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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.