1459±210	1228±175[a]	1043±112	848±78

Values are mean ± SEM. Significant difference between groups (within diet) [a]*P<0.05*; [b]*P<0.02*; [c]*P<0.005;* [d]*P<0.001*. Significant difference between diets (within group) [e]*P<0.05.* All differences were adjusted for body weight. [1]Sum of $VLDL_1$ and $VLDL_2$ –TAG.

Table 4: Effects of high and low sugars diets on lipoprotein kinetics and DNL

	NAFLD (n=11)		Controls (n=14)	
	High sugars	Low Sugars	High sugars	Low Sugars
$VLDL_1$-TAG production rate g/d	20.9±2.1	18.9±2.1[a]	16.6±1.4	12.4±1.2[e]
$VLDL_1$-TAG FCR pools/d[1]	9.0±0.9[a]	9.5±1.0	11.3±0.7	11.9±0.8
$VLDL_2$-TAG production rate g/d	4.90±0.59	3.70±0.43[d]	3.63±0.27	3.98±0.43
$VLDL_2$-TAG FCR pools/d	11.5±1.1	12.2±1.3	13.1±1.0	14.3±0.9
$VLDL_1$-apoB production rate mg/d	481±76	492±58	546±56	414±54
$VLDL_1$-apoB FCR pools/d	9.0±1.0	10.8±2.2	14.7±2.7	13.4±2.4
$VLDL_2$-apoB production rate mg/d	546±176	498±164	720±310	647±212
$VLDL_2$-apoB FCR pools/d	12.5±2.8	12.8±2.4	13.6±1.9	14.9±1.6
IDL-apoB production rate mg/d	609±122	391±69[f]	740±159	737±213
IDL-apoB FCR pools/d	9.5±1.9	8.7±0.9	12.2±1.1	12.1±1.2
LDL_2-apoB production rate mg/d	1452±277	858±101	1075±109	1176±118
LDL_2-apoB FCR pools/d	1.59±0.25	1.35±0.23	1.59±0.24	1.74±0.26
LDL_3-apoB production rate mg/d	2069±388	942±278	1518±237	1374±273
LDL_3-apoB FCR pools/d	1.01±0.15	0.46±0.09[b]	0.86±0.12	1.06±0.24
Contribution of DNL to:	1.66±0.39	1.59±0.34[e]	1.32±0.43	0.56±0.14[c]
$VLDL_1$-TAG production g/d	1.66±0.39	1.59±0.34[c]	1.32±0.43	0.56±0.14
$VLDL_2$-TAG production g/d	0.28±0.05	0.29±0.05	0.26±0.05	0.19±0.03

Values are mean ± SEM. Significant differences between groups (within diet) [a]*P*=0.01; [b]*P*<0.05, [c]*P*=0.003. Significant differences between diets (within group) [d]*P*=0.036; [e]*P*=0.02; [f]*P*=0.06. All differences were adjusted for body weight. For the IDL and LDL_2 kinetic data NAFLD (n=9), and n=8 for the LDL_3 kinetic data due to insufficient data for the model fit. [1]Analysed for first period only, so between group comparisons (within diet) only were analysed (NAFLD; high sugar n=7, low sugar n=4. Controls; high sugar n=7, low sugar n=7).

Table 5. Effects of high and low sugars diets on palmitate kinetics, post-heparin lipase activities, and plasma apoproteins

	NAFLD (n=11)		Controls (n=14)	
	High sugars	Low Sugars	High sugars	Low Sugars
Plasma NEFA μmol/l	658±30[c]	548±44	438±31	526±42[a]
Plasma Palmitate μmol/l	220±39	238±25	214±25	218±28
Palmitate production rate μmol/min	169±11	147±12[d]	168±15	168±17
Palmitate MCR ml/min	863±74	647±56[e]	863±110	850±101
Post heparin LPL pmol/ml/min	1.33±0.31	1.30±0.21	1.36±0.19	1.97±0.32
Post heparin HL pmol/ml/min	2.13±0.48[a]	1.43±0.38	1.01±0.17	0.90±0.18
Plasma apoE mg/l	33.3±3.7	30.2±2.7	29.1±1.4	27.7±1.4
Plasma apoC-III mg/l	112.2±9.8[a]	103.8±9.1[b]	86.0±7.5	73.5±5.4
Plasma apoC-II mg/l	82.7±9.2	77.0±8.0[a]	61.7±6.1	56.9±5.6

Values are mean ± SEM. Significant difference between groups (within diet); [a]*P*<0.05; [b]*P*<0.01; [c]*P*<0.001. Significant difference between diets (within group); [d]*P*<0.05; [e]*P*<0.01. All differences were adjusted for body weight.

Figure legends

Figure 1

Effects of high and low sugar diets (black and white bars, respectively) in men with NAFLD and low liver fat controls on the plasma concentrations of: **a)** $VLDL_1$-TAG, and **b)** $VLDL_2$-TAG. Effects of high and low sugar diets on the contribution of fatty acids from systemic (black bars), splanchnic (white bars) and DNL (grey bars) to: **c)** $VLDL_1$-TAG production rate and **d)** $VLDL_2$-TAG production rate. Significance of weight-adjusted differences between groups and diets are as shown, and for differences between groups; $P<0.05$; ******$P=0.006$; *******$P=0.003$. $^{\#}P=0.08$ denotes trend for difference between diets in controls.

Figure 2

Summary schematic of the relative effects of a high and low sugar diet (red hatched arrows) on lipoprotein metabolism in men with NAFLD and low liver fat controls. Thickness of black arrows represents the magnitude of pathway in men with NAFLD relative to controls (PR = production rate, FCR = fractional catabolic rate). *Significance of increases in liver fat in both NAFLD and controls, after the high sugar diet relative to the low sugar diet, were not maintained after adjustment for body weight.

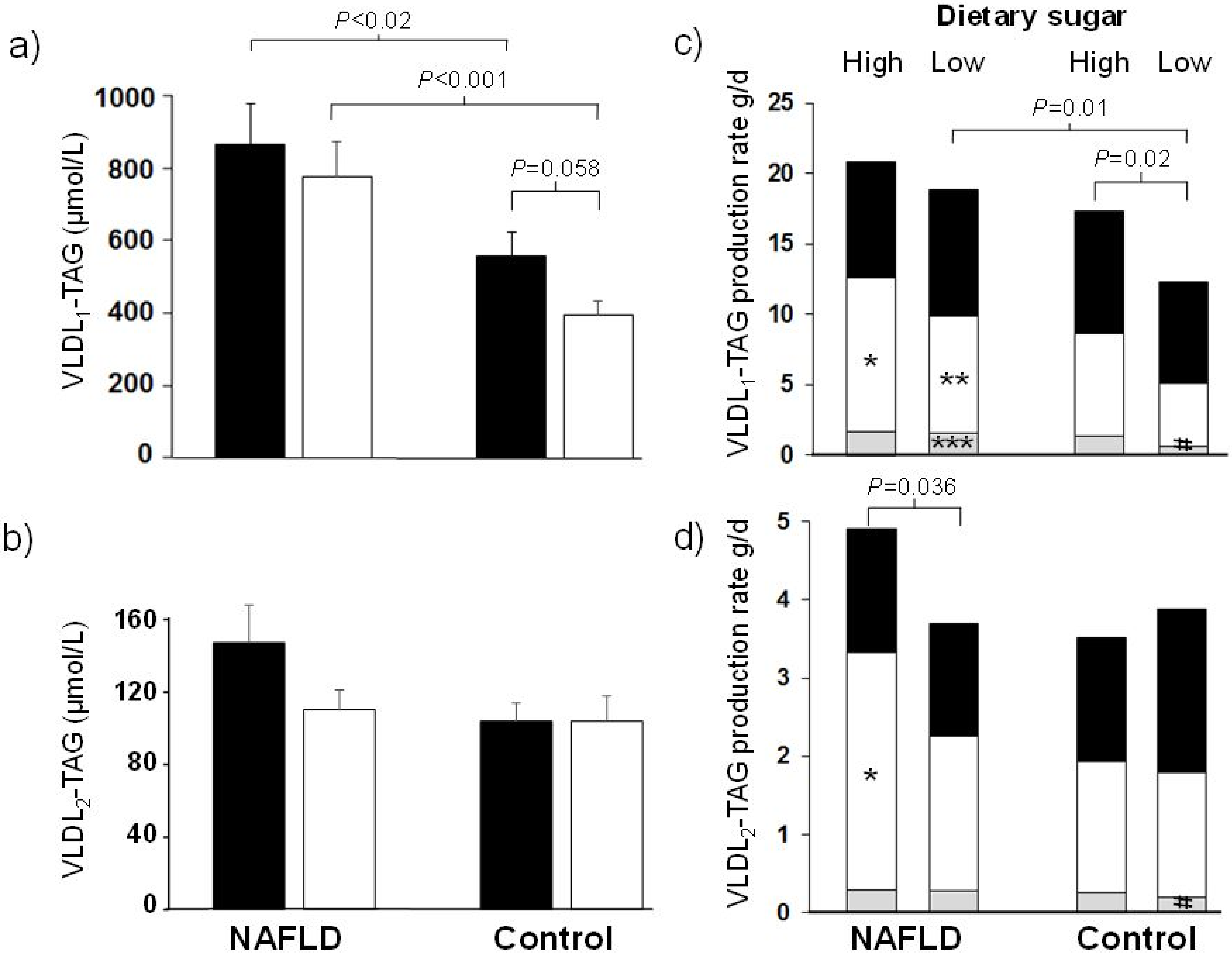
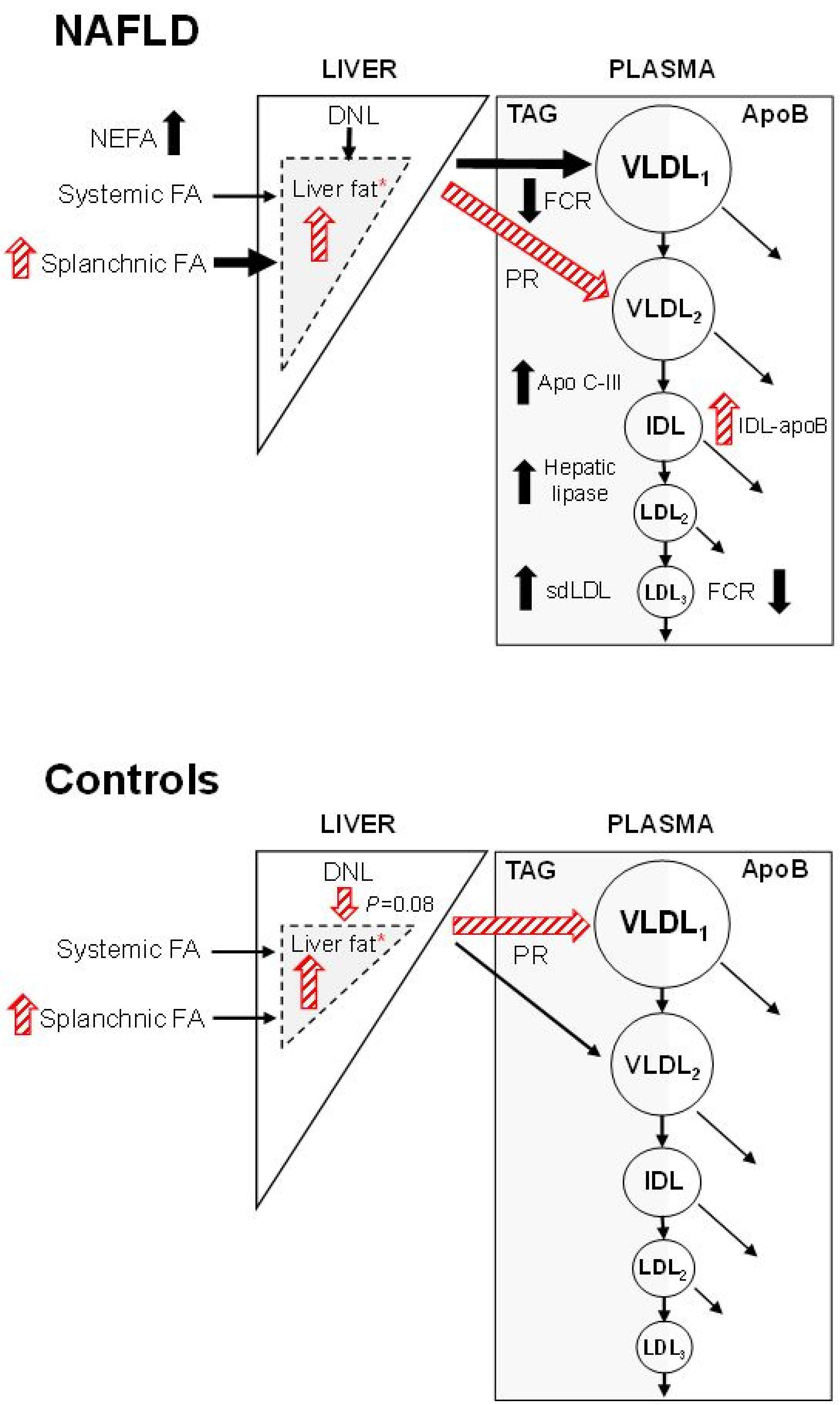

a)
P<0.02
P<0.001
P=0.058
VLDL1-TAG (µmol/L)
1000
800
600
400
200
0
b)
VLDL2-TAG (µmol/L)
160
120
80
40
0
NAFLD
Control
c)
Dietary sugar
High
Low
High
Low
VLDL1-TAG production rate g/d
25
20
15
10
5
0
P=0.01
P=0.02
*
**
***
#
d)
P=0.036
VLDL2-TAG production rate g/d
5
4
3
2
1
0
*
#
NAFLD
Control

Figure 2


NAFLD
LIVER
PLASMA
DNL
NEFA
Systemic FA
Splanchnic FA
Liver fat*
TAG
ApoB
VLDL1
FCR
PR
VLDL2
Apo C-III
IDL
IDL-apoB
Hepatic lipase
LDL2
sdLDL
LDL3
FCR
Controls
LIVER
PLASMA
DNL
P=0.08
Systemic FA
Splanchnic FA
Liver fat*
TAG
ApoB
VLDL1
PR
VLDL2
IDL
LDL2
LDL3

Supplementary Material

Impact of liver fat on the differential partitioning of hepatic triacylglycerol into very low density lipoprotein subclasses in response to high and low sugar diets

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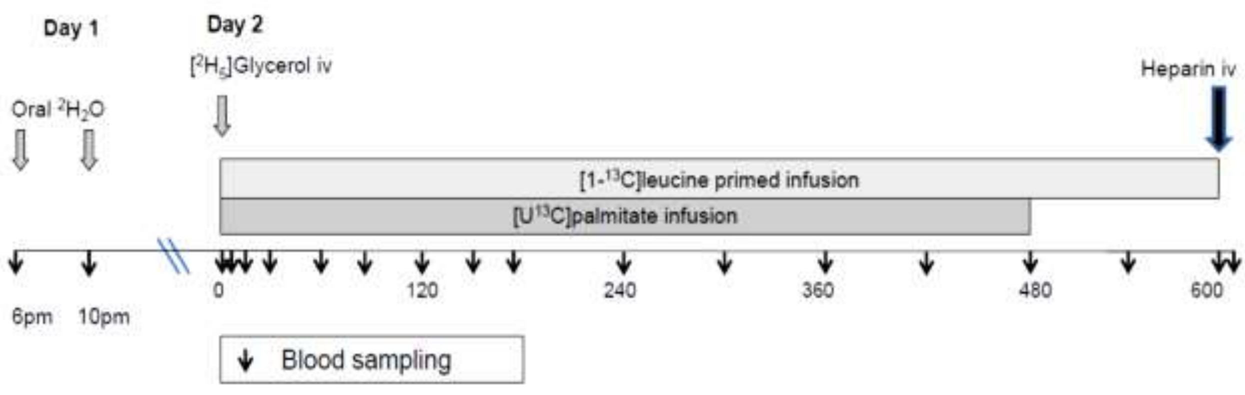
Dietary exchange model

The sugar content of the two diets was achieved by a dietary exchange of sugar for starch using foods that were either high or low in total sugars (≥ 40% or ≤ 10% of total carbohydrate (CHO), respectively). Foods with intermediate sugar content were excluded from the dietary exchange model, the aim of which was to replace two thirds of the habitual CHO intake with study foods (approximately 180 g/day) without changing other dietary components. Participants were required to exchange 6 portions of their habitual CHO per day (a portion representing 30g CHO) with either the high or low sugar foods, depending on their allocated diet. A number of different foods and drinks (containing either high sugar/low starch or low sugar/high starch) were supplied to the participants, which allowed dietary flexibility and aided compliance. The intervention diets were designed to be matched for total carbohydrate, protein and fat content and iso-energetic. Five home visits were made every 2 weeks to supply study foods, measure body weight, and to assess daily food and drink portion sheets to help maintain dietary compliance, and to maintain body weight to within ± 0.5 kg.

Figure 1: Protocol for metabolic study



Data analysis (Sources of fatty acids for VLDL production)

Palmitate production rate (PR) was calculated as: ***Palmitate PR (µmol/min) = Infusion rate of palmitate tracer (µmol/min) /*** TTR_{SS}. Where TTR = m/z 286/270 at time t minus m/z 286/270 at t=0 min, TTR_{SS} = mean TTR $_{(t=420-480\ min)}$ and SS= steady state.

The contribution of circulating palmitate (systemic contribution) to $VLDL_1$-TAG PR was calculated as: ***Systemic contribution of fatty acids to*** $VLDL_1$***-TAG PR (g/d) =*** $VLDL_1$***-TAG PR (g/d) x ((*** $VLDL_1$ ***TAG palmitate*** TTR_{SS} ***/ plasma palmitate*** TTR_{SS}***).*** This will include a contribution from visceral fat, since some labelled palmitate will be taken-up by this fat store and released into the portal vein.

The percent contribution of hepatic DNL-derived palmitate to $VLDL_1$ and $VLDL_2$-TAG PR was calculated from the deuterium enrichment in the palmitate of $VLDL_1$ and $VLDL_2$-TAG and in plasma water as previously described [1]. The calculation assumes that in all VLDL-TAG fatty acids derived from DNL, the enrichment in TG-palmitate (Maximum palmitate TTR) will be ***Maximum palmitate TTR =*** ^2H_2O ***TTR x N***, where ^2H_2O TTR is the enrichment of the plasma water, and N is the maximum number of deuterium atoms, that can be incorporated into a molecule of palmitate. In the present study N was 21, based on previous observations [1]. The percentage of palmitate derived from DNL in VLDL-TAG was calculated as: ***% hepatic DNL in VLDL-TAG palmitate = (VLDL-TG palmitate TTR / maximum palmitate TTR) / 100*** where TTR is m/z 271/270 at time 12 hour minus 271/270 at time 0 minutes. For details of the time course of deuterium incorporation into VLDL-TAG palmitate see Diraison *et al*. (1997) [2].

The contribution of DNL to $VLDL_1$-TAG PR was estimated as: ***DNL contribution to $VLDL_1$-TAG PR (g/d) = % hepatic DNL x $VLDL_1$-TAG PR (g/d) x 100.***

The splanchnic fat contribution was assumed to be all other sources of fatty acids and was calculated as: ***Splanchnic fat contribution of fatty acids to $VLDL_1$-TAG PR (g/d) = $VLDL_1$-TAG PR (g/d) - (DNL (g/d) + Systemic (g/d)).***

In these calculations it was assumed that palmitate is a representative of NEFAs. $VLDL_2$-TAG PR was substituted for $VLDL_1$-TAG PR in the above equations to calculate the contribution of different fatty acid sources to $VLDL_2$-TAG PR.

Kinetic modelling of $VLDL_1$ and $VLDL_2$-TAG

$VLDL_1$-TAG and $VLDL_2$-TAG FCR were calculated using a compartment model of $VLDL_1$-TG and $VLDL_2$-TG kinetics using SAAM II software. The model represents the kinetics of the tracer-to-tracee ratio (TTR) profiles which change as labelled glycerol is removed from plasma and incorporated into the TAG fractions. Plasma glycerol kinetics was described by a sum of three exponentials representing a three compartment model. A five-compartment chain described a time delay due to synthesis and secretion of $VLDL_1$ and $VLDL_2$-TAG. The model is schematically depicted in Fig. 2.

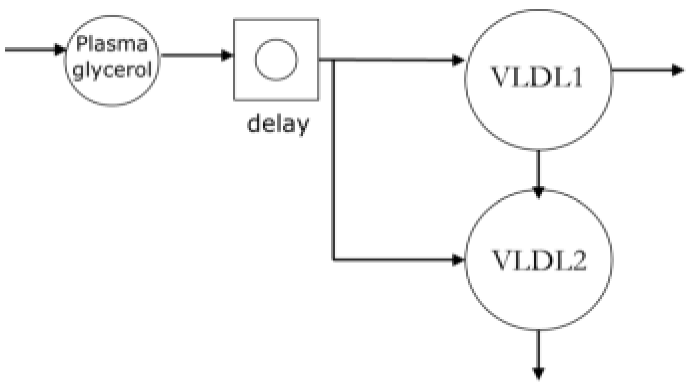


Figure 2. Schematic of model used to describe TTRs of $VLDL_1$ and $VLDL_2$-TAG.

The model assumes steady state of native (unlabelled) glycerol throughout the experimental period, i.e. a constant appearance, disappearance, and incorporation of native glycerol into the TAG fractions. The incorporation of glycerol into VLDL by the liver is subject to a delay. The model included a compartment for $VLDL_1$-TAG and a compartment for $VLDL_2$-TAG with an input into both compartments from the glycerol precursor pool, a loss from each compartment and a transfer from the $VLDL_1$-TAG compartment to the $VLDL_2$-TAG compartment. $VLDL_1$-TAG and $VLDL_2$-TAG production rates were calculated as the product of $VLDL_1$-TAG and $VLDL_2$-TAG FCR and their respective TAG pools. $VLDL_1$ and $VLDL_2$-TAG pools were calculated from $VLDL_1$ and $VLDL_2$-TAG concentration and plasma volume which was determined by the method of Pearson et al [2].

Kinetic modelling of apoB

$VLDL_1$, $VLDL_2$, IDL, LDL_2 and LDL_3 apoB FCR and production rate were determined using a multi-compartmental model using SAAM II software which incorporated a forcing function corresponding to precursor (α-KIC) enrichment and a delay function accounting for the amount of time required for synthesis and production rate of $VLDL_1$ and $VLDL_2$-apoB. Similar to the TAG model, a delay compartment consisting of a five-compartment chain was added to account for time required for the synthesis and secretion of VLDL1 and VLDL2-apoB. The model is schematically depicted in Figure 3. Production rate (mg/day) was calculated as the product of FCR and the apoB pool size. ApoB pool size (mg) was calculated as the product of apoB concentration and plasma volume (determined as described above)

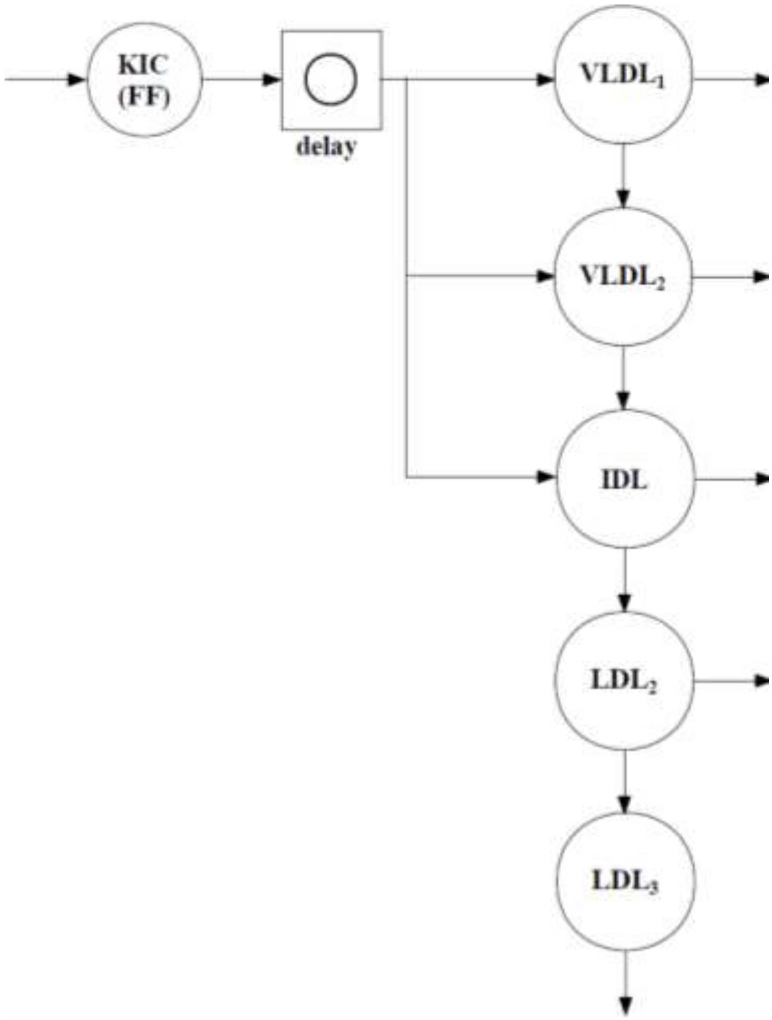


Figure 3. Schematic description of the model used to describe TTRs of $VLDL_1$ $VLDL_2$, IDL, LDL_2 and LDL_3-apoB.

In both models, the parameters were estimated using the weighted non-linear regression analysis. The weights were reciprocal to the variance of the measurement error. The measurement error was assumed uncorrelated with zero mean; a constant standard deviation of 0.005% below TTR of 0.1% and a constant coefficient of variation of 5% above TTR of 0.1%.

References

1. Diraison, F., Pachiaudi, C. and Beylot, M. (1996) In vivo measurement of plasma cholesterol and fatty acid synthesis with deuterated water: determination of the average number of deuterium atoms incorporated. Metabolism **45**, 817-821

2. Diraison, F. Pachiaudi, C. Beylot, M. (1997) Measuring lipogenesis and cholesterol synthesis in humans with deuterated water: use of simple gas chromatographic/mass spectrometric techniques. J. Mass Spectrom. **32**, 81-86.

3. Pearson, T.C., Guthrie, D.L., Simpson, J., Chinn, S., Barosi, G., Ferrant, A., Lewis S.M. and Najean, Y. (1995) Interpretation of measured red cell mass and plasma volume in adults: Expert Panel on Radionuclides of the International Council for Standardization in Haematology. Br. J. Haematol.**89**, 748-756

Table 1. Intake of energy and macronutrients

	NAFLD (n=11)		Controls (n=14)	
	High sugar	Low sugar	High sugar	Low sugar
Total energy MJ/d	10.6±8.6	9.6±6.2	10.6±5.5	10.1±4.3
Carbohydrate g/d	311±22	240±14	342±20	270±18
% energy	50±2	42±2[a]	54±2	44±2[b]
Total sugars g/d	168±15	53±6[b]	177±14	58±6[b]
% energy	27±2	9±1[b]	28±2	10±1[b]
Starch g/d	143±10	187±9[a]	165±11	212±14[a]
% energy	23±1	33±2[b]	26±1	35±1[b]
NMES g/d	152±13	31±2[b]	164±15	33±4[b]
% energy	25±2	6±0.4	26±2	5±0.5
Protein g/d	92±9	98±10	92±5	96±5
% energy	15±1	17±1	15±1	16±1
Total fat g/d	81±13	86±10	75±6	92±5
% energy	28±3	33±2	26±2	34±1[b]
SFA g/d	34±6	36±6	27±3	35±3
Fibre g/d	21±2	23±3	21±2	25±2
Sodium g/d	3.0±0.4	3.5±0.3	2.8±0.2	3.9±0.3[b]

Values are means ± SEM. high sugar versus low sugar $^{a}P < 0.01$, $^{b}P < 0.001$.

Table 2. Body fat distribution measured by MRS

	NAFLD (n=7)		Controls (n=10)	
	High sugar	Low sugar	High sugar	Low sugar
Total body fat (kg)	26.6±1.5	24.7±1.0	24.8±1.9	23.2±2.5
Total Subcutaneous fat (kg)	17.6±0.8	16.7±0.8	17.6±1.7	16.6±1.8
Total internal fat (kg)	9.0±0.9	8.0±0.5	7.3±0.5	6.6±0.8
Abdominal sub-cutaneous fat (kg)	5.2±0.5	4.8±0.3	5.2±0.7	4.8±0.7
Peripheral sub-cutaneous fat (kg)	12.4±0.6	11.9±0.3	12.4±1.0	11.8±1.2
Visceral fat (kg)	4.6±0.4	4.8±0.4	4.0±0.3	3.6±0.5
Non-visceral internal fat (kg)	4.4±0.9	3.2±0.2	3.3±0.3	3.0±0.3

Values are mean ± SEM