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Isocaloric manipulation of macronutrients within a high-carbohydrate/moderate-fat diet induces unique effects on hepatic lipogenesis, steatosis and liver injury

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Abstract

Diets containing excess carbohydrate and fat promote hepatic steatosis and steatohepatitis in mice. Little is known, however, about the impact of specific carbohydrate-fat combinations on liver outcome. This study was designed to determine whether high-energy diets with identical caloric density but different carbohydrate and fat composition have unique effects on the liver. Four experimental diets were formulated with 60% kcal carbohydrate and 20% kcal fat, each in nearly pure form from a single source: starch-oleate, starch-palmitate, sucrose-oleate and sucrose-palmitate. The diets were fed to mice for 3 or 12 wk for analysis of lipid metabolism and liver injury.

Results—All mice developed hepatic steatosis over 12 wk, but mice fed the sucrose-palmitate diet accumulated more hepatic lipid than those in the other 3 experimental groups. The exaggerated lipid accumulation in sucrose-palmitate-fed mice was attributable to a disproportionate rise in hepatic de novo lipogenesis. These mice accrued more hepatic palmitate and exhibited more evidence of liver injury than any of the other experimental groups. Interestingly, lipogenic gene expression in mice fed the custom diets did not correlate with actual de novo lipogenesis. In addition, de novo lipogenesis rose in all mice between 3 and 12 wk, without feedback inhibition from hepatic steatosis.

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Summary—The pairing of simple sugar (sucrose) and saturated fat (palmitate) in a high-carbohydrate/moderate-fat diet induces more de novo lipogenesis and liver injury than other carbohydrate-fat combinations.

Conclusion—Diet-induced liver injury correlates positively with hepatic de novo lipogenesis and is not predictable by isolated analysis of lipogenic gene expression.

Keywords

fatty liver; sugar; sucrose; saturated fat; palmitate; steatohepatitis; triglyceride

1. INTRODUCTION

Chronic overconsumption of calories is well known to result in obesity, insulin resistance and end-organ damage to the pancreas, heart and liver [1]. Obesity-related health complications, however, can vary widely among individuals, despite the presence of similar risk factors. This is certainly true of obesity-related fatty liver disease, in which hepatic steatosis and steatohepatitis do not always correlate directly with body mass index or insulin resistance [2]. Although some of this variability in liver outcome has been attributed to genetic differences, there is also evidence that the composition of the diet can have a unique influence on the liver. The latter point has been made in experimental animals in which diets enriched in specific macronutrients such as fructose, saturated fat, trans-fat or cholesterol cause more severe liver disease than diets without these ingredients [3-7]. Importantly, animal studies that have compared different diets side-by-side to address the effect of specific nutrients on the pathogenesis of fatty liver disease have not always been carefully controlled for energy content. This makes it difficult to dissect the impact of diet composition from that of overall energy intake.

One experimental model of fatty liver disease that has been used as a platform for evaluating the hepatotoxicity of individual macronutrients is the methionine-choline-deficient (MCD) diet model. MCD diets are completely devoid of methionine and choline, which leads to hepatic steatosis by inhibiting phospholipid synthesis, impairing VLDL assembly and preventing the normal export of hepatic lipids [8, 9]. MCD feeding also causes steatohepatitis, characterized by severe hepatocellular injury and inflammation; histologically, MCD-mediated liver disease resembles non-alcoholic steatohepatitis (NASH) in humans [10, 11]. Isocaloric manipulation of individual nutrients within the MCD diet has revealed that the type of dietary carbohydrate has a profound impact on liver disease. Specifically, simple sugar must be present in the MCD formula for it to induce steatohepatitis. When sugar is replaced with complex carbohydrate, the ability of the diet to induce liver injury is markedly reduced [12, 13].

Experiments have shown that dietary sugar promotes MCD-mediated liver injury by stimulating de novo lipogenesis (DNL) and the production of palmitate, a cytotoxic long-chain saturated fatty acid [12]. Because MCD-fed mice cannot efficiently export hepatic lipid, this newly synthesized palmitate accumulates in the liver and causes hepatocellular injury. Whether dietary sugar is more hepatotoxic than complex carbohydrate under non-MCD conditions has not been rigorously tested. Also uncertain is how the adverse effects of

dietary sugar on the liver are influenced by the accompanying type of dietary fat. The objective of this study was to address these questions by feeding mice diets similar in macronutrient content to the MCD formula (60% kcal carbohydrate, 20% kcal fat) but with methionine and choline repleted. The carbohydrate and fat composition of these diets was then customized to enable direct comparisons of liver outcome in response to 4 unique macronutrient pairs (2 different carbohydrates x 2 different fats). The results indicate that in the presence of adequate methionine and choline, all 4 custom diets induced hepatic steatosis, but the formula that paired simple sugar (sucrose) with saturated fat (palmitate) promoted the most hepatic lipid accumulation and liver injury. As in the MCD model, steatosis and liver injury correlated positively with the amount of hepatic DNL induced by the diet.

2. MATERIALS AND METHODS

2.1 Animals and diets

Adult male C3H/HeOuJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in the UCSF vivarium for a minimum of two weeks before starting experimental diets. The mean weight of the mice at the start of the diet study was 25.7 ± 0.3 g. In the study protocol, mice were fed either chow (PicoLab 5053, LabDiet, Inc., St. Louis, MO) or custom high-energy diets comprising 60% kcal carbohydrate, 20% kcal fat and 20% kcal protein (Dyets Inc., Bethlehem, PA). The detailed composition of the custom diets is shown in Table 1. Mice were maintained on experimental diets for 3 or 12 wk. Animals were weighed and food consumption measured twice weekly. Mice were fasted for 4 h prior to killing. Procedures for animal care and euthanasia followed the guidelines set by the American Veterinary Association, and all protocols were approved by the Committee on Animal Research at the University of California, San Francisco.

2.2 Serum chemistries

Alanine aminotransferase (ALT), glucose and lipids were measured in mouse serum using an ADVIA 1800 autoanalyzer (Siemens Healthcare Diagnostics, Deerfield, IL) in the clinical chemistry laboratory at San Francisco General Hospital.

2.2. Histology and immunohistochemistry

Paraffin sections of liver tissue were stained with hematoxylin and eosin. Slides were viewed blindly by a pathologist and scored for steatosis, ballooning and inflammation as described by Kleiner et al [14]. Cell death was evaluated in liver sections by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (ApopTag Plus Peroxidase *In Situ* Apoptosis Detection Kit, Millipore, Billerica, MA). Counting of TUNEL-positive cells was performed manually in 10 microscopic fields per liver, each measuring 0.4 mm^2. Data were reported as the average number of cells per microscopic field. Hepatocyte ballooning was demonstrated qualitatively by immunoperoxidase staining for cyokeratin-8 (Thermo Fisher Scientific, Rockford, IL) [15].

2.3. Quantitation of hepatic lipids

Lipids were extracted from fresh liver tissue by the Folch method [16]. Total triglyceride was measured spectrophotometrically as previously described (TR0100; Sigma Chemical Co., St. Louis, MO) [17]. Adjacent pieces of liver tissue were flash-frozen in liquid nitrogen for fatty acid analysis by the Mouse Metabolic Phenotyping Center at Vanderbilt University. Fatty acid profiles were analyzed in the free fatty acid (FFA), diacylglycerol (DAG) and triacylglycerol (TAG) compartments by gas liquid chromatography.

2.4. Measurement of DNL

DNL was measured in the liver and adipose tissue in vivo by the use of ^2H_2O labeling combined with mass isotopomer distribution analysis (MIDA) as described previously [18-20]. Mice were injected with 99.8% ^2H_2O in saline (30 mL/kg IP) and placed on drinking water containing 8% ^2H_2O for 3 days before killing. This yielded ^2H_2O enrichment in plasma of 4.2 ± 0.04%, a value which was constant across all dietary groups. At the time of killing, lipids were extracted from tissues and separated by thin-layer chromatography. Triglycerides were retrieved and transesterified by incubation with 3 N methanolic HCl. Fatty acid methyl esters were separated from the glycerol fraction and analyzed by gas chromatography-mass spectrometry (GC-MS) using Agilent (Santa Clara, CA) DB-225ms fused silica columns (30m length, 0.25mm ID, 0.25um film thickness) with helium carrier gas at 1 mL/min.

Methyl-palmitate and its isotopes (*m/z* 270–272 representing M_0-M_2) were quantified using electron ionization (EI) mode with selected ion-monitoring and MIDA calculations were based on 22 possible sites for deuterium incorporation [21, 22]. The proportion of deuterium-labeled palmitate in hepatic triglyceride was reported as fractional DNL. The absolute amount of palmitate retained in the tissue over the labeling period was calculated by multiplying fractional DNL by the total amount of palmitate in the triglyceride compartment of the tissue.

2.5. Incorporation of dietary palmitate into hepatic lipid

Twenty-four hours before killing, mice were gavaged with 10 mg $^2H_{31}$-palmitic acid (Cambridge Isotope Laboratories, Tewksbury, MA) dissolved in 200 μL olive oil and returned to their experimental diets. At the time of killing, liver triglycerides were extracted and processed as described above for DNL. $^2H_{31}$-labeled methyl-palmitate was measured by GC-MS as a percent of total palmitate [23]; m/z 301 was monitored to calculate the proportion of $^2H_{31}$-palmitate in the sample. The $^2H_{31}$-palmitate peak exhibited a shift in GC retention time relative to the natural palmitate-methyl ester and had to be monitored accordingly. Abundance-matched standard curves of $2H_{31}$-palmitate were run concurrently to quantify true isotopic enrichment. The raw data were then used to calculate the amount of $^2H_{31}$-labeled palmitate that would have been recovered from the liver if all dietary palmitate ingested in the last 24 h had been labeled. The results were reported as the absolute amount of dietary palmitate retained in the liver over 24 h.

2.6. Measurement of hepatic gene expression

RNA was extracted from whole liver in TRIzol (Life Technologies). RNA was purified and cDNA synthesized as previously described [12]. Gene expression was assessed by quantitative PCR using Assays-on-Demand® primer and probe sets (Applied Biosystems). The expression of each test gene was normalized to mouse β-glucuronidase as an internal control.

2.7. Statistical methods

Experiments included 10 mice per diet group, performed in 2 separate cohorts of 5. Some outcome measures were assessed in only one cohort as described in the figure legends. Results were compared by analysis of variance with Tukey post-hoc testing. P values < 0.05 were considered statistically significant.

3. RESULTS

3.1. Isocaloric high-carbohydrate diets cause similar weight gain but different degrees of hepatic steatosis

Four groups of mice were fed unique high-carbohydrate diets, each containing 60% kcal carbohydrate and 20% kcal fat but differing in macronutrient composition, for 3 or 12 wk. Chow-fed mice served as controls. Mice in all 4 experimental groups gained more weight than chow-fed mice (Figure 1A); food intake was comparable among all 5 groups (Figure 1B). Expansion of white adipose tissue was evident in all high-carbohydrate-fed mice by 3 wk and persisted at 12 wk (Figure 1C). At the 12-wk time point, the relative adipose tissue weight was similar in all 4 high-carbohydrate groups. Over the 12 weeks of study, fasting glucose levels rose only in the two sucrose-fed groups (Figure 1D).

In contrast to their similar effects on adipose tissue, the 4 high-carbohydrate diets induced different degrees of hepatic steatosis. At the 3-wk time point, triglyceride accumulation was detectable biochemically in the livers of high-carbohydrate mice even though steatosis was not evident histologically (Figure 2A, B). At this stage, all 4 high-carbohydrate livers contained similar amounts of triglyceride (Figure 2B). By 12 wk, mice fed sucrose-palmitate exhibited more histologic steatosis than the other 3 high-carbohydrate groups; this excess steatosis was confirmed by hepatic triglyceride measurement (Figure 2A, B). Notably, chow-fed control mice exhibited no increase in hepatic triglyceride content during the 12-wk study despite weight gain over this interval. In contrast to hepatic triglyceride levels, serum triglyceride levels remained constant from 3-12 wk. Values were similar among all 4 high-carbohydrate dietary groups at both time points (Figure 2C).

3.2. Hepatic expression of genes involved in lipid metabolism varies among the high-carbohydrate groups

Because the 4 high-carbohydrate diets induced different degrees of hepatic steatosis, we investigated their effects on the hepatic expression of genes pertinent to lipid metabolism. As expected, all 4 high-carbohydrate diets stimulated lipogenic gene expression above the levels observed in chow-fed mice. The degree of stimulation varied among the individual formulas (Figure 3); at the 3-wk time point, mRNAs encoding fatty acid synthase (FAS) and

stearoyl-CoA desaturase-1 (SCD1) were induced 3- to 6-fold in mice fed the starch-oleate, starch-palmitate and sucrose-oleate diets, whereas these genes were induced 10- to 15-fold in mice fed sucrose-palmitate. High-carbohydrate feeding also induced the expression of select genes involved in fatty acid oxidation such as carnitine palmitoyltransferase-1 (CPT1). In this case, all 4 high-carbohydrate diets had a similar effect. Interestingly, the pronounced induction of lipogenic gene expression in response to sucrose-palmitate feeding was not sustained through 12 wk. Indeed, by the 12-wk time point, FAS and SCD1 mRNA expression in the sucrose-palmitate group declined to the levels measured in the other high-carbohydrate groups.

3.3. Varying the macronutrient composition of the high-carbohydrate diets affects hepatic DNL

Having noted that the 4 high-carbohydrate diets induced marked differences in lipogenic gene expression at 3 wk and marked differences in hepatic steatosis at 12 wk, we performed direct measurements of hepatic DNL to determine whether gene expression was predictive of fatty acid synthesis at the two time points. Fractional DNL in the liver was high (> 50%) and fractional DNL in adipose tissue was low in all mice (< 5%), indicating that the vast majority of labeled palmitate recovered from the liver was produced in the liver and not in adipose tissue with subsequent trafficking to the liver. Absolute DNL, which corrects fractional DNL for the size of the hepatic lipid pool, varied among the dietary groups and was disproportionately elevated in mice fed sucrose-palmitate (Figure 4A). DNL-derived palmitate accumulated to a greater extent in the livers of mice fed sucrose in combination with palmitate than those fed sucrose in combination with oleate (Table 2). By contrast, diet-derived palmitate accumulated in the livers of all mice in proportion to the amount of palmitate in the diet, regardless of the accompanying carbohydrate (Table 2). Interestingly, absolute DNL rose in all high-carbohydrate-fed mice between 3 and 12 wk as they were developing hepatic steatosis. Moreover, the rise in DNL that occurred between 3 and 12 wk did not coincide with a rise in lipogenic gene expression. Instead, it was accompanied by either no change or a decrease in hepatic expression of FAS and SCD1 (Figure 3). The discordance between lipogenic gene expression and absolute DNL is underscored in the scattergrams in Figure 4B.

In addition to investigating the impact of the 4 experimental diets on hepatic lipid accumulation, we also examined how the diets influenced the fatty acid profile of the liver. Figure 5A demonstrates that regardless of macronutrient composition, all 4 diets caused relative expansion of the hepatic pool of monounsaturated fatty acids (MUFA) over time, with corresponding reductions in saturated and polyunsaturated fatty acids (SFA and PUFA). In palmitate-fed mice, the increase in hepatic MUFA predicted that SCD1 was highly active; indeed, at 3 and 12 wk, the desaturation index (C16:1/C16:0), an indicator of SCD1 activity [24, 25], was significantly higher in palmitate-fed mice than chow- or oleate-fed mice (Figure 5B). Despite evidence of high SCD1 activity, mice fed the sucrose-palmitate diet exhibited persistent elevation of hepatic palmitate levels above those in all other diet groups (Figure 5C).

3.4. Mice fed sucrose-palmitate exhibit features of liver injury

Based on prior experience [12] we theorized that the diet stimulating the most hepatic DNL, sucrose-palmitate, would also cause the most liver injury. This was supported by evidence that the livers of mice fed sucrose-palmitate exhibited the most hepatocyte ballooning and the largest number of TUNEL-positive cells of all 4 high-carbohydrate groups (Figure 6 A, B). The sucrose-palmitate diet also caused the greatest induction of C-C chemokine ligand 2 (CCL2) mRNA in the liver (Figure 6C), which encodes a chemokine linked to hepatic inflammation. Serum ALT levels were elevated in all high-carbohydrate-fed mice compare to chow, but there were no differences among the experimental groups (Figure 6D). Overall, liver injury in the high-carbohydrate-fed mice was at an early stage, reflecting the relatively brief exposure of the mice to caloric excess.

DISCUSSION

A number of high-energy diets such as the popular “Western” diet can induce hepatic steatosis and steatohepatitis [26-29]. These formulas are complex nutrient mixtures with varying ratios of sugar and starch, which are often combined with natural fats such as milkfat. The objective of the current study was to investigate which specific macronutrients within these mixtures are responsible for their adverse effects on the liver; we addressed this question by employing a reductionist strategy, feeding mice 4 custom high-energy diets formulated with nearly single sources of carbohydrate and fat. All 4 diets, regardless of nutrient composition, induced weight gain and hepatic steatosis. Three of the 4 diets caused comparable degrees of steatosis, but the fourth, which contained sucrose as the carbohydrate source and palmitate as the fat source, induced 50% more hepatic lipid accumulation. Mice fed the sucrose-palmitate diet also exhibited signs of liver injury and up-regulation of pro-inflammatory genes. The results indicate that in this experimental setting, no individual macronutrient provoked liver injury but the combination of refined sugar and saturated fat was harmful. Overall, the experiments provide direct evidence that these two classes of nutrients synergize to promote liver injury.

In humans, consumption of supplemental refined sugars, particularly fructose, has been implicated as a risk factor for hepatic steatosis and fatty liver disease [30-32]. Still, there is a dearth of evidence that simple sugars are more harmful to the liver than other carbohydrates as integral components of standard diets [33-37]. Likewise, consumption of supplemental saturated fat induces more hepatic steatosis in humans than unsaturated fat [38], but evidence is inconsistent whether saturated fat within a standard diet poses a greater risk factor for fatty liver disease than unsaturated fat [39-42]. The current study supports the concept that chronic overnutrition in any form yields hepatic steatosis, but specific macronutrient combinations are capable of inducing excess steatosis and promoting liver disease. These adverse liver outcomes coincide with high rates of hepatic DNL. Recent studies have documented a positive correlation between DNL and hepatic steatosis in human subjects with fatty livers [43, 44]. Our experiments implicate diet composition as a factor in this outcome, and suggest that excess DNL is a risk factor not only for hepatic steatosis, but also NASH.

The results of the current experiments mirror those from recent studies involving nutrient manipulation in the MCD model of fatty liver disease [12, 13, 45]. Indeed, in the setting of an MCD diet, pairing sucrose with palmitate induced the worst liver disease of any carbohydrate-fat combination [12, 13, 17, 45]. In the current study, sucrose-palmitate caused much milder liver disease than it did in the MCD model; this is likely because the diets contained methionine and choline, and thus eliminated the confounding effect of MCD-related hepatic lipid trapping. Even so, the fact that sucrose-palmitate caused the worst liver outcome in non-MCD as well as MCD settings suggests that the underlying mechanism of nutrient-related liver disease is similar in both situations. To this point, DNL was disproportionately increased in the livers of mice fed sucrose-palmitate in the current study; DNL also correlates positively with the degree of liver injury in MCD-fed mice [12]. DNL can promote liver disease through its end product, palmitate, which plays an important role in hepatic lipotoxicity [46-48]. Interestingly, our findings suggest that DNL is a more potent inducer of hepatic palmitate accumulation than direct consumption of palmitate as dietary fat (Figure 5C). Further study will be required to confirm this theory, but at present, the data are consistent with the known inverse relationship between lipid synthesis and oxidation regulated at the level of CPT1 by malonyl-CoA [49, 50]. Overall, the fact that a sucrose-palmitate diet induces excess DNL and liver injury under both MCD and non-MCD conditions indicates that hepatic accumulation of DNL-derived palmitate is central to the pathogenesis of NASH.

Notably, our results differ from those of Sampath and colleagues [51], who reported that saturated fat induced less hepatic steatosis than unsaturated fat when incorporated isocalorically into a rodent diet. One reason for the difference may be that their diets contained 36% kcal fat, whereas ours contained 20% kcal fat. The lower fat content of our diet was offset by a higher carbohydrate content; moreover, in the sucrose formula, virtually all the carbohydrate was sucrose, which provided a large amount of substrate for DNL. Also relevant is that dietary saturated fat stimulates lipogenic gene expression [52] and SCD1activity [53], which together enhance the formation of hepatic triglyceride. Thus, in the context of a dietary formula with 60% carbohydrate:20% fat, sucrose and palmitate provided an ideal combination of lipogenic substrate and machinery to maximize hepatic lipid accumulation.

One important observation stemming from our study is that lipogenic gene expression in the liver is not reliable as a surrogate measure of hepatic lipogenesis. This was evident from our time-course data, in which hepatic DNL increased in all mice fed high-energy diets between 3 wk and 12 wk, whereas lipogenic gene expression either remained stable or decreased over the same interval. A potential explanation for this phenomenon is that lipogenic genes and proteins can vary in expression over a wide range in the liver, beyond that required for most metabolic challenges. If true, then lipogenic gene expression could “overshoot” in response to a dietary stimulus without producing as great a change in hepatic lipid synthesis. Such an overshoot could result in gross overestimation of hepatic lipogenesis on the basis of gene expression alone. Our data indicate that hepatic DNL measured by stable isotope incorporation is much different than that predicted by lipogenic gene expression. Similar discrepancies have been reported in adipose tissue [54]. These findings underscore the need to assess DNL directly when examining lipid regulation in the liver and other tissues.

Also noteworthy in our study was that hepatic DNL increased significantly in all mice fed high-energy diets from the 3-wk to the 12-wk time point. This rise in DNL occurred even as the livers were accumulating triglyceride, indicating that under the current dietary conditions, hepatic lipid accumulation provides no feedback inhibition to DNL. A number of groups have studied the effects of high-energy diets on DNL; high-fat diets tend to decrease DNL [55-57], whereas low-fat, high-carbohydrate diets tend to increase DNL [58] compared to chow. The present data underscore that diet composition is a major factor underlying DNL, and supplement this with evidence that the end products of DNL have little influence over the process.

Despite the short-term nature of our experiments, mice fed the sucrose-palmitate diet exhibited several signs of early steatohepatitis. High-carbohydrate, low-fat formulas tend to induce milder liver disease in mice than formulas with more fat [59], and thus the emergence of early liver disease after only 12 weeks on the sucrose-palmitate diet underscores the toxic potential of this macronutrient combination. Our goal for this study was to determine whether the macronutrient combination that induced the worst liver disease in the MCD setting also induces liver disease in the presence of methionine and choline. Our results confirm that sucrose-palmitate causes the same disproportionate steatosis, hepatocyte injury and induction of pro-inflammatory genes in the non-MCD setting as it does in the MCD model.

In summary, the current study demonstrates that the macronutrient composition of a high-energy diet is an important determinant of liver outcome. Although all high-energy formulas induced hepatic steatosis, the diet containing a combination of simple sugar and saturated fat promoted more hepatic lipid accumulation and greater signs of hepatocellular injury and inflammation than all others. This macronutrient pair stimulated hepatic DNL, which, importantly, was not predicted by lipogenic gene expression. Excess DNL led to disproportionate hepatic accumulation of palmitate, a toxic long-chain saturated fatty acid. Overall, our observations indicate that accumulation of DNL-derived palmitate in the liver is an important contributor to the pathogenesis of NASH.

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Abbreviations

ACOX1	acyl-CoA oxidase-1
ALT	alanine aminotransferase
CCL2	CC chemokine ligand 2
CPT1	carnitine palmitoyltransferase-1
DAG	diacylglycerol
DNL	de novo lipogenesis

EI	electron ionization
FAS	fatty acid synthase
FFA	free fatty acid
GC-MS	gas chromatography-mass spectrometry
MCD	methionine-choline-deficient
MIDA	mass isotopomer distribution analysis
MUFA	monounsaturated fatty acid
NASH	non-alcoholic steatohepatitis
PGC1a	peroxisome proliferator activated receptor-gamma co-activator-1α
PUFA	polyunsaturated fatty acid
SCD1	stearoyl-CoA desaturase-1
SFA	saturated fatty acid
SREBP1	sterol regulatory element binding protein-1
TAG	triacylglycerol
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labeling

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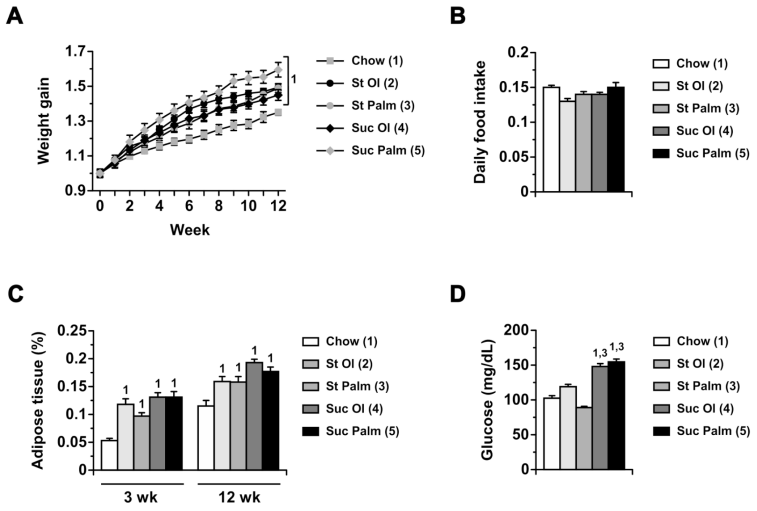

Figure 1. Metabolic features of mice fed custom high-carbohydrate diets

(A) Graph depicts the weight gain (relative to initial body weight) of mice fed chow or one of four custom isocaloric diets. (B) Graph illustrates average daily food intake (in grams per gram body weight), measured twice weekly over the duration of the 12-wk study. (C) Graph demonstrates white adipose tissue weight as a fraction of body weight at 3 wk and 12 wk. (D) Graph illustrates serum glucose levels in mice at 12 wk. Values represent mean ± SE for n = 10. Numerical symbols indicate significant differences compared to chow (1) or other diet groups as numbered in the legend. St Ol = starch-oleate; St Palm = starch-palmitate; Suc Ol = sucrose-oleate, Suc Palm = sucrose-palmitate.

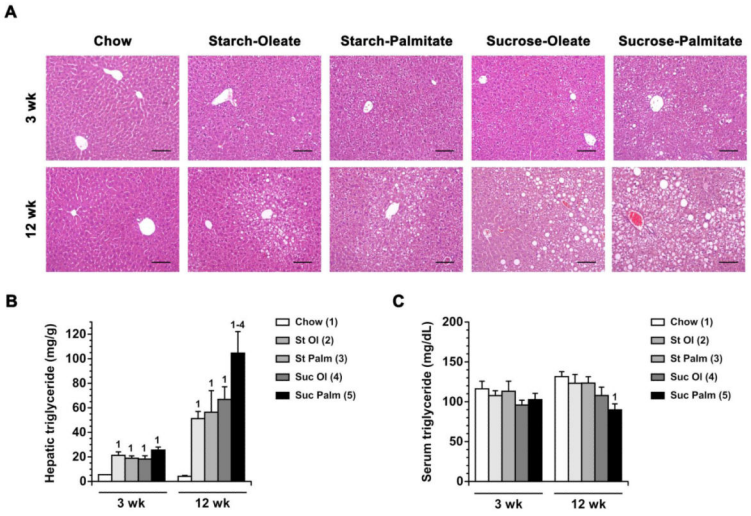

Figure 2. Hepatic steatosis in mice fed custom high-carbohydrate diets

(A) Photomicrographs illustrate liver histology in mice fed chow or a custom high-carbohydrate diet for 3 or 12 wk. Hematoxylin and eosin stain; bar = 100 μm. (B) Hepatic triglyceride measurements at 3 and 12 wk. (C) Serum triglycerides at 3 and 12 wk. Values represent mean ± SE for n = 10. Numerical symbols indicate significant differences compared to chow (1) or other diet groups as numbered in the legend. St Ol = starch-oleate; St Palm = starch-palmitate; Suc Ol = sucrose-oleate, Suc Palm = sucrose-palmitate.

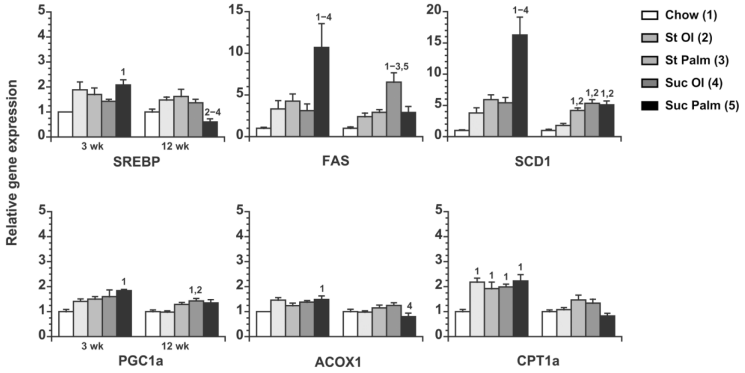

Figure 3. Hepatic gene expression in mice fed custom high-carbohydrate diets

Graphs depict mRNA encoding genes involved in hepatic lipid synthesis (SREBP1, sterol regulatory element binding protein-1; FAS, fatty acid synthase; SCD1, stearoyl Co-A desaturase-1) and oxidation (PGC1a, peroxisome proliferator activated receptor gamma co-activator-1α; ACOX1, acyl Co-A oxidase-1; CPT1, carnitine palmitoyltransferase-1) after 3 wk and 12 wk on chow or high-carbohydrate diets. Values represent mean ± SE for n = 10. Numerical symbols indicate significant differences compared to chow (1) or other diet groups as numbered in the legend. St Ol = starch-oleate; St Palm = starch-palmitate; Suc Ol = sucrose-oleate, Suc Palm = sucrose-palmitate.

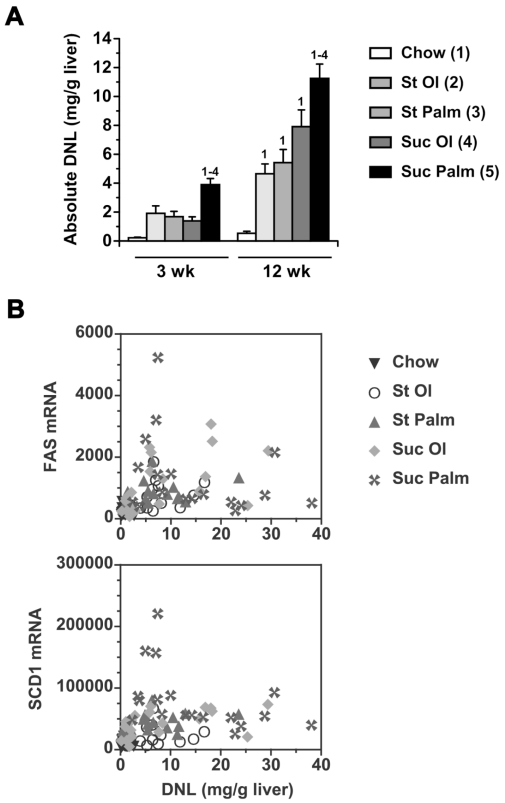

Figure 4. Hepatic DNL in mice fed custom high-carbohydrate diets

(A) Graph depicts hepatic DNL as the amount of newly synthesized palmitate measured by stable isotope incorporation as described in Methods. Values represent mean ± SE for n = 10. Numerical symbols indicate significant differences compared to chow (1) or other diet groups as numbered in the legend. St Ol = starch-oleate; St Palm = starch-palmitate; Suc Ol = sucrose-oleate, Suc Palm = sucrose-palmitate. (B) Scattergrams depict the relationship between hepatic DNL and gene expression of FAS and SCD1.

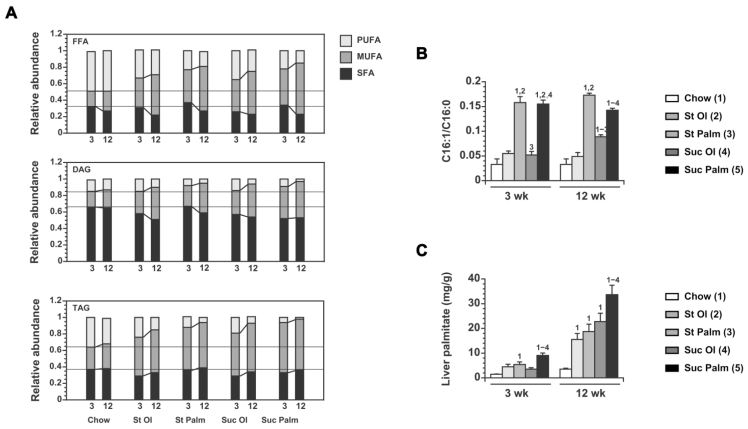

Figure 5. Fatty acid profile of the hepatic lipids in mice fed custom high-carbohydrate diets

(A) Histograms depict the proportion of SFA, MUFA, and PUFA in the free fatty acid (FFA), diacylglycerol (DAG) and triacylglycerol (TAG) compartments of mouse liver after 3 or 12 wk on the designated diets. (B) Graph illustrates the desaturation index (C16:1/C16:0), an indicator of SCD1 activity, in hepatic triglycerides from mice on the custom diets for 3 or 12 wk. (C) Histograms depict the absolute levels of hepatic palmitate in mouse livers at 3 and 12 wk on the custom diets. Values represent mean ± SE for n = 10. Numerical symbols indicate significant differences compared to chow (1) or other diet groups as numbered in the legend. St Ol = starch-oleate; St Palm = starch-palmitate; Suc Ol = sucrose-oleate, Suc Palm = sucrose-palmitate.

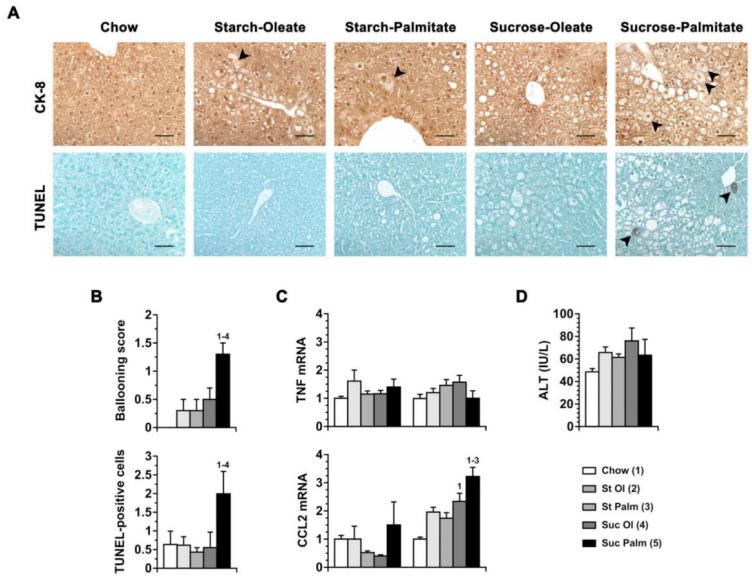

Figure 6. Markers of liver injury in mice fed custom high-carbohydrate diets for 12 wk

(A) Immunohistochemistry for cytokeratin-8 (CK-8) demonstrates brown staining in most hepatocytes but no staining in ballooned hepatocytes in the dietary groups (arrowheads). TUNEL staining shows positive cells only in the sucrose-palmitate group (arrowheads). Bar = 50 μm. (B). Graph depicts histologic ballooning scores (0-3 as described in Methods) at 12 wk. (C). Graph depicts the number of TUNEL-positive cells (counted as described in Methods) in the liver at 12 wk. (D) Graph illustrates serum ALT levels at 12 wk. Values represent mean ± SE for n = 10. Numerical superscripts indicate significant differences compared to individual group(s) as numbered in the legend. St Ol = starch-oleate; St Palm = starch-palmitate; Suc Ol = sucrose-oleate, Suc Palm = sucrose-palmitate.

Table 1

Composition of the 4 custom dietary formulas.

	Starch-Oleate	Starch-Palmitate	Sucrose-Oleate	Sucrose-Palmitate
Protein (g/kg)				
L-arginine (free base)	**6.3**	**6.3**	**6.3**	**6.3**
L-histidine (free base)	**4.5**	**4.5**	**4.5**	**4.5**
L-lysine	**16.1**	**16.1**	**16.1**	**16.1**
L-methionine	**2.0**	**2.0**	**2.0**	**2.0**
L-tyrosine	**9.2**	**9.2**	**9.2**	**9.2**
L-tryptophan	**2.1**	**2.1**	**2.1**	**2.1**
L-phenylalanine	**8.7**	**8.7**	**8.7**	**8.7**
L-cysteine	**3.7**	**3.7**	**3.7**	**3.7**
L-threonine	**6.6**	**6.6**	**6.6**	**6.6**
L-leucine	**15.3**	**15.3**	**15.3**	**15.3**
L-isoleucine	**8.4**	**8.4**	**8.4**	**8.4**
L-valine	**9.9**	**9.9**	**9.9**	**9.9**
Glycine	**3.1**	**3.1**	**3.1**	**3.1**
L-proline	**20.4**	**20.4**	**20.4**	**20.4**
L-glutamic acid	**36.2**	**36.2**	**36.2**	**36.2**
L-alanine	**4.5**	**4.5**	**4.5**	**4.5**
L-aspartic acid	**11.3**	**11.3**	**11.3**	**11.3**
L-serine	**9.4**	**9.4**	**9.4**	**9.4**
Carbohydrate (g/kg)				
Cornstarch	**587.9**	**587.9**	**0.0**	**0.0**
Dyetrose	**50.0**	**50.0**	**50.0**	**50.0**
Sucrose	**0.0**	**0.0**	**587.9**	**587.9**
Cellulose	**30.0**	**30.0**	**30.0**	**30.0**
Fat (g/kg)				
Tripalmitin	**0.0**	**100.0**	**0.0**	**100.0**
High-oleate (85%) sunflower oil	**100.0**	**0.0**	**100.0**	**0.0**
Additives (g/kg)				
Salt mix #210030	**35.0**	**35.0**	**35.0**	**35.0**
Sodium bicarbonate	**7.4**	**7.4**	**7.4**	**7.4**
Vitamin Mix #310025	**10.0**	**10.0**	**10.0**	**10.0**
Choline chloride	**2.0**	**2.0**	**2.0**	**2.0**
Total (g/kg)	**1000.0**	**1000.0**	**1000.0**	**1000.0**
Protein	**18%**	**18%**	**18%**	**18%**
carbohydrate	**64%**	**64%**	**64%**	**64%**
Fat	**10%**	**10%**	**10%**	**10%**
Fiber	**3%**	**3%**	**3%**	**3%**

Table 2

Origin of hepatic palmitate after 3 wk on experimental diets.

Experimental Group	Palmitate from diet (mg/g liver)	Palmitate from DNL (mg/g liver)
Chow	0.07 ± 0.01	0.22 ± 0.04
Starch-Oleate	0.16 ± 0.04	1.91 ± 0.52
Starch-Palmitate	1.53 ± 0.25	1.68 ± 0.37
Sucrose-Oleate	0.18 ± 0.04	1.39 ± 0.28
Sucrose-Palmitate	1.43 ± 0.21	3.90 ± 0.42

The amount of liver palmitate deriving from the diet or DNL was assessed by stable isotope labeling as described in Methods. Values represent mean ± SE for n = 10.