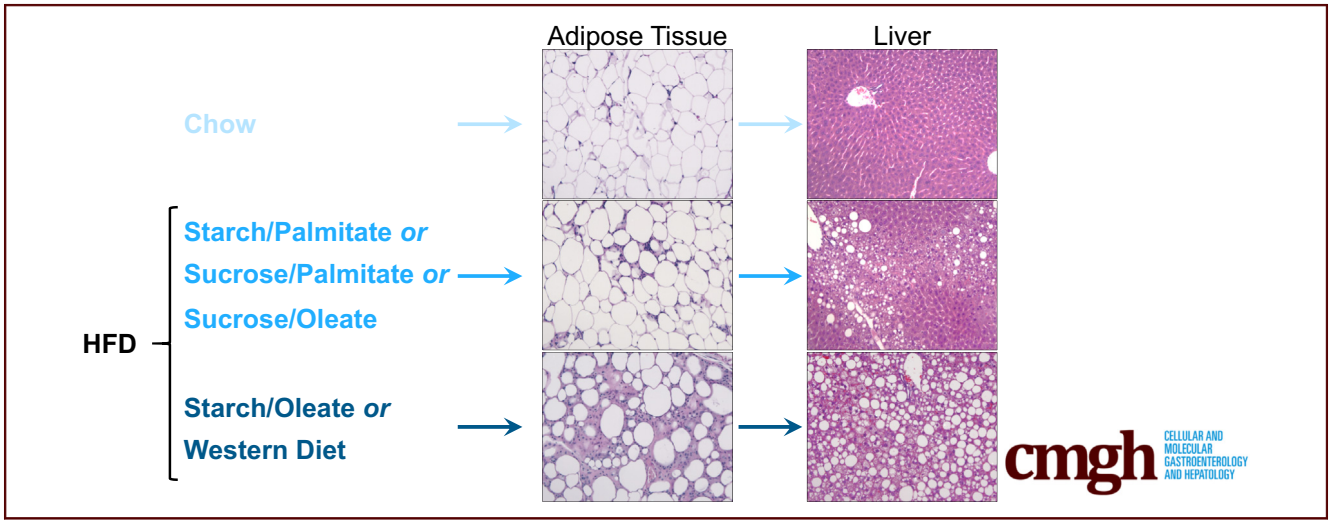
cmgh
CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Specific Macronutrients Exert Unique Influences on the Adipose-Liver Axis to Promote Hepatic Steatosis in Mice

Caroline C. Duwaerts,[1,2] Amin M. Amin,[1,2] Kevin Siao,[1,2] Chris Her,[1,2] Mark Fitch,[3] Carine Beysen,[4] Scott M. Turner,[4] Amanda Goodsell,[1,2] Jody L. Baron,[1,2] James P. Grenert,[2,5] Soo-Jin Cho,[5] and Jacquelyn J. Maher[1,2]

[1]Department of Medicine, University of California, San Francisco, California; [2]The Liver Center, University of California, San Francisco, California; [3]Department of Nutritional Sciences and Toxicology, University of California, Berkeley, California; [4]KineMed, Inc, Emeryville, California; and [5]Department of Pathology, University of California, San Francisco, California



SUMMARY

Testing the effect of specific macronutrient combinations on mouse metabolism in vivo, the authors determined that diets pairing starch as carbohydrate with oleate as fat (42%:42% kcal) induced significant white adipose tissue pathology and hepatic steatosis compared with other isocaloric combinations.

BACKGROUND & AIMS: The factors that distinguish metabolically healthy obesity from metabolically unhealthy obesity are not well understood. Diet has been implicated as a determinant of the unhealthy obesity phenotype, but which aspects of the diet induce dysmetabolism are unknown. The goal of this study was to investigate whether specific macronutrients or macronutrient combinations provoke dysmetabolism in the context of isocaloric, high-energy diets.

METHODS: Mice were fed 4 high-energy diets identical in calorie and nutrient content but different in nutrient composition for 3 weeks to 6 months. The test diets contained 42% carbohydrate (sucrose or starch) and 42% fat (oleate or palmitate). Weight and glucose tolerance were monitored; blood and tissues were collected for histology, gene expression, and immunophenotyping.

RESULTS: Mice gained weight on all 4 test diets but differed significantly in other metabolic outcomes. Animals fed the starch-oleate diet developed more severe hepatic steatosis than those on other formulas. Stable isotope incorporation showed that the excess hepatic steatosis in starch-oleate–fed mice derived from exaggerated adipose tissue lipolysis. In these mice, adipose tissue lipolysis coincided with adipocyte necrosis and inflammation. Notably, the liver and adipose tissue abnormalities provoked by starch-oleate feeding were reproduced when mice were fed a mixed-nutrient Western diet with 42% carbohydrate and 42% fat.

CONCLUSIONS: The macronutrient composition of the diet exerts a significant influence on metabolic outcome, independent of calories and nutrient proportions. Starch-oleate appears to cause hepatic steatosis by inducing progressive adipose tissue injury. Starch-oleate phenocopies the effect of a Western diet; consequently, it may provide clues to the mechanism whereby specific nutrients cause metabolically unhealthy obesity. *(Cell Mol Gastroenterol Hepatol 2017;4:223–236; http://dx.doi.org/10.1016/j.jcmgh.2017.04.004)*

Keywords: Steatohepatitis; Oleate; Starch; Adipose Tissue.

See editorial on page 301.

Obesity affects nearly 38% of adults in the United States and more than 10% of adults worldwide.[1,2] Although obesity is often accompanied by dysmetabolism and inflammation in the form of insulin resistance, type 2 diabetes, hypertension, and cardiovascular disease, not all obese individuals display these comorbidities. Experts now separate obese persons into 2 categories based on the presence or absence of metabolic derangements: the metabolically healthy obese (MHO) and the metabolically unhealthy obese (MUO). The stringency by which metabolic "health" is defined is not yet completely standardized. Accordingly, somewhere between 10% and 50% of obese individuals are classified as MHO, whereas the remainder are considered MUO.[3]

Hepatic steatosis is an important feature of the MUO phenotype.[4] Scientists have recently reported that hepatic steatosis predates other features of dysmetabolism in obesity,[5] suggesting it is a predictor of more advanced metabolic disease. Given the importance of hepatic steatosis to the definition and possibly the pathogenesis of MUO, there is great interest in defining the factors that promote metabolic deterioration in the obese. One variable that has been scrutinized as a possible determinant of MUO is diet. Although some large population studies have identified subtle differences in diet as a determinant of the MUO phenotype,[6,7] others have not been able to detect a difference in either calorie or nutrient consumption between MHO and MUO subgroups.[8–10] This may be due to the imperfect quality of dietary data in population studies, which in general rely on self-report and recall.

Despite the paucity of epidemiologic evidence that diet is a risk factor for MUO, carefully controlled intervention studies argue that manipulation of individual nutrients can impact metainflammation.[11–13] Still, it is uncertain whether specific carbohydrate (CHO)-fat combinations, when tested within the context of calorically matched diets, have unique effects on metabolic health over the long term.[14,15] The goal of this study was to determine whether diets similar in nutrient content to the typical American diet, but differing in their sources of CHO and fat, exert different effects on the livers of mice. The results indicate that 4 different dietary formulas, matched in calories, CHO, and fat content but with different macronutrient composition, did indeed induce significantly different degrees of hepatic steatosis over 6 months. The 4 formulas also induced significantly different degrees of adipose tissue injury and inflammation, and demonstrated a striking direct relationship between adipose tissue damage and hepatic lipid accumulation. Surprisingly, the single macronutrient that promoted the worst metabolic phenotype in mice was oleate, a monounsaturated fat.

Methods

Animals and Diets

Adult male mice (C57BL/6J and C3H/HeOuJ, The Jackson Laboratory, Bar Harbor, ME) were used for all studies. Some were fed standard laboratory chow as a control diet (PicoLab Diet # 5053, St. Louis, MO). Others were randomly assigned to 1 of 4 isocaloric high-energy diets comprising 15% kcal protein, 42% kcal CHO, and 42% kcal fat (Dyets, Inc, Bethlehem, PA) (Table 1). The groups were named for their primary ingredients: starch-palmitate, sucrose-palmitate, starch-oleate, and sucrose-oleate. A separate group of mice was fed a Western diet (TD.88137, Envigo Teklad, Madison, WI) comprising 42% kcal CHO and 42% kcal fat of mixed sources. Animals were group-housed with ad libitum access to food and water and were treated in accordance with Institutional Animal Care and Use Committee guidelines at the University of California, San Francisco.

Serum Chemistries

Serum alanine aminotransferase was measured on an ADVIA 1800 autoanalyzer (Siemens Healthcare Diagnostics, Deerfield, IL) in the clinical chemistry laboratory at the Zuckerberg San Francisco General Hospital.

Glucose Tolerance Test

Glucose tolerance tests were performed 1 week prior to the end of each dietary study as previously described.[16]

Measurement of Hepatic Lipids

Lipids were extracted from liver tissue employing the Folch method.[17] Liver triglyceride (TG) was quantified biochemically as described previously.[18]

Histology and Immunohistochemistry

Sections of liver and epididymal white adipose tissue (eWAT) were stained with H&E and blindly scored by a pathologist (J.P.G., liver; S.J.C., adipose tissue). Liver sections were scored for steatosis, ballooning, and inflammation using the criteria developed by Kleiner et al.[19] Adipose tissue was scored for necrosis on a 0 to 3 scale as follows: 0 = scattered crown-like structures only, 1 = focal areas of necrosis affecting <5% of the total tissue, 2 = 5%–25% necrosis, 3 = >25% necrosis. Adipose tissue necrosis is defined as anuclear or disintegrated adipocytes, of varying size, surrounded by aggregates of inflammatory cells with or without multinucleate giant cells present.[20] Additional sections of eWAT were subjected to immunohistochemistry for perilipin 1 (#9349, Cell Signaling Technology, Danvers, MA) and NKp46 (#137615, Biolegend, San Diego, CA).

Abbreviations used in this paper: **CHO, carbohydrate; DNL, de novo lipogenesis; eWAT, epididymal white adipose tissue; MHO, metabolically healthy obese; MUO, metabolically unhealthy obese; NK, natural killer; TG, triglyceride.**

Most current article

2352-345X
http://dx.doi.org/10.1016/j.jcmgh.2017.04.004