



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

Jose O. Leite^a, Ryan DeOgburn^a, Joseph Ratliff^a, Randy Su^a, Joan A. Smyth^b, Jeff S. Volek^{a,c}, Mary M. McGrane^a, Alan Dardik^d, Maria Luz Fernandez^{a,c,*}

^a Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA

^b Department of Pathobiology and Veterinary Science, University of Connecticut, Storrs, CT 06269, USA

^c Department of Kinesiology, University of Connecticut, Storrs, CT 06269, USA

^d Department of Surgery, Yale University School of Medicine, New Haven, CT 06520, USA

ARTICLE INFO

Article history:

Received 3 July 2009

Received in revised form

22 September 2009

Accepted 1 October 2009

Available online 12 October 2009

Keywords:

Low-carbohydrate diet

Dietary cholesterol

Atherosclerosis

Cytokines

Guinea pig

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.

© 2009 Elsevier Ireland Ltd. All rights reserved.

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 vege-

tal 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.

* Corresponding author at: University of Connecticut, Department Nutritional Sciences, 3624 Horsebarn Road Ext., U-17, Storrs, CT 06269, USA.
Tel.: +1 860 486 5547; fax: +1 860 486 3674.

E-mail address: maria-luz.fernandez@uconn.edu (M.L. Fernandez).

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 overlaid 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 metalloproteinase-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.

Table 1

Plasma total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG), VLDL cholesterol (VLDL-C) and non-esterified fatty acids (NEFA) of guinea pigs fed the LCD or LFD diets^a.

Lipids	LCD (n = 10)	LFD (n = 10)	p-Value
TC (mg/dL)	98.1 ± 25.6	148.9 ± 77.8	0.067
LDL-C (mg/dL)	71.9 ± 34.8	81.7 ± 26.9	0.039
HDL-C (mg/dL)	16.5 ± 5.4	15.5 ± 3.3	0.635
TG (mg/dL)	75.5 ± 29.4	47.9 ± 13.0	0.014
VLDL-C (mg/dL)	9.0 ± 2.5	6.6 ± 1.9	0.023
NEFA (mEq/L)	0.83 ± 0.30	0.63 ± 0.10	0.044
Aortic TC (mg/g)	4.98 ± 1.30	6.6 ± 2.00	0.040

^a Values are mean ± SD for the number of guinea pigs indicated in parenthesis. Unpaired *t*-test was used to evaluate statistical significance.

3. Results

3.1. Plasma and aortic lipids

Plasma and aortic lipids are presented in Table 1. There was a strong positive correlation between LDL-C and TC ($r=0.715$, $p<0.01$) (Fig. 1, panel A), and a strong negative correlation between LDL-C and HDL-C ($r=-0.518$, $p=0.019$) (Fig. 1, panel B). There was also a strong positive correlation between VLDL-C and TG ($r=0.691$, $p=0.01$) (Fig. 1, panel C). Finally, there was a strong positive correlation between plasma LDL-C and aortic TC ($r=0.701$, $p=0.01$) (Fig. 4, panel D). This reinforces the idea that in guinea pigs, similar to humans, it is the LDL that serves as the major contributor to cholesterol deposition in arteries [13].

3.2. Oxidized LDL

The plasma concentrations of oxLDL were not different between groups (Fig. 2, panel A). However, the oxLDL in the aorta of animals fed LCD was 61% lower than in the animals fed LFD (Fig. 2, panel B). In the LFD group, there was a strong correlation ($r=0.63$, $p=0.03$) between oxLDL and cholesterol concentration in the aorta. However, there was a lack of correlation between these two variables in LCD group ($r=-0.05$, $p=0.96$) (Fig. 2, panels C and D).

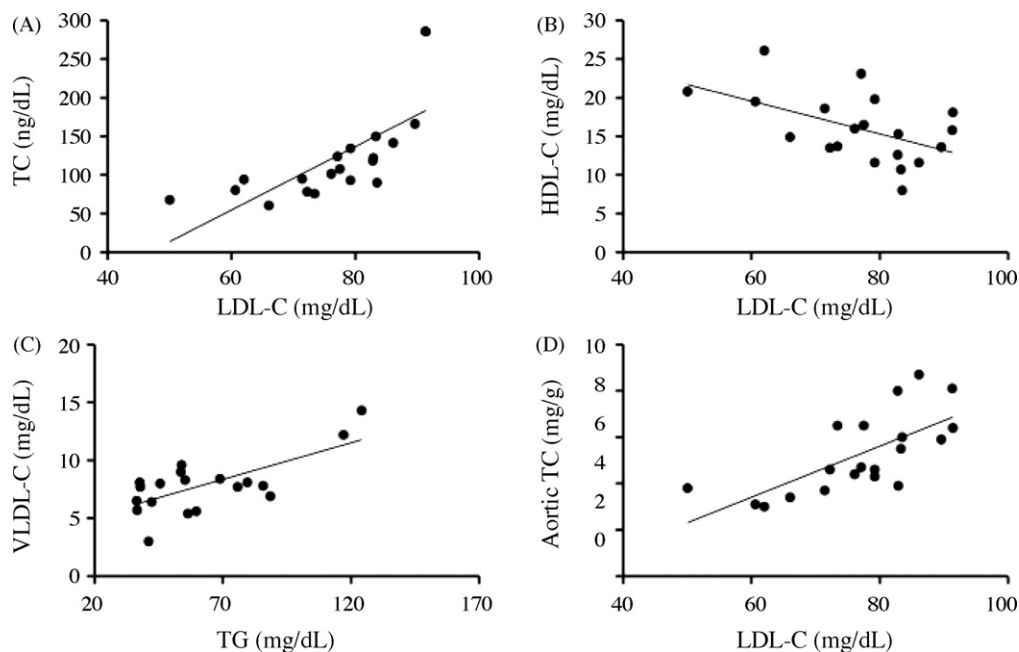


Fig. 1. Correlations between total cholesterol (TC) and LDL cholesterol (panel A); LDL cholesterol (LDL-C) and HDL cholesterol (HDL-C) (panel B); VLDL cholesterol (VLDL-C) and triglycerides (TG) (panel C) and aortic total cholesterol and LDL cholesterol (LDL-C) (panel D).

3.3. Aortic inflammatory cytokines

The immunohistochemistry analyses demonstrated that there was a positive staining for TNF- α of the aortic sinus (Fig. 3). The positive areas were close to the accumulation to the lipid-laden cells. The quantitative analyses in the MANOVA demonstrated that the LCD group had a lower level of inflammatory cytokines in the aorta than the LFD group ($p=0.03$). The results of the aortic inflammatory cytokines and their correlation with aortic oxLDL are presented in Table 2. There were also significant strong correlations among virtually all the inflammatory cytokines (data not shown).

3.4. Aortic sinus morphology

The formation of atherosclerotic plaque was not observed. Just some lipid-laden cells could be observed in both groups. Therefore, there was no difference between groups (Fig. 4, panels A–D).

3.5. Cholesterol accumulation in left carotid

The analyses of the percentage of accumulated cholesterol in the left carotid artery demonstrated that there was 54% less cholesterol deposition in LCD compared to LFD group (Fig. 5, panels A–C). In addition, there was a positive correlation between TC in the aorta and cholesterol accumulation in the left carotid artery ($r=0.630$, $p=0.036$) (data not shown).

4. Discussion

The results of this study clearly demonstrate that LCD is able to ameliorate the precursors of cardiovascular diseases in guinea pigs. In order to study the effects of macronutrient composition in atherosclerosis, we used a diet-induced model with high dietary cholesterol. The amount of cholesterol in the guinea pig diet was six times more than what humans usually eat, as this was necessary, to induce atherosclerosis in a short period of time. We postulate that the extremely high-cholesterol intake played a role in lipid metabolism generating features not usually observed in human conditions. The most frequent alteration observed after the

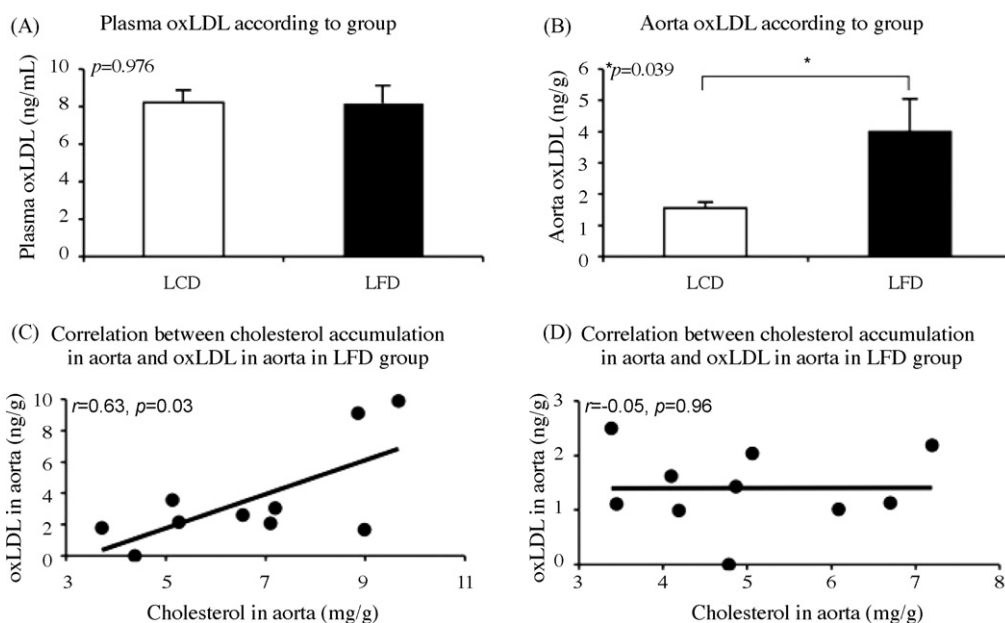


Fig. 2. Oxidized LDL in plasma (panel A) and in aorta (panel B) for guinea pigs the LFD or LCD diets. Correlation between aortic concentrations of oxidized LDL and accumulation of cholesterol in aorta for LFD group (panel C) and LCD group (panel D).

adoption of LCD by humans is the sharp reduction in TG when compared to LFD [20]. However, this was not observed in our study. We have had inconsistent findings regarding responses to low-carbohydrate in the guinea pig [21,28], which might be associated with the model and do not reflect the unfailing response in human studies [20]. The high correlation between TG and VLDL-C assures that these independent measurements were correctly performed. In addition, the lower concentrations of plasma LDL-C in the LCD group was also unexpected. We speculate that guinea pigs fed the LCD might have had an activation of hepatic LXR due to the high concentrations of dietary PUFA in this group, which may downregulate cholesterologenic enzymes and stimulates cholesterol excretion [22]. Consistent with the idea that cholesterol synthesis is reduced in LCD, the acetyl-CoA derived from fat oxidation is speculated to be used for ketone bodies synthesis inside the mitochondria, and therefore, is not shuttled to the cytoplasm to be used for cholesterol synthesis [23].

Plasma oxLDL levels were not different in guinea pigs fed LCD compared to those fed LFD, in agreement with what was found in humans [14]. However, there was significantly less oxidation of these particles in the aorta of animals fed LCD compared to the LFD animals. LDL can be oxidized in plasma [24], however, blood contains high concentrations of antioxidants and the half-life of LDL particles is usually not long enough to allow extensive oxidation in the intravascular compartment [25]. Furthermore, it has

been shown that the majority of LDL oxidation occurs in the arterial wall, where this lipoprotein is exposed to several oxidative agents for long periods of time [25]. The lack of correlation between aortic oxLDL and aortic TC suggests that the oxidation of LDL in the aorta was prevented, even with the greater accumulations of cholesterol in the aorta in LCD. These findings are supported by previous experiments from our laboratory [26], which demonstrated that LCD induces greater levels of vitamin E in tissues in guinea pigs, possibly because of the greater bioavailability of this vitamin in high fat diets [27].

The IHC for TNF- α , and its measurement by the immunoassay demonstrated the lower level of inflammation in the aorta of guinea pigs fed LCD. The MANOVA test also confirmed that the LCD group had lower levels of inflammatory cytokines in the aorta. This is consistent with previous experiments in guinea pigs [28]. The strong positive correlation between the aortic oxLDL and virtually all inflammatory cytokines supports the idea that the greater accumulation of this atherogenic form of LDL is associated with a greater inflammation in the arterial wall. In addition, the MANCOVA suggests that the aortic oxLDL is playing an important role in the production of inflammatory cytokines. Thus, the reduction in the accumulation of oxLDL in the aorta of animals fed LCD is associated with their lower inflammatory status. We have previously shown that there is a strong correlation between mRNA expression of inflammatory cytokines and protein levels in the aorta in guinea

Table 2

Concentration of aortic cytokines of guinea pigs fed the LCD or LFD diets plus correlations of these cytokines with oxidized LDL in the aorta^a.

Cytokine	LCD	LFD	p-Value	Aorta oxLDL
e-Selectin (pg/g)	5.27 \pm 4.87	11.07 \pm 8.38	0.075	$r = 0.818, p < 0.001^*$
GM-CSF (ng/g)	8.28 \pm 9.61	18.86 \pm 9.87	0.026 [†]	$r = 0.917, p < 0.001^*$
ICAM-1 (pg/g)	5.18 \pm 5.53	6.11 \pm 4.55	0.685	$r = 0.748, p < 0.001^*$
IL-b1 (ng/g)	10.69 \pm 4.21	16.45 \pm 10.59	0.128	$r = 0.739, p < 0.001^*$
MCP-1 (ng/g)	11.69 \pm 10.18	16.36 \pm 9.33	0.299	$r = 0.542, p = 0.020^†$
MMP-9 (pg/g)	28.75 \pm 25.39	39.90 \pm 30.83	0.389	$r = 0.663, p = 0.003^*$
PAI-1 (pg/g)	16.21 \pm 16.88	31.48 \pm 24.92	0.127	$r = 0.916, p < 0.001^*$
VCAM-1 (pg/g)	21.52 \pm 12.55	40.56 \pm 21.50	0.062	$r = 0.452, p = 0.059$
TNF-a (ng/g)	9.66 \pm 4.84	31.54 \pm 29.57	0.033 [†]	$r = 0.598, p = 0.009^*$

^a Values are mean \pm SD for the number of guinea pigs indicated in parenthesis.

[†] Indicates significantly different as evaluated by unpaired *t*-test. It also indicates a significant Pearson correlation.

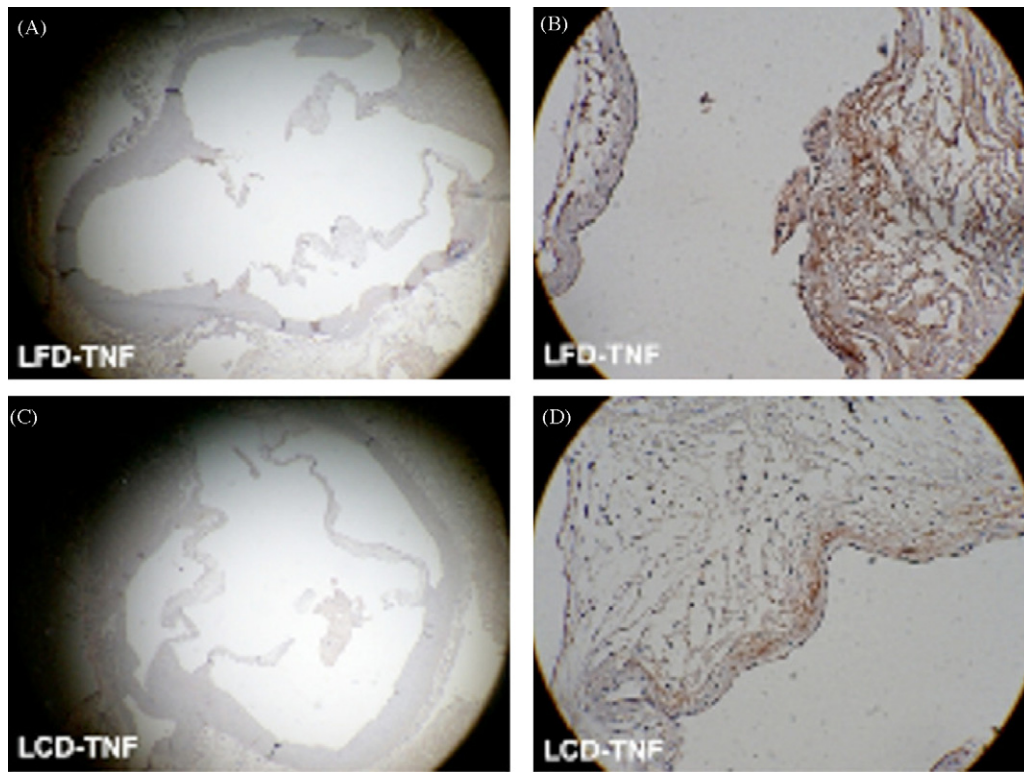


Fig. 3. IHC for TNF- α of the aortic sinus of guinea pigs fed the LFD (panel A, 40 \times magnification, panel B 400 \times magnification) or LCD (panel C, 40 \times magnification, panel D 400 \times magnification).

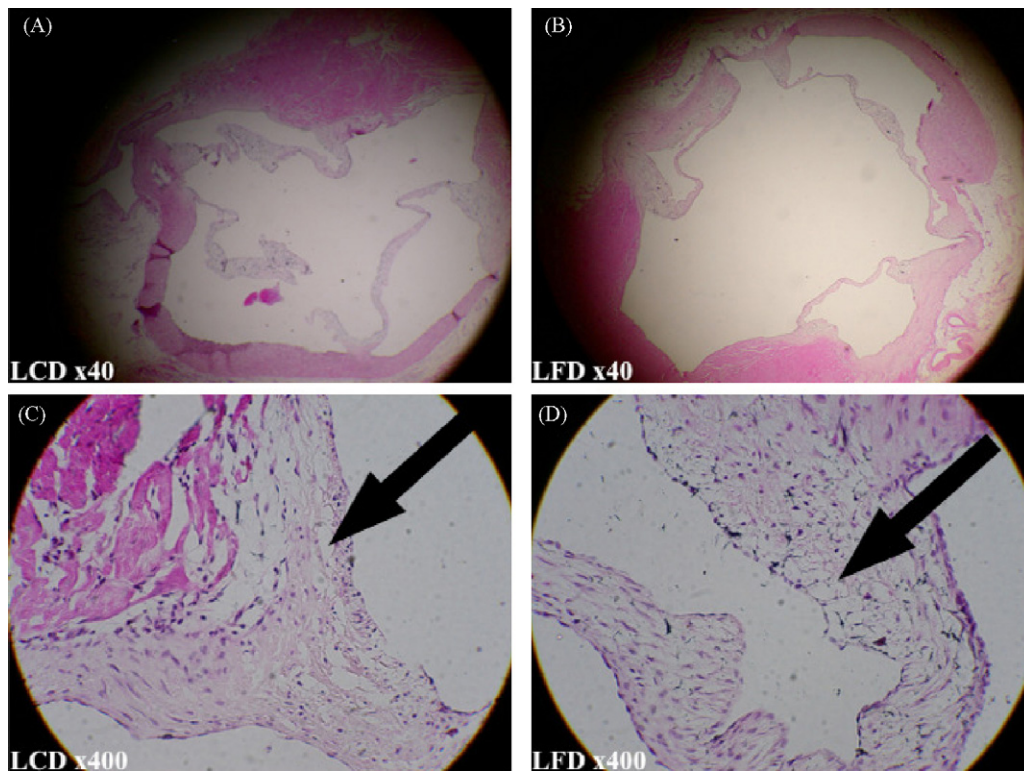


Fig. 4. Hematoxylin and eosin staining of the aortic sinus of guinea pigs fed the LCD (panel A, 40 \times magnification, panel C 400 \times magnification) or LFD (panel B, 40 \times magnification, panel D 400 \times magnification). Arrows point to the lipid-laden cells.

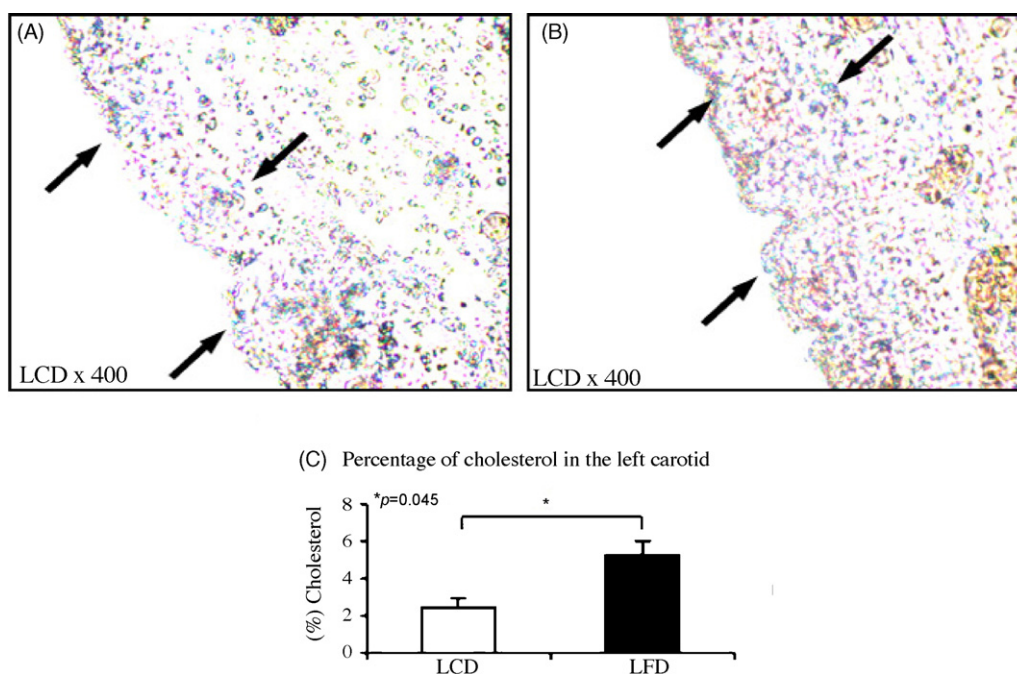


Fig. 5. Image processing of the cholesterol staining of left carotid in both groups and quantification of the blue-green stain—LCD: left carotid at 400× magnification (panel A). LFD: left carotid at 400× magnification (panel B). The arrows point to the positive staining for cholesterol. Panel C: statistical analysis of the proportion of the positive blue-green staining to the arterial area in the picture of the left carotid.

pigs [9]. We were not able to confirm these findings in this experiment because there was not sufficient aortic tissue to do these measurements.

The atherosclerotic lesions could not be observed in histopathological analyses, which is a common finding in short interventions. A study with a longer duration would be necessary to observe more advanced stages of atherosclerosis [29]. However, the deposit of cholesterol in the extracellular matrix can be observed in early stages of this disease, even before the development of foam cells [30]. The strong positive correlation between the aortic TC and cholesterol accumulation in the left carotid artery, demonstrates the systemic characteristic of atherosclerosis and that this method is valid to quantify incipient atherosclerotic modifications.

In conclusion, it is clear that animals fed LCD handled the very high amount of cholesterol ingested better. Altogether, a LCD reduced the levels of plasma LDL-C, prevented the accumulation of cholesterol in arteries, decreased the accumulation of oxLDL in the arterial wall, and mitigated the inflammation in the aorta of guinea pigs. Therefore, LCD may attenuate atherosclerosis in guinea pigs fed a high-cholesterol diet.

Acknowledgments

Special thanks to Michael Puglisi, who contributed to aortic cytokine analyses, and Dr. David J. Goldhamer and Dr. Cathy Cogswell, who contributed to the preparation of frozen sections.

References

- [1] Volek JS, Feinman RD. Carbohydrate restriction improves the features of Metabolic Syndrome. Metabolic Syndrome may be defined by the response to carbohydrate restriction. *Nutr Metab (Lond)* 2005;2:31.
- [2] Samaha FF, Iqbal N, Seshadri P, et al. A low-carbohydrate as compared with a low-fat diet in severe obesity. *N Engl J Med* 2003;348:2074–81.
- [3] Halton TL, Willett WC, Liu S, et al. Low-carbohydrate-diet score and the risk of coronary heart disease in women. *N Engl J Med* 2006;355:1991–2002.
- [4] Bantle JP, Wylie-Rosett J, Albright AL, et al. Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association. *Diabetes Care* 2008;31(Suppl. 1):S61–78.
- [5] Brehm BJ, Seeley RJ, Daniels SR, D'Alessio DA. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. *J Clin Endocrinol Metab* 2003;88:1617–23.
- [6] Yancy Jr WS, Olsen MK, Guyton JR, Bakst RP, Westman EC. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial. *Ann Intern Med* 2004;140:769–77.
- [7] Stern L, Iqbal N, Seshadri P, et al. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. *Ann Intern Med* 2004;140:778–85.
- [8] Fernandez ML, Sun DM, Montano C, McNamara DJ. Carbohydrate-fat exchange and regulation of hepatic cholesterol and plasma lipoprotein metabolism in the guinea pig. *Metabolism* 1995;44:855–64.
- [9] Sharman MJ, Fernandez ML, Zern TL, et al. Replacing dietary carbohydrate with protein and fat decreases the concentrations of small LDL and the inflammatory response induced by atherogenic diets in the guinea pig. *J Nutr Biochem* 2008;19:732–8.
- [10] Leite JO, Vaishnav U, Puglisi M, et al. A-002 (Varespladib), a phospholipase A2 inhibitor, reduces atherosclerosis in guinea pigs. *BMC Cardiovasc Disord* 2009;9:7.
- [11] West KL, Zern TL, Butteiger DN, Keller BT, Fernandez ML. SC-435, an ileal apical sodium co-dependent bile acid transporter (ASBT) inhibitor lowers plasma cholesterol and reduces atherosclerosis in guinea pigs. *Atherosclerosis* 2003;171:201–10.
- [12] Fernandez ML. Guinea pigs as models for cholesterol and lipoprotein metabolism. *J Nutr* 2001;131:10–20.
- [13] Fernandez ML, Volek JS. Guinea pigs: a suitable animal model to study lipoprotein metabolism, atherosclerosis and inflammation. *Nutr Metab (Lond)* 2006;3:17.
- [14] Volek JS, Sharman MJ, Gomez AL, et al. Comparison of a very low-carbohydrate and low-fat diet on fasting lipids, LDL subclasses, insulin resistance, and postprandial lipemic responses in overweight women. *J Am Coll Nutr* 2004;23:177–84.
- [15] Sharman MJ, Kraemer WJ, Love DM, et al. A ketogenic diet favorably affects serum biomarkers for cardiovascular disease in normal-weight men. *J Nutr* 2002;132:1879–85.
- [16] Fernandez ML, McNamara DJ. Regulation of cholesterol and lipoprotein metabolism in guinea pigs mediated by dietary fat quality and quantity. *J Nutr* 1991;121:934–43.
- [17] Fernandez ML, Sun DM, Tosca MA, McNamara DJ. Citrus pectin and cholesterol interact to regulate hepatic cholesterol homeostasis and lipoprotein metabolism: a dose-response study in guinea pigs. *Am J Clin Nutr* 1994;59:869–78.
- [18] Weber AF, Phillips MG, Bell Jr JT. An improved method for the Schultz cholesterol test. *J Histochem Cytochem* 1956;4:308–9.
- [19] Wadsworth MP, Sobel BE, Schneider DJ, Taatjes DJ. Delineation of the evolution of compositional changes in atheroma. *Histochem Cell Biol* 2002;118:59–68.

- [20] Sharman MJ, Gomez AL, Kraemer WJ, Volek JS. Very low-carbohydrate and low-fat diets affect fasting lipids and postprandial lipemia differently in overweight men. *J Nutr* 2004;134:880–5.
- [21] Torres-Gonzalez M, Volek JS, Sharman M, Contois JH, Fernandez ML. Dietary carbohydrate and cholesterol influence the number of particles and distributions of lipoprotein subfractions in guinea pigs. *J Nutr Biochem* 2006;17:773–9.
- [22] Baranowski M. Biological role of liver \times receptors. *J Physiol Pharmacol* 2008;59(Suppl. 7):31–55.
- [23] Leite JO, Deogburn R, Ratliff JC, et al. Low-carbohydrate diet disrupts the association between insulin resistance and weight gain. *Metabolism* 2009.
- [24] Hayek T, Oiknine J, Brook JG, Aviram M. Increased plasma and lipoprotein lipid peroxidation in apo E-deficient mice. *Biochem Biophys Res Commun* 1994;201:1567–74.
- [25] Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest* 1991;88:1785–92.
- [26] Bruno RS, Torres-Gonzalez M, Yeung SF, et al. Regulation of hepatic lipids and antioxidants by dietary carbohydrate restriction and cholesterol in guinea pigs. *FASEB J* 2008;22:1103.4.
- [27] Jeanes YM, Hall WL, Ellard S, Lee E, Lodge JK. The absorption of vitamin E is influenced by the amount of fat in a meal and the food matrix. *Br J Nutr* 2004;92:575–9.
- [28] Torres-Gonzalez M, Volek JS, Leite JO, Fraser H, Fernandez ML. Carbohydrate restriction reduces lipids and inflammation and prevents atherosclerosis in guinea pigs. *J Atheroscler Thromb* 2008;15:235–43.
- [29] Tararak EM. Regression of experimental atherosclerosis of the aorta in guinea pigs. *Bull Exper Biol Med* 1968;67:118–21.
- [30] Rosenfeld ME, Carew TE, von Hodenberg E, et al. Autoradiographic analysis of the distribution of 125I-tyramine-cellobiose-LDL in atherosclerotic lesions of the WHHL rabbit. *J Arterioscler Thromb* 1992;12:985–95.