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Dietary carbohydrate and cholesterol influence the number of particles and distributions of lipoprotein subfractions in guinea pigs

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Abstract

Guinea pigs ($n=10$/group) were fed one of three diets: a high carbohydrate (CHO) (42% energy), low cholesterol (0.04%) diet (LChHC), a diet with the same amount of CHO but with 0.25% cholesterol (HChHC) or a diet with 11% of energy from CHO and 0.25% cholesterol (HChLC) for 12 weeks. VLDL- and LDL cholesterol (LDL-C) were higher in the HChLC and HChHC groups than in the LChHC group ($P<.0001$). Lipoprotein subclasses and size were analyzed by nuclear magnetic resonance. Dietary cholesterol (HChHC and HChLC groups) resulted in larger VLDL particles (71.1±6.9, 78.9±3.33 nm, respectively) than those in the LChHC group (44.3±10.8 nm). In addition, there were higher concentrations of the large VLDL (>60 nm) and the medium VLDL (>35 nm) in the high cholesterol groups ($P<.01$). Similarly, the concentration of the medium (>8.2 nm) and small HDL (>7.2 nm) was higher in the HChHC and HChLC groups ($P<.001$). In contrast, CHO restriction affected the concentrations of LDL subfractions. The number of total LDL particles was lower in the HChLC (291.3±85.0 nmol/L) than in the HChHC group (467.6±113.1 nmol/L), indicating that the cholesterol in LDL was distributed in less particles in the former group. The concentrations of medium LDL (>19.8 nm) (98.4±90.8) and small LDL (>18 nm) (29.3±24.9 nmol/L) were lower in the HChLC group than in the HChHC group (261.8±105.8 and 64.9±27.9 nmol/L, respectively). These results indicate that dietary cholesterol increased the atherogenicity of both VLDL and HDL while CHO restriction increased the number of large LDL and decreased the concentrations of the more atherogenic smaller LDL subfractions.

Keywords: Dietary cholesterol; Carbohydrate restriction; Lipoprotein subclasses; Guinea pigs; VLDL particles; LDL size

1. Introduction

Macronutrient composition has varying effects on lipoproteins. In particular, low-fat diets are most effective at lowering LDL cholesterol (LDL-C), but may adversely affect triglyceride (TG) and HDL cholesterol (HDL-C). In contrast, diets restricted in carbohydrates have the opposite effect, primarily improving triglycerides and HDL cholesterol with more varying effects on LDL-C [1]. Although each of these major lipoprotein classes carries independent predictive value in determining a person's risk for cardiovascular disease (CVD), the relative importance of lowering LDL-C or TG vs. raising HDL-C on hard end points of morbidity and mortality is less clear. One approach to further characterize the clinical significance of changes in major lipoprotein classes to dietary alterations is to assess lipoprotein subfractions.

The major lipoproteins very low density lipoprotein (VLDL), LDL and HDL are heterogeneous, comprising particles of varying size, physical and chemical properties, and atherogenic potential [2]. For example, it has been shown that larger VLDL particles are more atherogenic than the smaller subfractions [3], and the major incidence of this VLDL subclass has been found when subjects consumed

Abbreviations: CVD, cardiovascular disease; HChHC, high cholesterol/high carbohydrate; HChLC, high cholesterol/low carbohydrate; HDL-C, HDL cholesterol; LChHC, low cholesterol/high carbohydrate; LDL-C, LDL cholesterol; NMR, nuclear magnetic resonance; TG, triglycerides; VLDL, very low density lipoprotein.

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Table 1
Composition of LChHC, HChHC and HChLC diets

Component	LChHC		HChHC		HChLC	
	g/100 g	% Energy	g/100 g	% Energy	g/100 g	% Energy
Protein (soybean)	22	23	22	23	37	34
Fat mix[a]	15.1	35	15.1	35	26	55
Corn starch/ sucrose[b]	41	42	41	42	12	11
Mineral mix[c]	8.2	–	8.2	–	8.2	–
Vitamin mix[c]	1.1	–	1.1	–	1.1	–
Cellulose	10	–	10	–	10	–
Guar gum	2.5	–	2.5	–	2.5	–
Cholesterol	0.04	–	0.25	–	0.25	–

[a] Fat mix contains olive oil–palm kernel oil–safflower oil (1:2:1.8), high in lauric and myristic acids.
[b] Corn starch–sucrose ratio (1:1.43).
[c] Mineral and vitamin mix adjusted to meet NRC requirements for guinea pigs.

diets high in carbohydrates [4]. Small LDL particles have been found to be more atherogenic than the larger ones because of a decreased binding to the LDL receptor leading to increased plasma residence time, becoming more susceptible to oxidation than large LDL particles [5,6]. Therefore a higher concentration of small LDL particles is associated with higher risk of CVD [7].

Numerous studies in humans have shown that diets rich in carbohydrates induce the formation of the smaller LDL subclass [8]. Consistent with this thesis, consuming diets low in carbohydrates increase large LDL and decrease the smaller LDL particles [9]. Finally, HDL subfractions also correlate with relative risk for CVD. Patients with type 2 diabetes and men with abdominal obesity have been reported to have higher concentration of small HDL particles, which are considered as another atherogenic feature [10–12]. Similar to LDL, HDL subfractions respond to decreases in fat and increases in carbohydrate by decreasing in size [13], whereas reductions in carbohydrate increase the larger more anti-atherogenic HDL_2 particles [14].

Because dietary interventions play a major role not only in determining plasma lipid levels, but also in promoting the formation of different atherogenic lipoprotein subfractions, this investigation was designed to compare diets varying in carbohydrate and cholesterol on lipoprotein size and distribution. There are inherent difficulties in accurately controlling food intake in human trials over long periods of time. Therefore we conducted this study in guinea pigs, which allowed us to precisely control the nutrient content of their diet. We have conducted several studies in guinea pigs and demonstrated they are an excellent animal model to study lipoprotein metabolism due to their similarities to humans in lipoprotein profile [15]. Also, guinea pigs develop hypercholesterolemia when challenged with high-cholesterol diets and lower their cholesterol with lipid-lowering drugs similar to humans [15].

The main aim of this study was to determine the influence of both dietary carbohydrate and cholesterol in the number of VLDL, LDL and HDL particles, and the distributions of lipoprotein subfractions. Another goal of this study was to evaluate the effects of carbohydrate restriction on LDL subclasses in the presence of a dietary cholesterol challenge. We hypothesized that dietary cholesterol would increase the atherogenicity of lipoprotein subclasses while carbohydrate restriction would attenuate this effect.

2. Materials and methods

2.1. Materials

Diets were prepared and pelleted by Research Diets (New Brunswick, NJ, USA). Kits to measure plasma triglycerides and cholesterol were purchased from Roche Diagnostics (Indianapolis, IN, USA). Quick-seal ultracentrifuge tubes were from Beckman (Palo Alto, CA, USA) and halothane from Halocarbon (Hackensack, NJ, USA).

2.2. Diets

Diets were designed to meet the nutritional requirements of the guinea pigs. The three diets were different in cholesterol, carbohydrate and/or fat content. The composition of the diets is shown in Table 1. Briefly, diet 1, low cholesterol/high carbohydrate (LChHC), was high in carbohydrate (42% energy) and low in cholesterol (0.04%) (LChHC). Diet 2 was high in cholesterol (0.25%) and had the same carbohydrate amount (HChHC). Diet 3 was high in cholesterol (0.25%) and low in carbohydrate (11% of total energy) and was defined as the carbohydrate-restricted (HChLC) diet. The level of cholesterol in diets 2 and 3 is known to cause hypercholesterolemia in guinea pigs. Dietary cholesterol at 0.25% in this model corresponds to an absorbed amount equal to 1.5 times the daily cholesterol synthesis rates [16] in guinea pigs and is equivalent to 1800 mg/day for a human diet. The fat mix was rich in lauric and myristic acids, known to cause endogenous hypercholesterolemia in guinea pigs [17].

Table 2
Total cholesterol, VLDL-C, LDL-C, HDL-C and TGs of guinea pigs fed LChHC, HChHC and HChLC diets for 12 weeks[1]

Parameter (mmol/L)	LChHC ($n=9$)	HChHC ($n=10$)	HChLC ($n=9$)
TC	3.5 ± 1.4^{a}	10.7 ± 3.4^{b}	14.1 ± 3.8^{c}
VLDL-C	0.8 ± 0.3^{a}	2.4 ± 2.4^{b}	3.5 ± 1.4^{b}
LDL-C	2.3 ± 1.2^{a}	7.9 ± 2.4^{b}	10.1 ± 3.3^{b}
HDL-C	0.3 ± 0.1	0.5 ± 0.3	0.6 ± 0.4
TG	0.32 ± 0.14^{a}	0.62 ± 0.27^{b}	0.83 ± 0.33^{b}

Values in the same row with different superscripts are significantly different ($P<.025$) as determined by one-way ANOVA and the least significant difference (LSD) test.
[1] Values are presented as mean±S.D. for the number of guinea pigs indicated in parentheses.

2.3. Animals

Male Hartley guinea pigs weighing between 250 and 300 g were purchased from Harlan Sprague-Dawley (Indianapolis, IN, USA). Animals, 10 guinea pigs per group, were randomly allocated to one of three treatments for 12 weeks: LChHC, HchHC or HchLC diets. Two guinea pigs were housed per cage, in a light cycle room (light from 7:00 a.m. to 7:00 p.m.), at 23°C. Diet and water were provided ad libitum. During this time, diets were weighed every 2 days to determine the amount of food consumed and guinea pigs were weighed weekly to ensure normal bodyweight gain. 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 Animal Care and Use Committee.

Fasted guinea pigs were anesthetized under isofluorane vapors, and blood was obtained via heart puncture. Plasma samples were collected and a preservation cocktail was added (aprotonin 0.5 ml/100 ml, phenyl methyl sulfonyl fluoride, 0.1 ml/100 ml and sodium azide 0.1 ml/100 ml). Plasma from each animal was stored at 4°C for analysis of plasma lipids and was used to quantify lipoprotein size and subfraction distributions.

2.4. VLDL-C determination

VLDL was isolated by sequential ultracentrifugation in an L8-M ultracentrifuge (Beckman Instruments, Fullerton, CA, USA) at a density range of 1.006–1.019 kg/L at 125,000×*g* at 15°C for 19 h in a Ti50 rotor. VLDL-C was calculated by enzymatic methods [18].

2.5. Plasma lipids

Plasma total cholesterol and HDL cholesterol were determined by enzymatic methods [18]. Plasma TG was determined by an enzymatic method, which blanks for free glycerol [19]. HDL cholesterol was analyzed after precipitation of apo B-containing lipoproteins with dextran sulfate [20] and was calculated using a modified method previously reported [21]. LDL-C was calculated by subtracting VLDL-C and HDL-C from total cholesterol.

Table 3
VLDL size and concentration of total VLDL particles and VLDL subfractions of guinea pigs fed LChHC, HChHC and HChLC diets for 12 weeks[1]

Parameter	LChHC ($n=9$)	HChHC ($n=10$)	HChLC ($n=9$)
VLDL size (nm)	44.3±10.8[a]	71.1±6.9[b]	78.9±3.33[c]
Total VLDL (nmol/L)	21.9±14.5[a]	54.3±11.1[b]	47.5±12.8[b]
Large VLDL (nmol/L)	0.40±0.55[a]	2.01±0.80[b]	2.14±0.85[b]
Medium VLDL (nmol/L)	4.92±3.80[a]	9.97±2.75[b]	6.37±2.68[b]
Small VLDL (nmol/L)	16.6±10.55[a]	42.33±10.65[b]	39.05±11.96[b]

Values in the same row with different superscripts are significantly different ($P<.01$) as determined by one-way ANOVA and the LSD test.

[1] Values are presented as mean±S.D. for the number of guinea pigs indicated in parentheses.

Table 4
LDL size and concentration of total LDL and LDL subfractions of guinea pigs fed LChHC, HChHC and HChLC diets for 12 weeks[1]

Parameter	LChHC ($n=9$)	HChHC ($n=10$)	HChLC ($n=9$)
LDL size (nm)	20.90±0.57	21.02±0.91	21.77±0.84
Total LDL (nmol/L)	288.9±101.7[a]	467.6±113.1[b]	291.3±85.0[a]
Large LDL (nmol/L)	99.7±38.4[a]	145.6±53.0[b]	136.0±45.4[ab]
Medium LDL (nmol/L)	156.2±88.1[a]	261.8±105.8[b]	98.4±90.8[a]
Small LDL (nmol/L)	28.1±13.8[a]	64.9±27.9[b]	29.3±24.9[a]
Very small LDL (nmol/L)	128.1±84.4[ab]	196.9±86.7[a]	69.0±67.7[b]

Values in the same row with different superscripts are significantly different ($P<.001$) as determined by one-way ANOVA and the LSD test.

[1] Values are presented as mean±S.D. for the number of guinea pigs indicated in parentheses.

2.6. VLDL, LDL and HDL size and subfractions

^{1}H NMR analysis was performed on a 400-MHz NMR analyzer (Bruker BioSpin, Billerica, MA, USA) as previously described [3,22]. 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 nine 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 triglycerides, total triglycerides and HDL-C) were estimated by assuming a typical lipid composition per particle and summing the concentrations in each subclass.

3. Results

No differences in weight gain or final body weights were observed among groups (data not shown). Similarly, food intake did not differ in the LChHC, HChHC or HChLC groups (data not shown).

Table 5
HDL size and concentration of total HDL and HDL subfractions of guinea pigs fed LChHC, HChHC and HChLC diets for 12 weeks[1]

Parameter	LChHC ($n=9$)	HChHC ($n=10$)	HChLC ($n=9$)
HDL size (nm)	10.56±0.96	8.87±0.58	9.38±1.13
Total HDL (nmol/L)	0.25±0.27[a]	2.09±1.01[b]	1.92±0.89[b]
Large HDL (nmol/L)	0.068±0.086	0.17±0.18	0.16±0.11
Medium HDL (nmol/L)	0.12±0.18[a]	0.88±0.77[b]	1.11±0.62[b]
Small HDL (nmol/L)	0.078±0.11[a]	1.04±0.51[b]	0.68±0.71[b]

Values in the same row with different superscripts are significantly different ($P<.001$) as determined by one-way ANOVA and the LSD test.

[1] Values are presented as mean±S.D. for the number of guinea pigs indicated in parentheses.

3.1. Plasma lipids

The main diet effects observed during the study were in total cholesterol, VLDL-C, LDL-C and TG (Table 2). Total cholesterol concentrations were highest in the HChLC group and statistically different from the other two groups (HChLC>HChHC>LChHC) ($P<.01$). Plasma VLDL-C concentrations were not different between HChHC and HChLC groups (92.0±94.0 and 133.6±52.9 mg/dl, respectively); however, they were both higher than in the LChHC group (33.4±12.9 mg/dl) ($P<.01$). Similarly, plasma LDL-C concentrations were not significantly different between HChHC and HChLC groups (305.3±91.1 and 390.2±127.3 mg/dl, respectively); however, they were higher ($P<.0001$) than in the LChHC group (88.9±45.9 mg/dl). Surprisingly, there were no differences in plasma TG between the HChHC and HChLC groups (54.1±24.3 and 73.0±29.4 mg/dl, respectively). Plasma TG was lower in the LChHC group (28.2±12.4 mg/dl) ($P<.01$) than in the high-cholesterol groups.

3.2. VLDL particles

The results of VLDL subclasses and size are presented in Table 3. The VLDL particle size was larger and significantly different in the HChLC group compared to that in the HChHC and LChHC groups ($P<.05$). The concentration of large VLDL particles was five times higher in guinea pigs fed the high-cholesterol diet than in the low-cholesterol group. Similarly, medium and small VLDL particle concentrations were higher in the HChHC and HChLC groups (high dietary cholesterol groups) than in the LChHC group ($P<.001$) (Table 3).

3.3. LDL particles

Carbohydrate restriction significantly affected the distribution of LDL subclasses in guinea pigs fed high cholesterol (Table 4). The total number of LDL particles was reduced by 48% in guinea pigs fed the HChLC diet compared to the HChHC diet. In spite of the huge differences in plasma LDL-C between the HChLC and the LChHC groups, guinea pigs from these two dietary groups had similar number of LDL particles, indicating that the HChLC group accommodated the extra plasma cholesterol in less LDL particles (Table 4). The lower number of total LDL particles in the HChLC group when compared to the HChHC group was associated with a reduction in number of medium, small and very small LDL subfractions. The HChHC group had 2.7-, 2.1- and 2.4-fold more medium, small and very small LDL than the HChLC group ($P<.001$) (Table 4).

3.4. HDL particles

Guinea pigs have low levels of plasma HDL-C; however, the number of HDL particles was affected by dietary treatments. The main changes in HDL particles were observed in the HChHC and HChLC groups, where total, medium and small HDL particle concentrations were higher and significantly different in these groups compared to those in the LChHC group (Table 5).

4. Discussion

In the present study, we have shown that high dietary cholesterol influences the size and subfraction distribution of plasma lipoproteins, and this effect is further modified by the carbohydrate content of the diet. Dietary cholesterol caused an increase in total and LDL-C, in the size and number of large and small VLDL particles, and in the number of medium and small HDL subclasses. In contrast, carbohydrate restriction in the presence of a high cholesterol challenge caused changes mostly in LDL particles and subfractions by decreasing the number of total LDL particles, mainly medium, small and very small LDL, and by increasing the number of large LDL particles. These results suggest that guinea pigs may also be a good model to study the effects of diets on LDL lipoprotein size and atherogenicity.

4.1. Plasma lipids

It is well known that guinea pigs respond to dietary cholesterol in a dose-dependent manner, increasing the plasma cholesterol associated with the LDL fraction [2,15]. In the present study, these results were observed. Dietary cholesterol produced changes mainly in total cholesterol, which were reflected by increases in LDL-C in the groups with high levels of cholesterol in the diet (HChHC and HChLC groups).

Human studies have consistently shown that low-carbohydrate diets significantly reduce fasting TG [1,23], presumably due to a decrease in VLDL production rate which contributes as well to the reduced postprandial lipidemia because a greater VLDL–TG pool size might compete with TG from intestinal origin for removal during the postprandial period [24]. In the current work, plasma VLDL-C and TG were higher in those groups consuming high cholesterol, and this was independent of the carbohydrate level, suggesting that VLDL-C and TG synthesis were up-regulated by dietary cholesterol rather than by the carbohydrate content. We hypothesized that reducing the carbohydrate content of a high cholesterol diet would lower TG. Failure to observe a hypotriglyceridemic response may have been due to the active growing phase of guinea pigs (body weight increased more than twofold) or the markedly high cholesterol content equivalent to about 1800 mg/day compared to carbohydrate-restricted diet studies in humans that involved cholesterol in the range of 500–750 mg/day [25,26]. In agreement with our data, the effect of very low fat–high carbohydrate and high fat (low carbohydrate) diets have shown no changes in plasma TG in healthy children during a 10-day study [27], which may explain that during the growing stage these diets have no effect on plasma TG.

4.2. VLDL and HDL particles

A predominance of smaller HDL particles [11] and large VLDL subfractions has been associated with an increased risk for coronary heart disease (CHD) independently of prevailing plasma lipid concentrations. Studies in humans have shown that carbohydrate-restricted diets affect all VLDL subfractions, reducing the concentrations of large, medium and small VLDL [28]. In the present study, HChLC did not decrease the number of VLDL particles, probably due to the high concentration of cholesterol in the diet, which masked the effects associated with carbohydrate restriction. High levels of cholesterol in the diet (HChHC and HChLC groups) caused an increase in the more atherogenic VLDL particle (the largest subfraction) compared with the LChHC group. Consistent with human studies, those guinea pigs with higher concentration of plasma TG also had higher concentrations of large VLDL particles. Some reports have mentioned that high hepatic TG leads to the synthesis of large VLDL particles, and this is reflected by higher plasma TG and therefore higher levels of large VLDL particles [24]. Abnormalities in VLDL particle size have been considered a major contributing factor of dysfunctional lipoprotein metabolism [29]. Large VLDL particles have been considered more atherogenic than the smaller subclasses. Studies have shown that elevation of TG-rich particles in the postprandial phase is mainly in the large VLDL subclass, and a greater postprandial elevation of chemically measured large VLDL is found in CVD patients than in control subjects [30]. Moreover, retinopathy and coronary artery calcification were associated with higher VLDL particle levels in diabetic patients. The diets tested in this work, which were high in cholesterol (HChHC and HChLC groups), also caused a higher increase in the concentration of the less atherogenic VLDL particle (smaller subfractions). However, some reports consider small VLDL particles more atherogenic since regression of atherosclerosis was more strongly related to reduction in small VLDL particles than in larger particles in an intervention study [31]. Although circulating large VLDL is considered atherogenic, its size makes penetration of the vascular wall unlikely.

HDL particles are also heterogeneous [10], and studies have found that smaller HDL particles may be atherogenic due to higher concentration in patients with diabetes type 2 [12,32] and in subjects with abdominal obesity [2]. In nondiabetic subjects, higher average HDL size has been associated with coronary artery calcification in that the prevalence of large HDL particles lowers the odds for coronary artery calcification in these subjects [2]. These results suggest that large HDL particles are more antiatherogenic than the smaller HDL particles. In addition, the larger HDL (cholesterol-rich) subclass has been inversely related with degree of stenosis at angiography, whereas the smaller HDL subclasses have been positively associated with stenosis [33]. In another study, smaller HDL were found in CHD cases than in control subjects [34]. Another lipid metabolism abnormality is related with hepatic lipase (HL) activity and HDL-C concentration. High HL activity has been considered as proatherogenic due to its positive correlation with plasma TG and inverse relationship to plasma HDL-C concentrations [35]. Some reports have shown that low HL activity leads to high plasma concentrations of the larger HDL particle subclass and a relative decrease in the concentration of the smaller HDL particle subclass [36]. Therefore smaller HDL particles have been considered atherogenic. In the current work, the guinea pigs that consumed the high cholesterol diets (HChHC and HChLC) presented high concentrations of smaller HDL particles, and also these groups had higher concentrations of plasma TG, which confirm the direct relationship between these two factors. However, the LChHC group, which consumed the high carbohydrate–low cholesterol diet, had the lowest HDL particle concentration. It has been mentioned that elevated TG leads to the generation of TG-rich HDL particles, which are more susceptible to modification by HL [37]. This modification leads to the formation of smaller HDL particles, which have a reduced plasma residence time, decreasing the reverse cholesterol transport [38,39].

4.3. LDL particles

Studies in humans indicate that a predominance of smaller LDL particles is associated with increased CHD [40]. In the last years, investigations about the incidence of CVDs have been related with LDL particle size [41,42]. Small LDL particles have been shown to be more atherogenic because of a decreased binding to the LDL receptor leading to increased plasma residence time [5] becoming more susceptible to oxidation than large LDL particles [6,43]. In the present work, diets with high cholesterol level (HChHC and HChLC groups) showed higher concentration of less atherogenic large LDL particle than diet with low cholesterol level (LChHC group). In humans, a predominance of smaller LDL particles signifies a higher risk for coronary artery disease than that associated with larger LDL particles [9]. Elevated TG are associated with a predominance of smaller, dense LDL particles that are more susceptible to oxidation and are more closely associated with atherosclerosis than are larger, buoyant LDL particles [7,44,45]. In type 2 diabetic patients using other methods for measuring LDL size, diabetic women were found to have smaller LDL size than diabetic men compared with the general population, and LDL size predicted CVD event rates in diabetic women, although not independently of other factors [46]. An interesting finding in the present work was that both groups of guinea pigs consuming the high cholesterol diets had high concentrations of LDL-C; however, the diets containing less carbohydrate and more protein and fat (HChLC group) resulted in higher concentration of the large LDL subclass and low concentrations of medium, small and very small LDL particles. These results suggest that the cholesterol in the HChLC group is

transported mainly in the less atherogenic particles, which is considered as a desirable effect. In addition, the distribution of cholesterol across LDL subclasses (more in large LDL and less in the smaller LDL subfractions) was similar between the low cholesterol group and the high cholesterol–low carbohydrate group, suggesting that carbohydrate restriction accompanied by increases in dietary fat and protein favors the distribution of less atherogenic LDL particles comparable to those seen in the absence of a dietary cholesterol challenge.

From these studies, we conclude that evaluating the physiology of different lipoprotein subclasses induced by dietary treatments provides additional information regarding diet and risk for CHD. For example, the potential of high dietary cholesterol to develop atherosclerosis can be visualized by the increases in the atherogenic lipoproteins (large VLDL and small HDL), whereas the protective effect of carbohydrate restriction (and increased dietary fat and protein) in the presence of a cholesterol challenge can be seen in the increased formation of large LDL and the reduction in the smaller LDL subfractions.

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