Intrahepatic fat and postprandial glycaemia increase after consumption of a diet enriched in saturated fat compared to free sugars.

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Study registration: www.clinicaltrials.org ID number NCT03145350.

Word count: 4197

References: 43

Tables: 1

Figures: 3

Supplementary Tables: 2

Supplementary Figures: 5

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Objective: Debate continues regarding the influence of dietary fats and sugars on the risk of developing metabolic diseases, including insulin resistance and nonalcoholic fatty liver disease (NAFLD). We investigated the effect of two eucaloric diets, one enriched with saturated fat (SFA), the other enriched with free sugars (SUGAR), on intrahepatic triacylglycerol (IHTAG) content, hepatic *de novo* lipogenesis (DNL) and whole-body postprandial metabolism in overweight males.

Research design and methods: Sixteen overweight males were randomized to consume the SFA or SUGAR diet for 4 weeks before consuming the alternate diet after a 7-week washout period. The metabolic effects of the respective diets on IHTAG content, hepatic DNL and whole-body metabolism were investigated using imaging techniques and metabolic substrates labelled with stable-isotope tracers.

Results: Consumption of the SFA diet significantly increased IHTAG by 39.0±10.0% (mean±SEM), whilst after the SUGAR diet IHTAG was virtually unchanged. Consumption of the SFA diet induced an exaggerated postprandial glucose and insulin response to a mixed test meal compared to SUGAR. Although whole-body fat oxidation, lipolysis and DNL were similar following the two diets, consumption of the SUGAR diet resulted in significant ($p<0.05$) decreases in plasma total, HDL and non-HDL cholesterol, and fasting β-hydroxybutyrate plasma concentrations.

Conclusions: Consumption of a SFA diet had a potent effect, increasing IHTAG together with exaggerating postprandial glycaemia. The SUGAR diet did not influence IHTAG, and induced minor metabolic changes. Our findings indicate that a diet enriched in SFA is more harmful to metabolic health than a diet enriched in free-sugars.

Introduction

Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of liver related conditions, ranging from steatosis (characterised by an accumulation of intrahepatic triacylglycerol (IHTAG)) to nonalcoholic steatohepatitis, cirrhosis, and hepatocellular carcinoma, and is the most prevalent liver disease worldwide (1). There appears to exist a bidirectional relationship between NAFLD and metabolic disease; the presence of NAFLD predicts the development of the metabolic syndrome/type 2 diabetes (T2D) and *vice versa* (2). Furthermore, the presence of NAFLD may exacerbate the metabolic abnormalities that occur with T2D (3). Obesity is a principal risk factor for NAFLD (1), and it is suggested that increased hepatic *de novo* lipogenesis (DNL) is an underlying cause in the development of NAFLD and/or insulin resistance (4; 5). As excess non-lipid precursors (*e.g.* sugars and protein) can exacerbate hepatic DNL, dietary composition may be an important mediator of NAFLD development.

Observational studies report diets high in fat and/or free sugars are associated with NAFLD and a consistent finding from interventional studies is that hypercaloric diets enriched in fat or sugars increased IHTAG (6). Recently, Luukkonen *et al.*, (7) reported that consuming 1000 excess kcal/day as SFA increased IHTAG content to a greater extent (55% relative increase) than consuming excess calories as unsaturated fatty acids (FA) (15% increase) or free sugars (33% increase); this effect was independent of changes in body weight. Others have reported IHTAG increased to a greater extent with overfeeding of SFA compared to diets overfeeding either fructose (8) or n-6 polyunsaturated fat (9).

Of the limited number of studies that have investigated the influence of macronutrient composition in eucaloric diets, findings for the effects on IHTAG are inconsistent, with some demonstrating diets enriched in fat/SFA (10), or sugars (11) increase IHTAG content, whereas others show no effect (12; 13). To date no study has directly compared eucaloric diets enriched in SFA or free sugars on IHTAG and few studies assess the effect specific diets have on postprandial metabolism and intrahepatic fatty acid (FA) synthesis and partitioning. Therefore, the aim of this study was to compare the effects of two eucaloric diets, one enriched in carbohydrate, specifically free sugars, and the other enriched in fat, specifically SFA, on IHTAG content, hepatic DNL and hepatic and whole-body postprandial metabolism in overweight males. Based on the available evidence, we hypothesized that diets enriched in SFA or free sugars would differentially influence whole-body and hepatic FA metabolism, with a SFA enriched diet increasing IHTAG to a greater extent than a sugar-enriched diet and this would be driven by an increase in adipose tissue lipolysis, whilst a sugar enriched diet would increase hepatic DNL.

Methods

Participants

Participants were recruited from the Oxford BioBank (www.oxfordbiobank.org.uk) (14) (supplementary Figure S1). All volunteers were free from metabolic disease, had a body mass index (BMI) between 25-30kg/m^2, were not taking medication known to affect lipid or glucose metabolism, were non-smokers, and consumed alcohol within recommended limits (1). The study was approved by the North West, Lancaster Clinical Research Ethics Committee (16/NW/0751) and all participants gave written informed consent.

Experimental design

In a randomized crossover design, participants completed two 4-week dietary interventions separated by a 7-week washout period where they returned to their habitual diet. Participants also followed a one-week standardization diet, based on the UK EatWell plate, prior to starting the respective dietary interventions (*i.e.* before fasting study days). The two dietary interventions were i) a relatively high-fat diet enriched in SFA (referred to as SFA), and ii) a relatively high-carbohydrate diet enriched with free sugars (referred to as SUGAR). Participants were randomized to the order in which they undertook each intervention diet (e.g. SFA then SUGAR or SUGAR then SFA) prior to the first study day using a random number generator, by a statistician not involved in the running of the trial, in order to avoid any effects of dietary sequence. Participants completed 3-day diet diaries during all standardized and experimental diet periods. Before beginning each dietary intervention, participants underwent a fasting study day, and upon completion of the intervention diet, participants underwent a postprandial study day that utilized stable-isotope tracers to investigate postprandial metabolism (Figure 1).

Anthropometric measures, IHTAG content, and fasting plasma biochemistry and lipidomics were assessed across each of the respective interventions (pre vs. post diet), whilst others, including postprandial plasma biochemistry, isotopic analysis, bile acid species, and indirect calorimetry were compared between diets at the end of the respective dietary phase (post vs. post).

Fasting study day

Immediately before each dietary intervention, IHTAG was measured after an overnight fast by proton magnetic resonance spectroscopy (^{1}H-MRS) using a 3.0 Tesla MRI scanner (Siemens Healthineers, Erlangen, Germany). A single voxel ($20 \times 20 \times 20$ mm^3) was positioned in the posterior part of the left liver lobe and both water-suppressed and non-water-suppressed stimulated acquisition mode (STEAM) measurements performed (15). Sequence parameters were: TE=10ms, TM=7ms, TR was at least 2000ms for water-suppressed scans and TR was at least 4000ms for non-water-suppressed scans with acquisitions synchronized to ECG. At the analysis stage these two acquisitions were combined and the proportion of TAG in the liver tissue was determined using the OXSA toolbox (16). Following the MRI scan, blood samples were collected from an antecubital vein, body weight and waist circumference measurements were made, and a dual-energy X-ray absorptiometry (DEXA) scan performed to assess body composition. Participants then had a consultation with the study dietitian who provided diet sheets containing written and pictorial information about how to follow the respective experimental diets, including suggestions for suitable foods to be consumed. Participants were also provided with key foods to be consumed during experimental diets.

Experimental diets

The SUGAR diet was composed of 20% total energy (TE) fat, 65% TE carbohydrate and 15% TE protein, and was enriched in free sugars (20% TE). Participants were advised to adopt a low-fat, high-glycaemic index diet and were supplied with candy and sugarsweetened beverages providing ~100g free sugars daily. The SFA diet was composed of 45% TE fat, 40% carbohydrate and 15% protein, and was enriched in SFA (20% TE). On this diet, participants were advised to include red meat and meat products, full-fat dairy products and typical fast food items (e.g. hamburgers, pizza etc.) and were provided with foods (such as cheese/all-butter biscuits, and milk chocolate) which provided ~15g SFA daily. Participants were instructed to maintain their usual body weight, physical activity levels and alcohol intakes, and were contacted weekly by a member of the research team to support adherence.

Postprandial study day

The evening before the postprandial study day participants consumed 3g/kg of deuterated water (^2H_2O) (17). On the morning of the study day, participants arrived at the Clinical Research Unit after an overnight fast where a Teflon catheter was inserted into an antecubital vein for repeated blood sampling. A second catheter was inserted into the contralateral arm to allow for infusion of an isotopically-labelled FA. Prior to the start of the FA infusion, blood samples were collected to determine fasting metabolite concentrations and background isotopic enrichment, then the infusion of [$^2H_2$]palmitate (0.04μmol/kg/min) bound to human albumin started and continued for the duration of the study period. The infusion was continued for 30 minutes to enable isotopic equilibrium, after which blood and breath samples (*t*0) were taken before participants were fed a mixed test meal containing 40g carbohydrate, 40g fat, and 200mg of [U^{13}C]palmitic acid to trace the fate of dietary FAs. Repeated blood and breath samples were taken 30, 60, 90, 120, 180, 240, 300 and 360 minutes after meal consumption. Breath samples were collected in EXETAINER tubes (Labco Ltd, High Wycombe, UK) to determine $^{13}CO_2$ production. Indirect calorimetry was performed in the fasting state and 120 minutes after meal consumption using a GEM calorimeter (GEMNutrition Ltd., Cheshire, UK) to determine whole-body CO_2 production, whole-body respiratory exchange ratio (RER), and energy expenditure.

Analytical procedures

Whole blood was collected into heparinized tubes (Sarstedt, Leicester, UK), and plasma immediately separated for analysis by centrifugation. Plasma glucose, NEFA, total and HDL cholesterol, TAG, β-hydroxybutyrate, adiponectin and ALT were analyzed on a semi-automatic analyser (ILab 600/650 clinical chemistry. Warrington, UK). Plasma insulin levels were determined by radioimmunoassay as described (18). Analysis of plasma FGF-21 and Fetuin-A was performed via commercially available ELISAs (R & D Systems, Oxford, UK). Separation of chylomicrons (Svedberg flotation rate (S_f) >400) and VLDL-rich fractions (S_f 20-400) were made by sequential flotation using density gradient ultracentrifugation (17) and the S_f20-400 fraction separated by immunoaffinity chromatography (18).

FA composition and isotopic enrichment

Total lipids were extracted from plasma and lipoprotein fractions, and FA methyl esters prepared and FA compositions (µmol/100 µmol total FA) were determined by gas chromatography (GC) from which palmitate concentrations were calculated (18).

Tracer enrichment in plasma NEFA, TAG and lipoprotein fractions were determined by GC-mass spectrometry (19). Tracer-to-tracee ratio (TTR) for $[U^{13}C]$palmitate (M+16/M+0), and $[^2H_2]$palmitate (M+2/M+0) were calculated and multiplied by the corresponding palmitate concentration of the fraction to give tracer concentrations. The TTR of a fasting sample obtained prior to tracer administration was subtracted from each sample to account for natural isotopic abundance. Analysis of ^{13}C enrichment in breath CO_2 samples and the relative rate of whole-body meal-derived FA oxidation was calculated (19) and corrected for lean mass.

Fasting and postprandial hepatic *de novo* lipogenesis (DNL) was assessed by determining the incorporation of deuterium from $^{2}H_2O$ in plasma water (Finnigan gas bench II, Thermo Fischer Scientific, Paisley, UK) into VLDL-TAG palmitate using GC-mass spectrometry with monitoring ions with mass-to-charge ratios of 270 (M+0) and 271(M+1) (20).

Plasma lipidomics

Plasma lipidomics was performed as described previously (21). Quality criteria for the identified lipid metabolites were linearity $R^2>0.9$ and CV <20%.

Calculations and statistics

The rate of appearance of NEFA (Ra-NEFA) (22), the relative contribution of FA sources to VLDL-TAG (calculated at 360 minutes) (18), and HOMA-IR (23) were calculated as previously described. All data are presented as means±SEM. Statistical analysis was performed using SPSS (version 21.0) for windows (SPSS Inc.). Paired t-tests were used to make pre- to post-diet comparisons where appropriate (*i.e.* IHTAG, anthropometric measures and fasting plasma biochemistry). Postprandial data were compared using a two-way repeated measures ANOVA using time and experimental diet as within-subject effects, and Bonferroni post hoc analysis was performed where appropriate. Statistical significance was set at $p<0.05$.

Results

Anthropometric and fasting biochemical measures.

Sixteen males (age 47.9±1.1y, BMI 27.7±0.4kg/m^2) completed the study. Body weight, BMI and waist circumference significantly ($p<0.05$) increased after consumption of the SFA but not the SUGAR diet (Table 1). Neither fasting plasma glucose nor insulin concentrations were altered in response to either dietary intervention (Table 1). Plasma total-, HDL-, and non-HDL cholesterol, adiponectin, and β-hydroxybutyrate concentrations all significantly ($p<0.05$) decreased following the SUGAR diet, but remained unchanged in response to the SFA diet (Table 1). Plasma FGF-21 significantly ($p<0.05$) increased in response to both diets, whilst Fetuin-A was not affected by either diet (Table 1).

Dietary intakes

There was no difference in self-reported dietary intake between the two standardized run-in periods prior to the experimental interventions (Supplementary Table S1). Self-reported energy intake was greater during the SFA compared to the SUGAR diet (2697±126 kcal *vs.* 2405±88 kcal, respectively; $p<0.05$). During the SFA diet, participants reported consuming 46±1% TE from fat, and 21±1% TE from SFA, which was significantly ($p<0.05$) greater than the fat and SFA consumed during the SUGAR diet (20±2% total fat and 6±17% SFA). In contrast, the relative contribution of carbohydrates (62±2% TE) and free sugars (23±23% TE) were significantly ($p<0.05$) greater during the SUGAR compared to the SFA diet (35±13% and 6±1% for carbohydrate and free sugars, respectively) (Supplementary Table S1). There were no differences in the contribution of protein or alcohol between the two diets (Supplementary Table S1).

FA composition and lipid profile

The FA composition of VLDL-TAG was analyzed as a biomarker of dietary FA intake. The FA composition of VLDL-TAG was similar after the SUGAR and SFA diets (Supplementary Table S2), except for pentadecanoic acid (15:0), a marker of dairy fat intake, which was greater after the SFA compared to SUGAR diet (0.5±0.1% SFA vs. 0.3±0.1% SUGAR; $p<0.05$). We also assessed the overall lipid profile of plasma after the diets and although lipidomics analysis indicated lower acyl-carnitines after consumption of the SUGAR and SFA diets, there were no overt differences in the profile (supplementary figure S2 and S3).

IHTAG content

IHTAG significantly ($p<0.05$) increased by 39.0±10.0% following the SFA diet whilst it remained unchanged in response to the SUGAR diet (Figure 2A). Linear regression indicated the increase in body weight observed after the SFA diet explained only 17.2% (p=NS) of the variance in IHTAG, suggesting the increase in IHTAG following SFA occurred independently of changes in body weight (Supplementary Figure S4).

Postprandial biochemical measures.

As humans spend a large proportion of the day in the postprandial state (24), we assessed the metabolic response to a mixed test meal at the end of each dietary intervention phase. Although there were no differences in fasting plasma glucose or insulin concentrations in response to the two diets, the postprandial excursions were greater and more prolonged for plasma glucose (diet × time interaction; $p<0.05$; Figure 2B) and plasma insulin (main effect of diet, $p<0.05$ and diet x time interaction, $p<0.05$; Figure 2C) after consumption of the SFA compared to SUGAR diet. These differences remained significant ($p<0.05$) whether comparisons were made between the early (0-180min) postprandial responses, or entire postprandial period (0-360min). Postprandial plasma TAG concentrations were similar following the two diets (Figure 2D). However, there was a significant main effect ($p<0.05$) of diet for plasma NEFA concentrations across the postprandial period, where concentrations were greater after SUGAR compared to SFA (Figure 2E). There was no difference in postprandial β-hydroxybutyrate concentrations following the diets (Figure 2F), nor were there differences in the postprandial plasma chylomicron-TAG response (Figure 2G). Although not significantly different, fasting plasma VLDL-TAG concentrations were higher after the SUGAR diet, which may in part explain the significant ($p<0.05$) diet × time interaction for postprandial plasma VLDL-TAG (Figure 2H).

Ra-NEFA

Increased lipolysis of adipose tissue has previously been observed in response to an SFA-enriched diet (7). We therefore investigated postprandial plasma Ra-NEFA after SFA and SUGAR and found no significant difference between the diets (Figure 3A).

FA oxidation

The appearance of ^{13}C (from meal [U^{13}C]palmitate) in expired CO_2 was similar after consumption of both diets (Figure 3B), as was the recovery of tracer given (6.3±0.8% SFA vs. 6.0±0.6% SUGAR) indicating no difference in whole-body meal-derived FA oxidation. Similarly, there were no differences in fasting or postprandial respiratory quotient (RQ) or resting energy expenditure (REE) between the two diets (Table 1). We also calculated net substrate oxidation rates and found no significant difference in fasting or postprandial net carbohydrate or FA oxidation rate between the two diets (data not shown).

Intrahepatic DNL and FA partitioning

We assessed the contribution of different FA sources in VLDL-TAG as it has previously been suggested to reflect the contribution of different FA sources to IHTAG (25). Although previous studies have reported that diets enriched in carbohydrate increase hepatic DNL (26) we found no difference in fasting, or postprandial hepatic DNL between SFA and SUGAR (Figure 3C). There was also no difference in the relative contribution of systemic NEFA (from adipose tissue), meal-derived, and splanchnic FAs *(i.e.* FA derived from visceral adipose tissue and stored hepatic TAG) to VLDL-TAG between SFA and SUGAR (Figure 3D).

Systemic bile acids

There was no difference in total systemic bile acids, the concentration of specific bile acids or their relative contribution to total concentration, between the SFA and SUGAR diets (Supplementary Figure 5).

Discussion

Macronutrient composition may play a role in NAFLD development with increased consumption of SFA and/or free sugars being associated with NAFLD (6). A large proportion of experimental evidence is derived from overfeeding studies, and although they suggest increased SFA intakes exaggerate IHTAG accumulation compared to unsaturated fat and dietary sugars (7-9), it is challenging to disentangle the effects of excess energy from those of the macronutrients *per se*. By utilizing a combination of methodologies, we investigated the effect of two diets, one enriched in carbohydrate, specifically free sugars, and the other enriched in fat, specifically SFA, on IHTAG content, hepatic DNL and hepatic and whole-body postprandial metabolism in overweight males. We found consumption of a SFA diet increased IHTAG, whereas consumption of the SUGAR diet did not. Despite no changes in fasting plasma glucose and insulin concentrations, we found consumption of the SFA diet resulted in exaggerated postprandial plasma glucose and insulin excursions compared to consumption of the SUGAR diet

The effect of SFA and free sugars on glycaemic control

Dietary composition has previously been reported to influence markers of glycaemic control/whole-body insulin sensitivity, with SFA-induced impairments being reported by some (7; 27) but not all (9; 28). Although we found that consumption of SFA or SUGAR for 4-weeks had a negligible effect on fasting plasma glucose and insulin concentrations, by feeding a mixed test meal at the end of the respective interventions we are able to demonstrate that consumption of the SFA compared to SUGAR diet led to exaggerated postprandial glucose and insulin excursions. The increased postprandial insulin concentrations following SFA, may in part be explained by a reduced hepatic or peripheral insulin sensitivity resulting in increased endogenous glucose production/reduced peripheral glucose uptake and a compensatory increase in insulin secretion (29). Alternatively, the elevated insulin concentrations may be due to impaired hepatic insulin extraction, which has previously been associated with increased IHTAG and peripheral insulin resistance (30; 31). Proposed mechanisms underpinning SFA-induced reductions in insulin sensitivity include increased ceramide production (32), and/or induction of metabolic endotoxemia and associated inflammation (7).

IHTAG and dietary SFA and free sugars

We found IHTAG content increased by ~37% after consumption of the SFA diet whilst IHTAG was not significantly altered in response to the SUGAR diet. The lack of change in IHTAG in response to the SUGAR diet is in-line with others who have fed sugar-enriched eucaloric diets for 4-10 weeks (13; 33). In contrast, hypercaloric sugar-enriched diets, which result in weight gain are associated with increased IHTAG (6). Although participants were instructed to maintain body weight during the dietary interventions, this was not achieved during the SFA diet, where on average participants gained ~1.5kg; linear regression indicated that the change in body weight in response to the SFA diet was not associated with IHTAG accumulation. This is in agreement with observations from hypercaloric studies, who found a notably greater increase in IHTAG after overfeeding SFA compared to overfeeding free sugars and unsaturated fats after matching for increases in body weight (7; 9; 10). Taken together these data indicate that diets enriched in SFA increase IHTAG independent of weight gain. Why SFA has a profound effect on IHTAG accumulation remains to be elucidated but it has been hypothesized that the change is due to an increased endogenous NEFA flux to the liver and/or increased ceramide synthesis which has been suggested to induce hepatic insulin resistance (7; 9).

The lack of change in IHTAG in response to SUGAR, is notable as it has previously been suggested that a diet enriched in sugars would increase IHTAG content as a result of increased hepatic DNL (5). Hypercaloric feeding of carbohydrate/sugar enriched diets for 4-days to 3-weeks upregulates DNL (7; 34; 35). In contrast, findings from isocaloric interventions are inconsistent, with one study suggesting a 4-fold increase in fasting DNL after a high-sugar compared to low-sugar diet (36), whilst others have observed no significant difference between individuals with and without NAFLD in response to a 12-week eucaloric diet enriched in free-sugars (26% TE) (11). We observed a non-significant increase in both fasting and postprandial hepatic DNL after SUGAR compared to SFA, despite increasing the intake of free sugars to a level equivalent to the 90th percentile of intake in UK populations (37). It is possible the lack of difference in hepatic DNL between the two diets is attributable to an adaptive response whereby differences may have been apparent earlier in the intervention period. Moreover, it is plausible that under conditions of energy balance other disposal pathways (*e.g.* storage as glycogen, oxidative glucose disposal etc.) are sufficient.

Hepatic FA input and disposal

By using stable-isotope methodologies in combination with a mixed test meal we were able to investigate intrahepatic FA partitioning across the postprandial period. As dietary composition has been suggested to influence adipose tissue TAG hydrolysis, we assessed Ra-NEFA and found no difference between the dietary interventions, suggesting a similar level of exposure of the liver to endogenous systemic NEFA. Within the liver, FA can be broadly partitioned into either oxidation or esterification pathways. We assessed FA oxidation in two ways: i) via plasma β-hydroxybutyrate concentrations as a marker of hepatic FA oxidation, and ii) the appearance of ^{13}C (from the mixed test meal) in expired CO_2 as a marker of whole-body FA oxidation, and found no difference for either between diets. Although there was no difference in the incorporation of either adipose tissue-derived or meal derived FAs into VLDL-TAG, there was a significant diet x time interaction for plasma VLDL-TAG concentrations, which may in part be explained by fasting VLDL-TAG concentrations being non-significantly higher at the end of the SUGAR compared to SFA diet.

Whilst there were no significant differences in hepatic DNL, evidence from animal studies has suggested newly synthesised FA are preferentially partitioned towards secretory pathways, which would lead to an increase in VLDL-TAG production and secretion. Others have found that a diet enriched in sugars increases IHTAG and upregulates VLDL-TAG secretion in overweight men, and in those with NAFLD this occurs alongside a concomitant reduction in the fractional catabolic rate of plasma VLDL-TAG (11). We did not measure hepatic VLDL-TAG production or clearance, and differences between diets could be due to differences in either of these processes.

Adherence and Biomarkers

Food diaries completed during the intervention periods indicate participants closely adhered to the experimental dietary interventions. We investigated a number of biomarkers that have been suggested to reflect changes in dietary intake and/or metabolism. We found a greater relative abundance of pentadecanoic acid (15:0), a marker of dairy fat intake, in VLDL-TAG after the SFA compared to SUGAR diet. There is currently no universally accepted biomarker for dietary sugar, although the hepatokine FGF-21 has been shown to increase in response to sucrose consumption (38). Although we observed an increase in fasting plasma FGF-21 concentrations in response to SUGAR, we also found an increase in fasting plasma FGF-21 in response to SFA, with the latter corresponding with reports that IHTAG is the strongest predictor of FGF-21 production (39). We found no difference in Fetuin-A, which has previously been associated with hepatic steatosis (40) after either dietary intervention.

Limitations

Our study has a number of limitations. We did not provide all food to participants as others have done (28). Rather, we educated participants on how to meet the targeted dietary intakes for the interventions, allowing us to investigate participants in a real-world setting. However, this resulted in participants gaining weight during the SFA diet, likely explained by participants being encouraged to increase their consumption of energy-dense (*i.e.* high-fat) foods. For logistical reasons, we did not undertake postprandial study days at the start of the intervention, it would be of interest to compare postprandial responses across the interventions (pre vs. post). We only studied overweight males, who were representative of the UK adult population and considered to be at increased risk of NAFLD relative to females (41). As sexual dimorphism exists in the development of NAFLD, T2D and intrahepatic FA metabolism (41; 42) it is plausible that findings in females may differ from what we report here.

Conclusions

There has been much controversy about the role of SFA and free sugars in metabolic disease, and recently, low-carbohydrate, high-fat diets have been promoted for weight loss and for the management of T2D (43). The evidence suggests that these diets are safe and effective over the short-term, but are not superior to other dietary strategies (43). However, in all studies conducted to date, hypocaloric diets specifically designed to induce weight loss were studied, and there is little evidence for eucaloric diets that are high in SFA. Our findings suggest that consumption of a SFA enriched diet, in the absence of weight loss, had adverse metabolic effects (including increased IHTAG and exaggerated postprandial plasma glucose and insulin responses) when compared to a diet enriched in free sugars; this may have implications for those who are not aiming for weight loss but choose to adopt a relatively high-fat diet. Moreover, despite careful monitoring and support, small weight gains were noted with the

SFA as opposed to the SUGAR diet, suggesting that weight maintenance is challenging with diets high in SFA. The lack of substantial metabolic changes after consumption of the SUGAR diet for 4 weeks, may be, in part explained by participants being metabolically healthy, remaining weight-stable and maintaining energy balance over the course of the SUGAR diet. Others have reported an increase in IHTAG when a hypercaloric diet, high in sugar is consumed (7); thus suggesting that the proposed unfavourable metabolic effects of a high sugar diet are mediated through excess energy intake. Taken together our findings indicate that a diet enriched in SFA is more harmful to metabolic health than a diet enriched in free-sugars.

Acknowledgements

Author contributions: LH designed research and gained research funding; LH, SAP, TC, MH, FR, MEP and PD conducted the research; SAP, PD, FEM and LH analyzed data; SAP, FR, PD and LH wrote the paper; LH helped with data interpretation and revisions of the manuscript; LH had primary responsibility for final content. All authors read and approved the final manuscript.

Acknowledgements

We thank Louise Dennis, Rachel Craven-Todd and all CRU staff for excellent nursing provision, Ruth Coleman for helpful statistical advice and technical assistance, and Niall Dempster and Lia Anguelova for providing medical support during study days. We thank Naira Beraza of the Quadram Institute for assistance with the analysis of bile acids. We thank the volunteers from the Oxford Biobank (www.oxfordbiobank.org.uk) for their participation in this recall study. The Oxford BioBank and Oxford Bioresource are funded by the NIHR Oxford Biomedical Research Centre (BRC). The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social care.

Financial support: LH is a British Heart Foundation (BHF) Senior Fellow in Basic Science. This study was funded by the World Sugar Research Organization and BBSRC (BB/N005600/1) and the British Heart Foundation FS/15/56/31645 (LH). The conduct of the trial, data analyses, and writing of the manuscript were all undertaken by the authors and were completely independent from World Sugar Research Organization. FR is supported by Henning and Johan Throne-Holsts Foundation, Swedish Society for Medical Research, Swedish Society of Medicine and The Foundation Blanceflor. MEP was the recipient of a fellowship grant from the Fonds de Recherche du Québec-Santé (FRQS) and the Heart and Lung Institute Foundation.

Conflict of Interest: PD is a member (unpaid) of the joint SACN/NHS England/Diabetes UK working group to review the evidence on lower carbohydrate diets compared to current government advice for adults with type 2 diabetes. All other authors have no conflict of interests to declare.

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Table 1. Characteristics of study participants and fasting biochemistry.

	SFA		**Sugar**	
	Pre	Post	Pre	Post
Weight (kg)	89.3±2.6	90.8±2.8*	89.8±2.5	90.1±2.6
Body mass index (kg/m^2)	27.7±0.6	28.1±0.6*	27.9±0.5	28.0±0.5
Waist (cm)	98±2	99±2*	99±2	99±2
Fasting Plasma Biochemical Parameters				
Glucose (mmol/L)	5.3±0.1	5.5±0.1	5.3±0.1	5.2±0.1
Insulin (mU/L)	10.7±0.9	9.2±1.2	10.3±1.4	9.3±1.0
HOMA-IR	2.5±0.3	2.2±0.3	2.5±0.3	2.2±0.2
NEFA (µmol/L)	429±56	378±27	396±42	410±42
Total cholesterol (mmol/L)	4.8±0.2	4.7±0.2	5.0±0.2	4.4±0.2*
HDL cholesterol (mmol/L)	1.2±0.1	1.2±0.1	1.3±0.1	1.0±0.1*
Non-HDL cholesterol (mmol/L)	3.6±0.2	3.5±0.2	3.7±0.2	3.4±0.1*
TAG (mmol/L)	1.0±0.1	1.0±0.1	1.0±0.1	1.1±0.1
3OHB (µmol/L)	85.7±33.1	45.1±6.5	73.1±16.5	39.6±5.0*
Adiponectin (µg/mL)	8.1±0.9	8.8±1.0	9.5±0.9	7.2±0.9*
ALT (IU/L)	11±1	12±2	10±1	9±1
FGF-21 (pg/mL)	138.7±13.6	197.3±24.3*	193 ±25.6	248.1±37.7*
Fetuin-A (µg/mL)	1160.3±39.1	1119.4±50.8	1199.4±61.9	1154.1±67.5
Indirect calorimetry measures				
Fasting RQ		0.73±0.02		0.76±0.01
Postprandial RQ		0.81±0.03		0.82±0.02
Fasting REE (kcal)		1817.5±81.2		1665.0±56.6

Postprandial REE (kcal)	1829.6±54.7	1741.8±84.8

HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; NEFA, non-esterified fatty acids; HDL, high-density lipoprotein; TAG, triglyceride; 3OHB, β-hydroxybutyrate; ALT, alanine transaminase; FGF-21, fibroblast growth factor 21; *t*, time; RQ, respiratory quotient; REE, resting energy expenditure. n = 16. Data are presented as means ± SEM. *, $p<0.05$ pre vs post diet.

Figure Legends

Figure 1. Overview of study design.

Abbreviations: SFA, saturated fat-enriched; SUGAR, free sugar-enriched.

Figure 2. Intrahepatic triacylglycerol (IHTAG) percentage, before (pre) and after (post) consumption of a saturated fat (SFA), or free-sugar (SUGAR) enriched diet for 4-weeks (A). Systemic plasma glucose (B), insulin (C), TAG (D), non-esterified fatty acid (NEFA) (E), β-hydroxybutyrate (3-OHB) (F), chylomicron-TAG (G), and VLDL-TAG (H) following a standardised test meal conducted after consumption of a saturated fat (SFA), or free-sugar (SUGAR) enriched diet for 4-weeks. Data presented are means ± standard error of the mean (SEM). n = 16 (A, B, C, D, E, F, G), n = 13 (H). * $p<0.05$ pre- to post-diet. Dotted line indicates consumption of test meal. Shading on figure B and C refers to additional statistical analysis performed due to the dynamic glucose and insulin response known to occur during the first 180 minutes of the postprandial period.

Figure 3. Plasma NEFA rate of appearance (Ra) (A), expired $^{13}CO_2$ (B), hepatic DNL (C), and the relative contribution of FA derived from systemic NEFA, diet, and splanchnic sources (*i.e.* from visceral adipose tissue and the intrahepatic pool) to VLDL-TAG (calculated at 360 minutes) (D) following a standardised test meal conducted after consumption of a saturated fat (SFA), or free-sugar (SUGAR) enriched diet for 4-weeks. Data presented are means ± standard error of the mean (SEM). n = 16. Dotted line indicates consumption of test meal.