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# Carbohydrate restriction and dietary cholesterol distinctly affect plasma lipids and lipoprotein subfractions in adult guinea pigs

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**Abstract**

To evaluate the effects of carbohydrate restriction (CR) and dietary cholesterol on lipoprotein metabolism, adult male guinea pigs (10 guinea pigs/diet) were fed either low (0.04 g/100 g) or high (0.25 g/100 g) amounts of dietary cholesterol, in combination with either low (10% total energy) or high (54.2% total energy) dietary carbohydrate (control groups) for a total of four groups: high carbohydrate–low cholesterol (control-L), high carbohydrate–high cholesterol (control-H), low carbohydrate–low cholesterol (CR-L) and low carbohydrate–high cholesterol (CR-H). Plasma triglyceride concentrations were lower ($P<.01$%), while high-density lipoprotein cholesterol concentrations were higher ($P<.05$) in the CR groups compared to the control groups. In contrast, high dietary cholesterol (CR-H and control-H) resulted in higher concentrations of total and low-density lipoprotein (LDL) cholesterol compared to those guinea pigs fed the low-cholesterol diets ($P<.01$). Dietary cholesterol significantly increased the total number of LDL particles ($P<.001$) and the number of small LDL ($P<.001$), as determined by nuclear magnetic resonance. In contrast, carbohydrate restriction (CR-L and CR-H) resulted in lower concentrations of medium very-low-density lipoprotein and small LDL particles compared to the high-carbohydrate groups. Plasma lecithin:cholesterol acyltransferase (LCAT) activity was decreased and cholesterol ester transfer protein activity was increased by dietary cholesterol, whereas carbohydrate restriction increased LCAT activity ($P<.05$). These findings are similar to those observed in humans, thus validating the use of adult guinea pigs to study lipid responses to carbohydrate restriction. The results also indicate that the atherogenicity of lipoproteins induced by high dietary cholesterol is attenuated by carbohydrate restriction in guinea pigs.


*Keywords:* Carbohydrate restriction; Dietary cholesterol; LDL subfractions; LCAT; CETP; Guinea pigs

## 1. Introduction

Coronary heart disease (CHD) is increasingly becoming a worldwide epidemic that encompasses several physiological and metabolic abnormalities such as obesity, metabolic syndrome, diabetes, atherosclerosis and high blood pressure [1,2]. These health complications are interconnected and can be closely related with dysfunctions in lipoprotein metabolism. Diet is one of the major determinants of the onset of disturbances in lipoprotein metabolism and hence also of CHD [3]. Thus, numerous investigations have focused on studying the effects of different types of diet to reduce the risk of CHD. Carbohydrate-restricted diets (CRDs) have been shown to positively affect lipoprotein metabolism, in addition to being very effective in reducing body weight [4]. The most consistent beneficial effects of CRD are improvement in atherogenic dyslipidemia, including a decrease in plasma triglycerides (TG) and very-low-density lipoprotein cholesterol (VLDL-C), an increase in high-density lipoprotein

*Abbreviations:* CETP, cholesterol ester transfer protein; CHD, coronary heart disease; control-H, high carbohydrate–high cholesterol; control-L, high carbohydrate–low cholesterol; CR-H, low carbohydrate–high cholesterol; CR-L, low carbohydrate–low cholesterol; HDL-C, high-density lipoprotein cholesterol; LCAT, lecithin:cholesterol acyltransferase; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol.

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Table 1
Composition of experimental diets: control-L, CR-L, control-H and CR-H

| | Control-L | | CR-L | | Control-H | | CR-H | |
|---|---|---|---|---|---|---|---|---|
| | g/100 g | % Energy | g/100 g | % Energy | g/100 g | % Energy | g/100 g | % Energy |
| Soybean protein | 22.5 | 25.5 | 33.0 | 30 | 22.5 | 25.5 | 33.0 | 30 |
| Carbohydrate | 48.0 | 54.2 | 11.0 | 10 | 48.0 | 54.2 | 11.0 | 10 |
| Fat mix [a] | 8.0 | 20.3 | 30.0 | 60 | 8.0 | 20.3 | 30.0 | 60 |
| Cellulose | 10.0 | | 12.5 | | 10.0 | | 12.5 | |
| Guar gum | 2.5 | | 3.1 | | 3.1 | | 3.1 | |
| Mineral mix [b] | 8.0 | | 8.2 | | 8.0 | | 8.2 | |
| Vitamin mix [b] | 1.0 | | 1.2 | | 1.0 | | 1.2 | |
| Cholesterol | 0.04 | | 0.04 | | 0.25 | | 0.25 | |

[a] The fat mix was high in lauric and myristic acids, which are known to cause endogenous hypercholesterolemia in guinea pigs.
[b] Mineral and vitamin mixes are formulated to meet NRC requirements for guinea pigs [13].

cholesterol (HDL-C), a reduction in the levels of the more atherogenic smaller low-density lipoprotein (LDL) particles and an increase in the formation of the more antiatherogenic larger HDL particles [5–7].

Our laboratory group has been working extensively with guinea pigs as a suitable animal model for studying lipoprotein metabolism because of their similarities with humans. Guinea pigs, in addition to carrying the majority of their cholesterol in LDL, have high LDL/HDL ratios and respond to dietary interventions similar to humans [8,9]. We used this animal model previously to evaluate the effects of CRD during a cholesterol challenge [9]. In this previous work, growing guinea pigs were used, and the main findings regarding plasma lipoprotein metabolism were found in the levels of small LDL particles. The CRD group had lower levels of the smaller LDL particles compared with the high-carbohydrate group. Similar responses to CRD have been reported in children [10].

However, studies evaluating the effects of CRD have normally been carried out in adult humans due to the fact that they are at higher risk for developing CHD. Consequently, the main objective of this study was to evaluate the effects of CRDs during a cholesterol challenge on plasma lipids and lipoprotein distribution in adult guinea pigs. Consistent with human studies [11,12], in the current work, a similar energy contribution of macronutrients for CRDs was used (10% fat, 54% carbohydrate and 35% protein). We hypothesized that adult guinea pigs would have similar plasma lipid responses to CRD as adult humans and that carbohydrate restriction would attenuate the plasma lipid response to dietary cholesterol, resulting in less atherogenic lipoproteins.

## 2. Materials and methods

### 2.1. Materials

Enzymatic cholesterol and TG kits, cholesterol oxidase, cholesterol esterase and peroxidase were purchased from Roche-Diagnostics (Indianapolis, IN). Quick-seal ultracentrifuge tubes were obtained from Beckman Instruments (Palo Alto, CA).

### 2.2. Diets

Diets were prepared and pelleted by Research Diets (New Brunswick, NJ). Isocaloric diets were designed to meet all the nutritional requirements of guinea pigs. The four diets varied in the amount of macronutrients and in the concentrations of dietary cholesterol. Vitamins, minerals and fiber were adjusted for the increased caloric content of the low-carbohydrate diets. The composition of the diets and the energy contribution of the macronutrients are indicated in Table 1. The high carbohydrate–low cholesterol (control-L) diet and the high carbohydrate–high cholesterol (control-H) diet had the following energy contributions: 25.5% protein, 20.2% fat and 54.3% carbohydrate. The low carbohydrate–low cholesterol (CR-L) diet and the low carbohydrate–high cholesterol (CR-H) diet had the following energy contribution: 30% protein, 10% carbohydrate and 60% fat. The amount of cholesterol was either low (0.04 g/100 g) or high (0.25 g/100 g), being equivalent to 300 or 1800 mg/day in the human situation [13]. The high-cholesterol diets were used to develop atherosclerosis in guinea pigs [14]. The fatty acid composition of the fat mix was as follows: 23.8% lauric acid, 7.8% myristic acid, 9.2% palmitic acid, 8.6% stearic acid, 19.9% oleic acid and 26.4% linoleic acid.

### 2.3. Animals

Forty male adult guinea pigs with a stable weight between 800 and 1000 g were assigned to the four dietary treatments (10 animals/group). Guinea pigs were housed individually in a metal cage in a light cycle room (lights on from 0700 to 1900 h) and had free access to food and water. Food consumption was monitored every other day, and the guinea pigs were weighed weekly to ensure stable weight. After 12 weeks of treatment, the guinea pigs were deprived of food for 12 h and sacrificed by heart puncture after isoflurane anesthesia. All animal experiments were conducted in accordance with US Public Health Service/US Department of Agriculture guidelines. Experimental protocols were approved by the University of Connecticut Institutional Care and Use Committee.

### 2.4. Plasma isolation and plasma lipids

Plasma was obtained after 20 min of centrifugation at 2000×*g*. A preservation cocktail of aprotonin, phenyl methyl sulfonyl fluoride and sodium azide was added to plasma samples to minimize proteolytic enzymes and microbial contamination. Plasma was distributed in several aliquots for plasma lipid analysis, lipoprotein isolation and determination of lipoprotein subfraction, lecithin:cholesterol acyltransferase (LCAT) and cholesterol ester transfer protein (CETP).

Plasma cholesterol [15] and TG [16] were analyzed by previously described methods. HDL-C was also determined in accordance with the method of Fernandez et al. [17], with modifications, which consisted of 2 mol/L $MgCl_2$ for precipitation of apoB-containing lipoproteins. VLDL was isolated by sequential ultracentrifugation in an LE-80K ultracentrifuge (Beckman Instruments). VLDL was isolated at *d*=1.006 kg/L at 125,000×*g* and 15°C for 3 h in a VTi-60 rotor. VLDL-C was determined enzymatically.

### 2.5. VLDL, LDL and HDL size and subfractions

$^1$H nuclear magnetic resonance (NMR) analysis was performed on a 400-MHz NMR analyzer (Bruker BioSpin Corp., Billerica, MA), as previously described [9,18]. Briefly, lipoprotein subclasses of different sizes produce a distinct lipid methyl signal whose amplitude is directly proportional to lipoprotein particle concentration. NMR simultaneously quantifies >30 lipoprotein subclasses that are empirically grouped into 9 smaller subclasses based on particle diameters: large VLDL (>60 nm), medium VLDL (27–60 nm), small VLDL (23–27 nm), large LDL (21.2–23 nm), medium LDL (19.8–21.2), small LDL (18–19.8 nm), large HDL (8.8–13 nm), medium HDL (8.2–8.8 nm) and small HDL (7.3–8.2 nm). Weighted average lipoprotein particle sizes in diameters were calculated based on the diameter of each lipoprotein subclass multiplied by its respective relative concentration. Lipid concentrations (VLDL TG, total TG and HDL-C) were estimated by assuming a typical lipid composition per particle and by summing the concentrations in each subclass. Lipoprotein subclasses using NMR techniques have been published in guinea pigs [9].

### 2.6. LCAT and CETP activities

Plasma LCAT activity was determined using a previously described method [19], by measuring the reduction in the mass of endogenous free cholesterol after a 6-h incubation, and was expressed as the molar esterification rate (μmol decrease in unesterified cholesterol L plasma$^{-1}$ h$^{-1}$). Free cholesterol concentrations were measured by an enzymatic method.

Plasma CETP activity was determined using a CETP activity assay kit following the manufacturer's instruction. Briefly, 3 μl of plasma sample (as the source of CETP) was added to the reaction mixture containing a fluorescent self-quenched neutral lipid as the donor molecule and an acceptor molecule. A CETP-mediated transfer of the fluorescent-neutral lipid to the acceptor molecule results in an increase in fluorescence, which was read in a fluorescence plate reader at 456 nm excitation and 535 nm emission. CETP activity was expressed as millimoles of neutral lipid transferred per liter of plasma per hour (mmol L$^{-1}$ h$^{-1}$). This method has been previously standardized in our laboratory [20].

Table 2
Plasma TC, VLDL-C, LDL-C, HDL-C and TG of guinea pigs fed high-carbohydrate diets (control) and CRDs, in combination with low (L; 0.04 g/100 g) or high (H; 0.25 g/100 g) cholesterol

| | TC [a] (mg/dl) | VLDL-C [a] (mg/dl) | LDL-C [a] (mg/dl) | HDL-C [a] (mg/dl) | TG [b] (mg/dl) |
|---|---|---|---|---|---|
| Carbohydrate restricted | | | | | |
| CR-L | 71.7±17.6 | 4.6±2.2 | 56.5±20.7 | 10.6±6.0 | 49.0±13.2 |
| CR-H | 110.0±17.6 | 5.1±5.7 | 96.1±18.1 | 8.8±4.4 | 64.0±22.1 |
| Control | | | | | |
| Control-L | 63.5±21.2 | 3.2±0.7 | 53.1±21.4 | 7.1±3.6 | 71.5±18.0 |
| Control-H | 123.4±26.9 | 2.6±1.6 | 114.2±27.2 | 6.6±2.4 | 141.3±71.7 |
| Cholesterol effect (*P*) | <.001 | NS | <.001 | NS | <.001 |
| Carbohydrate effect (*P*) | NS | NS | NS | <.05 | <.01 |
| Interaction (*P*) | NS | NS | NS | NS | <.01 |

Values are presented as mean±S.D. for *n*=10 guinea pigs per group.
[a] To convert to millimoles per liter, multiply by 38.67.
[b] To convert to millimoles per liter, multiply by 88.54.

### 2.7. Statistics

One-way analysis of variance was used to determine the food consumption and final weights of the guinea pigs from the four dietary treatments. Two-way analysis of variance was used to evaluate carbohydrate and cholesterol effects, interactions on plasma lipids and cholesterol distribution among lipoproteins, lipoprotein subfractions, LCAT and CETP activities. $P<.05$ was considered significant.

## 3. Results

Guinea pigs maintained a stable weight during the 12 weeks of treatment. There were no differences in final weights among groups. The average final weight for the four groups was 950.8±76.6 g. Guinea pigs also consumed similar amounts of food, with an average of 206.9±65.7 g/week per group during the experimental period.

### 3.1. Plasma lipids and lipoproteins

Table 2 shows the plasma lipid results. Dietary cholesterol affected plasma total cholesterol (TC) and LDL-C levels. Total plasma cholesterol values were higher and statistically different in the high-cholesterol groups (3.18±0.69 and 2.83±0.45 mmol/L, respectively) than in the low-cholesterol groups (1.85±0.70 and 1.63±0.55 mmol/L, respectively) ($P<.001$). The same pattern was observed in LDL-C levels. The high-cholesterol groups had higher and significantly different LDL-C levels (CR-H: 2.47±0.47 mmol/L; control-

Table 3
Total number of VLDL, IDL, LDL and HDL particles of guinea pigs fed high-carbohydrate (control) diets and CRDs, in combination with low (L; 0.04 g/100 g) or high (H; 0.25 g/100 g) cholesterol

| | Total VLDL (nmol/L) | Total IDL (nmol/L) | Total LDL (nmol/L) | Total HDL (nmol/L) |
|---|---|---|---|---|
| Carbohydrate restricted | | | | |
| CR-L | 63.5±18.8 | 48.3±29.5 | 330.1±122.6 | 480±290 |
| CR-H | 66.3±20.0 | 33.8±17.8 | 194.3±81.5 | 210±170 |
| Control | | | | |
| Control-L | 63.4±11.4 | 67.8±26.1 | 430.1±122.7 | 270±230 |
| Control-H | 79.5±25.4 | 41.3±21.8 | 255.5±132.7 | 440±570 |
| Cholesterol effect (*P*) | NS | <.01 | <.0001 | NS |
| Carbohydrate effect (*P*) | NS | .08 | <.05 | NS |
| Interaction (*P*) | NS | NS | NS | NS |

Values are presented as mean±S.D. for *n*=10 guinea pigs per group.

Table 5
Concentration of LDL subfractions of guinea pigs fed high-carbohydrate diets (control) and CRDs, in combination with low (L; 0.04 g/100 g) or high (H; 0.25 g/100 g) cholesterol

| | Large LDL (nmol/L) | Medium LDL (nmol/L) | Small LDL (nmol/L) |
|---|---|---|---|
| Carbohydrate restricted | | | |
| CR-L | 88.9±34.7 | 42.8±25.2 | 150.8±94.8 |
| CR-H | 46.4±24.4 | 36.7±28.6 | 77.3±55.7 |
| Control | | | |
| Control-L | 99.1±40.8 | 52.9±22.2 | 210.2±125.6 |
| Control-H | 44.9±25.7 | 50.5±44.1 | 143.3±103.1 |
| Cholesterol effect (*P*) | <.0001 | NS | <.05 |
| Carbohydrate effect (*P*) | NS | NS | <.05 |
| Interaction (*P*) | NS | NS | NS |

Values are presented as mean±S.D. for *n*=10 guinea pigs per group.

H: 2.94±0.70 mmol/L) than the low-cholesterol groups (CR-L: 1.45±0.53 mmol/L; control-L: 1.37±0.55 mmol/L) (*P*<.001). Plasma HDL-C was affected by dietary carbohydrate. The high-carbohydrate groups (control groups) had lower concentrations of HDL-C than the carbohydrate-restricted groups (*P*<.05). High-carbohydrate diets also affected plasma TG (*P*<.01). High-carbohydrate intake caused higher levels of plasma TG compared with the carbohydrate-restricted groups (Table 2). The results observed for plasma HDL-C and TG indicate that adult guinea pigs respond to CRDs in a similar way to adult humans.

In regards to the effects of dietary cholesterol and carbohydrate on the different lipoprotein particles, the major effects were seen in IDL and LDL (Table 3). The levels of IDL particles were higher and statistically different (*P*<.01) in guinea pigs fed the low-cholesterol diets (control-L and CR-L) than in those fed the high-cholesterol diets (control-H and CR-H). The total LDL particles were increased by both dietary cholesterol and carbohydrate. The low-cholesterol groups had higher concentrations of LDL particles than the high-cholesterol groups (*P*<.0001), while the carbohydrate-restricted groups had lower concentrations of LDL particles (CR-L: 330.1±122.6 nmol/L; CR-H: nmol/L) than the control groups (control-L: 430.1±122.7 nmol/L; control-H:255.5±132.7 nmol/L) (*P*<.05). Neither dietary cholesterol nor dietary carbohydrate affected the number of total HDL particles (Table 3).

The major changes in the different lipoprotein subfractions are shown in Tables 4 and 5. The changes were seen mainly in the medium VLDL, as well as in the large and small LDL subfractions. Guinea pigs fed CRDs had a higher concentration of medium VLDL subclasses than those fed the high-carbohydrate diets (Table 4). With respect to large LDL subclass, a cholesterol effect was observed. The low-cholesterol groups (CR-L and control-L) induced more than twice the formation of larger LDL particles (88.9±34.7

Table 4
Concentration of VLDL subfractions of guinea pigs fed high-carbohydrate diets (control) and CRDs, in combination with low (L; 0.04 g/100 g) or high (H; 0.25 g/100 g) cholesterol

| | Large VLDL (nmol/L) | Medium VLDL (nmol/L) | Small VLDL (nmol/L) |
|---|---|---|---|
| Carbohydrate restricted | | | |
| CR-L | 1.4±1.4 | 8.4±5.0 | 15.7±9.5 |
| CR-H | 1.3±1.0 | 9.0±4.4 | 11.7±4.9 |
| Control | | | |
| Control-L | 2.2±2.8 | 5.2±6.0 | 20.1±10.8 |
| Control-H | 0.8±0.6 | 3.9±3.3 | 14.4±6.8 |
| Cholesterol effect (*P*) | NS | NS | NS |
| Carbohydrate effect (*P*) | NS | <.002 | NS |
| Interaction (*P*) | NS | NS | NS |

Values are presented as mean±S.D. for *n*=10 guinea pigs per group.

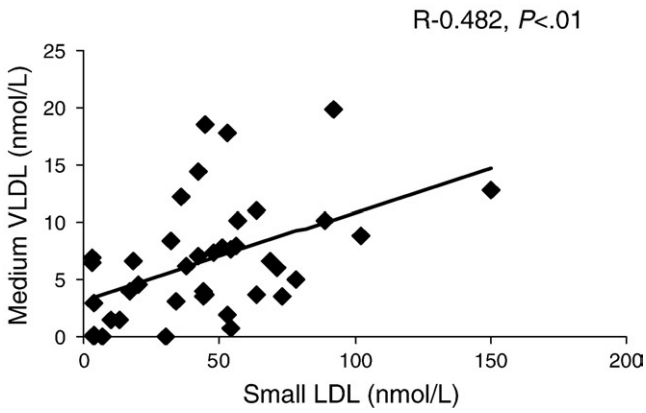


Fig. 1. Correlation between small LDL particles and medium VLDL particles of guinea pigs fed high (54.2% energy) and low (10% energy) carbohydrates, in combination with low (0.04g/100g) or high (0.25 g/100 g) dietary cholesterol.

Table 6
LCAT and CETP activities of guinea pigs fed high-carbohydrate diets (control) and CRDs, in combination with low (0.04 g/100 g) or high (0.25 g/100 g) cholesterol

| | LCAT activity (mmol $L^{-1}$ $h^{-1}$) | CETP activity (mmol $L^{-1}$ $h^{-1}$) |
|---|---|---|
| Carbohydrate restricted | | |
| CR-L | 58.5±23.9 | 28.1±1.0 |
| CR-H | 25.7±28.6 | 29.5±2.2 |
| Control | | |
| Control-L | 43.3±25.8 | 28.9±1.3 |
| Control-H | 14.1±8.3 | 30.1±1.5 |
| Cholesterol effect (*P*) | <.0001 | <.015 |
| Carbohydrate effect (*P*) | <.05 | NS |
| Interaction (*P*) | NS | NS |

Values are presented as mean±S.D. for $n$=10 guinea pigs per group.

and 99.1±40.8 nmol/L, respectively) compared with their counterpart high-cholesterol groups (CR-H: 46.4±24.4 nmol/L; control-H: 44.9±25.7 nmol/L) ($P$<.0001). The formation of the smaller LDL subfractions was affected by both dietary cholesterol and carbohydrate. Guinea pigs fed low-cholesterol diets had higher levels of smaller LDL particles than the guinea pigs fed high-cholesterol diets ($P$<.05). In contrast, carbohydrate restriction reduced the formation of the more atherogenic LDL particles, since the low-carbohydrate groups had lower levels of the smaller LDL subclass than their counterpart high-carbohydrate groups ($P$<.05). A significant positive correlation was found between medium VLDL particles and small LDL for all groups ($r$=.482, $P$<.01) (Fig. 1). Neither dietary cholesterol nor dietary carbohydrate affected the distribution of HDL subclasses (data not shown).

### 3.2. LCAT and CETP

Table 6 presents the results obtained in the measurements of LCAT and CETP activities. LCAT was affected by dietary cholesterol ($P$<.0001) and carbohydrate restriction ($P$<.05). Both high-cholesterol and high-carbohydrate levels affected LCAT activity. High-cholesterol intake resulted in lower LCAT activity compared to low-cholesterol intake. Similarly, high-carbohydrate levels in the diet reduced LCAT activity compared to carbohydrate restriction (Table 6). CETP activity was mainly affected by high-cholesterol diets, since the CR-H and control-H groups presented greater CETP activity (29.5±2.2 and 30.1±1.5 mmol $L^{-1}$ $h^{-1}$, respectively) than the low-cholesterol groups (CR-L: 28.10±1.0 mmol $L^{-1}$ $h^{-1}$; control-L: 28.9±1.3 mmol $L^{-1}$ $h^{-1}$) ($P$<.015).

## 4. Discussion

CRDs have been recommended for people at higher risk for developing some form of CHD because of their beneficial effects on improving plasma lipid profiles [6,21]. We have previously reported the effect of these diets on plasma lipids in growing guinea pigs. In our previous report, we observed that growing guinea pigs [9] responded to CRD similarly to children [10]. In the current work, we determined whether adult guinea pigs responded to CRD similarly to adult humans, and we investigated changes induced by carbohydrate restriction and dietary cholesterol in the intravascular compartment.

The number of HDL particles for guinea pigs using NMR techniques is lower than what has been reported for humans [11],which is not surprising due to the low levels of HDL-C in this animal model [22]. However, mice, rats, rabbits, hamsters and nonhuman primates have much lower LDL/HDL ratios compared to humans [8,23], and they are still considered useful models for explaining dietary effects on lipoprotein metabolism [24,25]. The fact that guinea pigs have a higher LDL/HDL ratio than humans does not change the biological significance of carbohydrate restriction and dietary cholesterol in lipoprotein metabolism observed in this study.

### 4.1. Carbohydrate effects

Studies in adult humans following CRDs have consistently shown a reduction in plasma TG and VLDL-C [26]. Moreover, an increase in HDL-C, a reduction in the more atherogenic LDL subfractions, has also been observed [11,27]. In this study, we report that adult guinea pigs have similar responses to CRD in terms of their plasma lipid metabolism. Hence, guinea pigs fed CRDs had higher levels of HDL-C, lower levels of plasma TG and lower concentrations of the smaller LDL subclasses compared to the high-carbohydrate groups. Numerous studies have demonstrated that high-carbohydrate intake stimulates the de novo synthesis of fatty acids and TG; therefore, high-carbohydrate diets may, in this way, induce high plasma TG [28,29]. In the present study, the higher plasma TG could be linked to the increased number of medium VLDL particles. It has been shown that medium VLDL particles are associated with visceral fat area and fasting insulin in obese individuals [30]. It has also been shown that large and medium VLDL particles are precursors of small dense LDL [31]. Thus, CR reduced VLDL particles associated with insulin resistance and formation of atherogenic LDL. We found a significant correlation between medium VLDL particles and small LDL in this study.

The presence of high levels of plasma TG has normally been associated with low HDL-C. This association between high plasma TG and low HDL-C levels is recognized as an important atherogenic feature. Obese people, diabetics and people with metabolic syndrome commonly present this feature and are thus at higher risk for developing any form of CHD [32]. It has been hypothesized that low HDL-C due to high plasma TG is linked to VLDL metabolism. When VLDL is excreted by

the liver, it is normally rich in TG. In plasma, VLDL can exchange TG for CE with HDL, a process mediated by CETP [33]. The exchange of lipids between these two lipoproteins leads to the production of TG-rich HDL particles [34]. Subsequently, hepatic lipase hydrolyzes TG in the HDL, decreasing the larger HDL particles (the more antiatherogenic HDL subclass) and generating higher concentrations of the smaller HDL particles, which can be removed faster from the circulation, thus ending up with lower levels of HDL-C [35]. Nevertheless, in accordance with the mechanism hypothesized, in the present work, high CETP activity in the high-carbohydrate groups would have been expected. However, CETP activity was increased only by dietary cholesterol, suggesting that CETP activity responds more to high plasma cholesterol rather than to high plasma TG.

Nowadays, LDL size and subfractions are considered more important clinical markers for CHD. Epidemiological studies have shown that smaller LDL particles increased the risk of CHD independently of the levels of HDL-C, non-HDL-C and TG, smoking, systolic blood pressure and body mass index [36]. LDL subclasses differ in their metabolic behavior and pathological role. It has been suggested that small dense LDL has a longer residence time in plasma, making it more susceptible to oxidative modification [37]. It is believed that in the formation of smaller denser LDL particles, the exchange of CE and TG between LDL and VLDL, respectively, has a major role [38]. The TG-rich LDL particles formed then become substrates for hepatic lipase, which hydrolyzes TG, generating smaller LDL particles [39]. In the current study, we observed that guinea pigs eating the CRD had lower concentrations of the smaller LDL subfractions, as has been observed in human studies. In contrast, the high-carbohydrate groups, in addition to having higher concentrations of total LDL particles, also had higher levels of smaller LDL particles, indicating that their cholesterol is carried mainly in the most atherogenic LDL subfractions. Thus, these results suggest that the CRD reduced the formation of atherogenic LDL in adult and growing guinea pigs [9].

As explained previously, it has been hypothesized that CETP may be implicated both in lowering the HDL-C levels and in playing an important role in the formation of smaller more atherogenic LDL particles; this can be linked to high plasma TG [38]. However, in this work, we did not observe higher CETP activity in the high-carbohydrate groups, but we did observe that CR increased LCAT activity, lowered plasma TG and increased HDL-C, in addition to reducing the concentration of the smaller LDL particles. It is well known that high-carbohydrate diets induce de novo synthesis of fatty acids and TG, so we might therefore suggest that a continuous intake of high amounts of carbohydrates stimulates the formation of VLDL, mainly the medium-sized VLDL whose atherogenic potential has been previously discussed.

### 4.2. Cholesterol effects

Our laboratory group has previously reported that guinea pigs respond to dietary cholesterol in a dose-responsive manner [8,17]. Thus, high-cholesterol intake has been reflected in high plasma total and LDL cholesterol levels. In the current work, we did see this effect. The increases in plasma cholesterol observed in guinea pigs are independent of the amount of fat, the type of fatty acids or the different levels of protein in this study. Studies have shown that the amount of linoleic acid [40] or soybean protein influences plasma cholesterol [41]. However, plasma total and LDL cholesterol were clearly influenced only by dietary cholesterol and not by the macronutrient composition of the diet in this study. The high-cholesterol groups had higher plasma cholesterol concentrations (TC and LDL-C) than the low-cholesterol groups. It was also seen that the combination of high cholesterol and high carbohydrate further increased plasma TG. Reports have shown that cholesterol can induce the expression of lipogenic enzymes [42], but other reports have also found that high-carbohydrate intakes induce the lipogenic enzymes as well [43,44]. This may explain why the control-H group had higher plasma TG levels than the other three groups. Interestingly, high-cholesterol diets reduced the formation of smaller LDL particles. This may indicate that even when the high-cholesterol groups had higher LDL-C levels than the low-cholesterol groups, the cholesterol is carried mainly in the medium and small LDL particles.

Another important effect caused by cholesterol was found in LCAT and CETP activities. The high-cholesterol groups had lower LCAT and higher CETP activities than the low-cholesterol groups. It is known that LCAT and CETP are responsible for the esterification of cell-derived cholesterol and for the transfer of newly synthesized cholesteryl ester from HDL to TG-rich lipoproteins in human plasma [45]. LCAT and CETP activities are key components for HDL remodeling by which HDL particles with a high capacity for cell cholesterol uptake are generated in plasma. In other words, they have an important role in reverse cholesterol transport [34]. Because high cholesterol lowered LCAT activity in the current work, we may suggest that LCAT and CETP are not in coordination to remove excess cholesterol from the body. It has been shown that LCAT activity appears to have a major role in decreasing atherosclerotic potential [46]. In fact, deletion of LCAT results in increased atherosclerotic plaques in LDL receptors and apoE knockout mice [47]. The lower LCAT activity observed in guinea pigs fed high cholesterol in the present study suggests the potential of increased atherosclerotic lesions in these guinea pigs.

In summary, in these studies, we have demonstrated that adult guinea pigs respond to carbohydrate restriction similarly to humans by increasing HDL-C, reducing TG and increasing the size of LDL. In addition, we have shown that carbohydrate restriction attenuated the effects

of dietary cholesterol on the atherogenicity of lipoproteins by decreasing the number of medium VLDL and small LDL particles.

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