Diet-induced dyslipidemia leads to nonalcoholic fatty liver disease and oxidative stress in guinea pigs

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Chronic dyslipidemia imposed by a high-fat and high-caloric dietary regime leads to debilitating disorders such as obesity, nonalcoholic fatty liver disease (NAFLD), and insulin resistance. As disease rates surge, so does the need for high validity animal models to effectively study the causal relationship between diet and disease progression. The dyslipidemic guinea pig displays a high similarity with the human lipoprotein profile and may in this aspect be superior to other rodent models. This study investigated the effects of 2 long-term Westernized diets (0.35% cholesterol, 18.5% vegetable oil and either 15% or 20% sucrose) compared with isocaloric standard chow in adult guinea pigs. Biochemical markers confirmed dyslipidemia in agreement with dietary regimens; however, both high-fat groups displayed a decreased tissue fat percentage compared with controls. Macroscopic appearance, histopathologic evaluation, and plasma markers of liver function confirmed NAFLD in high-fat groups, supported by liver redox imbalance and markers suggesting hepatic endothelial dysfunction. Plasma markers indicated endothelial dysfunction in response to a high-fat diet, although atherosclerotic lesions were not evident. Evaluation of glucose tolerance showed no indication of insulin resistance. The 5% increase in sucrose between the 2 high-fat diets did not lead to significant differences between groups. In conclusion, we find the dyslipidemic guinea pig to be a valid model of diet imposed dyslipidemia, particularly with regards to hepatic steatosis and endothelial dysfunction. Furthermore, the absence of obesity supports the present study setup as targeting NAFLD in nonobese individuals. (Translational Research 2016;168:146–160)

Abbreviations: ADMA = asymmetric dimethylarginine; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BH_2 = dihydrobiopterin; BH_4 = tetrahydrobiopterin; BW = body weight; CTRL = isocaloric control standard chow diet; DEXA = dual-energy x-ray absorptiometry; eNOS = endothelial nitric oxide synthase; GSH = glutathione; GSSG = oxidized glutathione; H&E = Mayer's hematoxylin and eosin; HDL = high-density lipoprotein; HF = high fat + 15% sucrose; HFS = high fat + 20% sucrose; HPLC = high-performance liquid chromatography; L-Arg = L-arginine; LDL = low-density lipoprotein; MDA = malondialdehyde; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis; NO = nitric oxide; OGTT = oral glucose tolerance test; Ox-LDL = oxidized low-density lipoprotein; SOD = superoxide dismutase; TC = total cholesterol; TG = triglyceride; VitC = vitamin C; VLDL = very low-density lipoprotein

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AT A GLANCE COMMENTARY

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Background

As the obesity pandemic continues to expand, the need for valid animal models is increasing. Owing to a high similarity with the human lipoprotein profile, the guinea pig may prove to be superior compared with mice and rat models of diet-induced dyslipidemia and subsequent debilitating disease.

Translational Significance

This study shows extensive liver damage and oxidative stress as well as indicates endothelial dysfunction imposed by a diet-induced dyslipidemia. Our findings support the applicability of the guinea pig model in future in vivo studies, particularly targeting the nonobese form of nonalcoholic liver disease.

INTRODUCTION

The obesity pandemic and increase in debilitating chronic diseases such as cardiovascular disease, type 2 diabetes, and metabolic disorders, as a consequence of a high-fat—"Westernized"—diet, have highlighted the need for accurate animal models. In this aspect, the guinea pig (*Cavia porcellus*) may be a natural and superior model because of a unique similarity with the human lipoprotein profile and the enzymatic processing of lipids in vivo. On the contrary, both mice and rats display a large proportion of high-density lipoprotein (HDL) and comparatively low levels of low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL), thus showing the almost inverse pattern compared with humans and guinea pigs in response to a fatty meal.[1]

Deposition of lipids (in the form of triglycerides [TGs]) in the liver and subsequent development of nonalcoholic fatty liver disease (NAFLD) is linked to the intake of excess calories and is regarded as a hepatic consequence of, but not limited to, obesity and the metabolic syndrome.[2,3] In humans, NAFLD currently constitutes the most prevalent liver disease in the industrialized world, surveys estimating as much as 20% of the population to be affected and encompassing both obese and nonobese individuals.[4,5] Although early stages of NAFLD comprise the reversible changes of simple steatosis, the disease may progress to include nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma imposing irreversible damage and ultimately liver failure.[4,6]

The mechanisms governing the accumulation of TGs in hepatic cells include changes in hepatocellular metabolism,[7-10] propagating an imbalance between uptake and de novo fatty acid synthesis, VLDL formation and subsequent export, and oxidation capacity.[3,11] The resulting hepatic TG accumulation leads to the imbalance in redox homeostasis and increased free radicals with negative consequences for hepatocytes including mitochondrial dysfunction.[12,13] Hepatocellular distress in turn induces the release of inflammatory cytokines and eventually also fibrosis. A reduced insulin response disturbs hepatic autoregulation increasing endogenous glucose production from fatty acids and depleting glycogen reserves.[14,15] In addition, insulin resistance in adipocytes may increase free fatty acid influx to the liver enhancing NAFLD and subsequent hepatocellular damage potentially developing into NASH.[11,16] Hyperplasia and hypertrophy of adipocytes are followed by macrophage activation and infiltration, reprogramming of adipocytes (adipocyte dysfunction) leading to a deviated secretion of anti-inflammatory cytokines and adiponectin, as well as systemic redox imbalance and low-grade inflammation.[5,17,18] This creates a self-propagating viscous circle in which cellular metabolism is distorted and fueled by an excess caloric intake.[16,19] Apart from the association between dyslipidemia and NAFLD induced in experimental models by high-fat meals,[20-22] the composition of dietary fatty acids and the intake of refined carbohydrates (such as fructose) have been suggested as comorbidity in NAFLD disease development.[11,23,24]

Using guinea pigs as animal models of diet-induced dyslipidemia, we investigated the effects of 2 high-fat Westernized diets (0.35% cholesterol, 18.5% vegetable oil and either 15% sucrose [HF diet] or 20% sucrose [HFS diet]) compared with an isocaloric control standard chow diet (CTRL). We hypothesize that high-fat feeding would induce NAFLD in guinea pigs and that a 5% increase in sucrose would accelerate disease progression.

MATERIALS AND METHODS

Animals. Animals were treated in accordance with the Animal Experimentation Act of Denmark, which is in accordance with the Council of Europe Convention ETS 123. The study was approved by the National Animal Experimentation Board.

Twenty-one female Hartley guinea pigs (aged 12 weeks; Charles River Laboratories, Kisslegg, Germany) were tagged with a 12-mm microchip subcutaneously in the neck for identification (Pet ID, West