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Low-carbohydrate diets reduce lipid accumulation and arterial inflammation in guinea pigs fed a high-cholesterol diet

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ABSTRACT

Introduction: Low-carbohydrate diets (LCD) efficiently induce weight loss and favorably affect plasma lipids, however, the effect of LCD on atherosclerosis is still argued.
Objective: To evaluate the effect of LCD on the prevention of atherosclerosis.
Methods: Twenty guinea pigs were fed either a LCD or a low-fat diet (LFD) in combination with high-cholesterol (0.25 g/100 g) for 12 weeks. The percentage energy of macronutrient distribution was 10:65:25 for carbohydrate:fat:protein for the LCD, and 55:20:25 for the LFD. Plasma lipids were measured using colorimetric assays. Plasma and aortic oxidized (oxLDL) were quantified using ELISA methods. Inflammatory cytokines were measured in aortic homogenates using an immunoassay. H&E stained sections of aortic sinus and Schultz stained sections of carotid arteries were examined.
Results: LDL cholesterol was lower in the LCD compared to the LFD group (71.9 ± 34.8 vs. 81.7 ± 26.9 mg/dL; $p = 0.039$). Aortic cholesterol was also lower in the LCD (4.98 ± 1.3 mg/g) compared to the LFD group (6.68 ± 2.0 mg/g); $p < 0.05$. The Schultz staining method confirmed less aortic cholesterol accumulation in the LCD group. Plasma oxLDL did not differ between groups, however, aortic oxLDL was 61% lower in the LCD compared to the LFD group ($p = 0.045$). There was a positive correlation ($r = 0.63$, $p = 0.03$) between oxLDL and cholesterol concentration in the aorta of LFD group, which was not observed in LCD group ($r = -0.05$, $p = 0.96$). Inflammatory markers were reduced in guinea pigs from the LCD group ($p < 0.05$) and they were correlated with the decreases in oxLDL in aorta.
Conclusion: These results suggest that LCD not only decreases lipid deposition, but also prevents the accumulation of oxLDL and reduces inflammatory cytokines within the arterial wall and may prevent atherosclerosis.

1. Introduction

Several studies have suggested that a low-carbohydrate diet (LCD) is an effective alternative to treat the metabolic syndrome [1], mitigate obesity [2], and prevent atherosclerosis [3]. Data from retrospective analyses of the Nurses' Health Study with more than 80,000 subjects suggest that lower proportion of carbohydrate intake, at the expense of a greater intake of animal fat and protein, is not associated with a greater risk of coronary heart events [3]. In addition, a LCD associated with a greater intake of vegetal fat and protein reduced the risk for coronary heart diseases when compared to subjects who had proportionally greater intake of carbohydrate [3]. The recognition of the deleterious effect of high carbohydrate diets can be noted by the change in the American Diabetes Association dietary recommendations [4]. Although they still do not recommend a LCD, the most recent guidelines' [4] recommended a reduction in carbohydrate intake compared to the previous guidelines.

Epidemiological data and measurements of the risk factors for cardiovascular disease suggest that a LCD can be successfully used to prevent and treat atherosclerosis [3,5–7]. However, the mechanism by which this diet may mitigate this disease remains unclear. Therefore, to clarify this issue, an animal study is proposed. The use of surrogates for atherosclerosis is advantageous because animals can reach hard endpoints in a short period of time with a reduced cost and target tissues can be collected for analyses.

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Guinea pigs have been successfully used to study the effects of both LCD and low-fat diet (LFD) [8,9]. In addition, it is possible to quickly induce the initial stages of atherosclerosis by feeding them high levels of cholesterol [10,11]. These animal model, unlike others, transports cholesterol mainly in LDL particles [12,13], which make them a good model to study risk factors for cardiovascular diseases [10,14]. The recognition of this model as an adequate model to study atherosclerosis is driving pharmaceutical companies to adopt guinea pigs as a surrogate to study new anti-atherosclerotic medications [10,11].

Despite favorable evidence that LCD could be a suitable alternative to treat atherosclerosis [14,15], there is still a concern that the high fat intake could favor the development of cardiovascular diseases. In order to evaluate whether this concern is pertinent, we performed a study in which guinea pigs were fed either LCD or LFD associated with a high-cholesterol content to induce atherosclerosis.

This study directly compares the effect of these diets on the main stages of the natural history of atherosclerosis. We evaluated how these diets differ in the modulation of risk factors for cardiovascular diseases by assessing plasma lipids, lipoprotein remodeling, oxidized lipoproteins, and arterial inflammation. We also compared the effect of these diets on atherosclerosis itself. We evaluated the initial stages of disease, measuring the deposition of cholesterol in arteries by biochemical, histochemical, and histological analyses. Ultimately, we evaluated whether beneficial effects of diets in the prevention of atherosclerosis.

2. Methods

Twenty male guinea pigs, aged 18 months old, were randomly assigned to be fed either a LCD or a LFD in combination with high-cholesterol (0.25 g/100 g) for 12 weeks. The percent energy distribution of the diets were 10:65:25 (carbohydrate:fat:protein) for the LCD and 55:20:25 for the LFD. The fatty acid composition of experimental diets for LCD and LFD groups was identical and contained 46.3:28.0:25.7% (SFA:MUFA:PUFA). The micronutrient composition was formulated to meet the National Research Council requirements for guinea pigs [16]. The caloric density was 4.46 kcal/g for the LCD and 3.58 kcal/g for the HCD. However, dietary fiber and micronutrient composition were adjusted for the high fat diet as previously reported [9]. The experiment was conducted according to Institutional Animal Care and Use Committee (IACUC) guidelines from University of Connecticut. After the feeding period, the animals were sacrificed, and blood, heart, and the left carotid artery, were collected for analysis.

2.1. Plasma lipids

Enzymatic methods were used to measure the plasma lipids after the samples were mixed with aprotinin (0.5 mL/100 mL), sodium azide (0.1 mL/100 mL), and phenylmethylsulfonyl fluoride (0.1 mL/100 mL) to prevent degradation. For total cholesterol a Roche-Diagnostics (Indianapolis, IN) cholesterol assay was used. Plasma triglycerides (TG) were measured using Trig/GB assay from Roche-Diagnostics (Indianapolis, IN). HDL cholesterol (HDL-C) was measured after apo-B containing lipoproteins were precipitated by adding a solution made up of an equal volume of magnesium chloride (2 M) and dextran sulfate (0.025 mg/mL). For VLDL cholesterol (VLDL-C), 1.2 mL of plasma were overlayered with 4 mL of NaCl–KBr solution (density = 1.006 g/mL) in an ultracentrifugation tube [17]. This was placed in a rotor for 45 min at 200,000 × *g* at 10 °C. The upper layer with VLDL was removed and it was used to measure cholesterol. LDL-C was calculated following the formula: TC − (HDL-C + VLDL-C).

2.2. Plasma and aortic oxidized LDL (oxLDL)

Plasma oxLDL was measured using a mouse sandwich ELISA from Mercodia (Mercodia oxidized LDL ELISA; Mercodia AB, Uppsala, Sweden). This assay is based on the recognition of oxidized Apo-B100 by monoclonal antibody 4E6. OxLDL was evaluated in homogenized descendent thoracic aorta, as described elsewhere [10].

2.3. Cholesterol accumulation in aorta and arterial morphology

The measurement of cholesterol in the abdominal aorta was performed using the Folch method, and then analysed as described by Leite et al. [10]. Hematoxylin and eosin (H&E) stained formalin-fixed paraffin embedded sections of the aortic sinus were examined for atherosclerotic features by a board certified pathologist in a blind fashion.

2.4. Histochemical analyses of the cholesterol in left carotid artery

Frozen sections (20 μm thick) of the left carotid artery were stained for cholesterol using the Schultz method [18]. This assay is based on the incubation of the target tissue with iron and acid solution, which led to a formation of a blue-green stain of cholesterol.

2.5. Quantitative analyses of the cholesterol staining in the left carotid artery

The spot with greatest positive staining on each slide was chosen in a blinded fashion. Using Adobe Photoshop CS2 version 9.0.2 for Macintosh (San Jose, CA), the threshold for positive blue-green staining was set and it was quantified proportionally to the total arterial area in the picture, such as suggested by Wadsworth et al. [19].

2.6. Immunohistochemistry (IHC) of the aortic sinus

In order to assess inflammation in the arterial wall, IHC of the aortic sinus was performed. For this analysis, an anti-human TNF-α rabbit polyclonal antibody (Abcam, Cambridge, MA, USA) at a 1:100 dilution was used.

2.7. Inflammatory cytokine concentration in the aorta

Cytokines were evaluated from homogenates of the descendent thoracic aorta, such as described elsewhere [10] using the LINCOplex™ Cytokine Kit (Linco Research Inc, St. Charles, MO, USA) and Luminex system (Luminex 200 System, Austin, TX) according to the manufacturers' specifications. The following aortic cytokines were measured: TNF-α, e-selectin, granulocyte–macrophage colony-stimulating factor (GM-CSF), inter-cellular adhesion molecule-1 (ICAM-1), interleukin-β1 (IL-β1), monocyte chemoattractant protein-1 (MCP-1), matrix metallopeptidase-9 (MMP-9), plasminogen activator inhibitor-1 (PAI-1), and vascular cell adhesion molecule-1 (VCAM-1).

2.8. Statistical analyses

Independent *t*-tests, mixed analyses of variance, multivariate analyses of variance (MANOVA), multivariate analyses of covariance (MANCOVA) and bivariate correlations were used when appropriate. Significant values were considered when the *p*-value was less than 0.05. All the results are expressed as mean ± standard deviation.