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High-carbohydrate Diets Affect the Size and Composition of Plasma Lipoproteins in Hamsters (*Mesocricetus auratus*)

Limin Wang,[1,†,*] Jun Yu,[2] and Rosemary L Walzem[1,2,*]

High-carbohydrate diets reduce plasma low-density lipoprotein (LDL)–cholesterol but also provoke the appearance of an atherogenic lipoprotein profile (ALP). Characterized by high plasma triglyceride, small dense LDL, and reduced high-density lipoprotein (HDL) cholesterol, an ALP is associated with insulin resistance. Despite extensive use of the fructose-fed hamster as a model of insulin resistance, little is known about changes that occur in the physical properties of circulating lipoproteins. Therefore, we investigated the metabolic and physical properties of lipoproteins in hamsters fed high-carbohydrate diets of varying complexity (60% carbohydrate as chow, cornstarch, or fructose) for 2 wk. Hamsters fed the high-fructose diet showed significantly increased very- low-density lipoprotein (VLDL)–triglyceride (92.3%), free cholesterol (68.6%), and phospholipid (95%), whereas apolipoprotein B levels remained unchanged. Median diameter of circulating VLDL was larger in fructose-fed hamsters (63 nm) than in cornstarch-fed hamsters. Fructose feeding induced a 42.5% increase LDL–triglyceride concurrent with a 20% reduction in LDL–cholesteryl ester. Compositional changes were associated with reduced LDL diameter. In contrast, fructose feeding caused elevations in all HDL fractions. The physical properties of apolipoprotein-B-containing lipoprotein fractions are similar between fructose-fed hamsters and humans with ALP. However, metabolism of high-density lipoprotein appears to differ in the 2 species.

Abbreviations: ALP, atherogenic lipoprotein profile; ApoB, apolipoprotein B; C, cholesterol; CE, esterified cholesterol; Fch, free cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PL, phospholipid; SREBP, sterol regulatory element binding protein; TG, triglyceride; VLDL, very-low–density lipoprotein

An effect of diet on lipoprotein metabolism and atherosclerosis is well documented.[4,13,34,35] Dietary macronutrient composition can influence lipoprotein synthesis, metabolism clearance, and arterial retention. Targeting specific dietary habits can lead to reductions in morbidity and mortality associated with atherosclerosis. Low-fat diets often are advocated to reduce atherosclerotic risk. However, low-fat diets have increased carbohydrates, which are used to replace fat calories in the diet. However, it is increasingly recognized that high–carbohydrate diets are accompanied frequently by hypertriglyceridemia, which is associated with insulin resistance and the appearance of atherogenic lipoprotein profile (ALP). The ALP is characterized high plasma concentrations of triacyglyceride (TG), low concentration of high-density lipoproteins (HDL), and small, dense low-density lipoprotein (LDL).[2] The pathogenic ALP is a major contributing factor for the pathogenesis of atherosclerosis and coronary artery disease in obesity and type 2 diabetes.

Consistent with observations in humans,[56] diets high in simple monosaccharides increase hepatic lipid synthesis and changes in whole body lipid metabolism in animals. Notably, feeding high-fructose diets to various animal species is associated with several metabolic and cardiovascular adverse effects: a high-fructose diet for several weeks leads to the development of the features of the metabolic syndrome, such as dylipidemia[45,58] and insulin resistance.[58,69] Several recent reviews addressed the metabolic effects of fructose in animal models and unravel the mechanism by which high-fructose diets affect intestinal lipoprotein metabolism and ALP.[5,14,44,62] Evidence from rat studies demonstrated that diets high in fructose elevate plasma TG concentration and lower HDL concentration.[28,60] The hamster has proven to be a useful animal model to study effects of the diet on lipoprotein and lipid metabolism.[11] The plasma lipoprotein profile and cholesterol ester transfer protein activity of the hamster resemble those of humans more closely than do those of the rat or mouse.[3,55] The hamster also exhibits a metabolic response to dietary lipids that is similar to the response exhibited by humans.[55]

The effects of a high-fructose diet on lipid metabolism have been investigated in the hamster. Several investigators have noted that diets containing 60% fructose elevate plasma TG concentration in hamsters and cause insulin resistance.[31,46,58] Distinct from humans and rats, however, hamsters fed a high-fructose diet exhibit increased HDL–cholesterol (C) concentration in plasma.[37,46] Elevated plasma HDL-C has been associated with a reduction in coronary events that is independent of changes in LDL-C and TG.[39] Whether hamsters fed high-fructose diet exhibit changes in lipoprotein particle diameter and chemical composition indicative of ALP is unknown. An objective of this study was to establish the features of the plasma lipoprotein profile in hamsters fed a diet rich in the monosaccharide fructose and compare them

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[1]Intercollegiate Faculty of Nutrition, [2]Poultry Science Department, Texas A & M University, College Station, TX
[†]Current address: Department of Internal Medicine, University of California, Davis, CA
[*]Corresponding authors. Emails: rwalzem@poultry.tamu.edu; lwang@ucdavis.edu

with those of hamsters fed diets containing a similarly high carbohydrate content as more complex carbohydrate in the form of either mixed whole feeds or cornstarch.

Previous studies of plasma lipoprotein profiles[46,58] compared the effects of a control rodent chow diet with those of a purified fructose-rich diet. Chow is a nonpurified diet composed of a mixture of intact feed. In contrast, purified diets provide macronutrients as purified ingredients. For example, carbohydrate in chow diets is derived from complex mixtures of corn and wheat flakes, wheat middlings, ground corn, and dried whey. In addition to carbohydrate, these ingredients provide variable amounts of protein, fat, vitamins, minerals, and various phytochemicals and other nutrients. However, carbohydrate in a fructose-rich diet only consists of a single simple monosaccharide-fructose in combination with purified sources of protein, fat, and micronutrients. Therefore, the carbohydrate composition of any chow diet is quantifiably but variably different from that of purified diet. Starches can be highly purified, but because they are polysaccharides, they retain unique chemical and physical characteristics. Starch can be a straight or branched chain polymer comprising thousands of glucose molecules. Starch polymers must be digested to liberate individual glucose molecules for transport into the bloodstream. Therefore, glucose from starch is absorbed more slowly than is glucose that is consumed as mono- or disaccharide, such that starch from glucose produces a lower postprandial rise in insulin and glucose.[61] In the present study, a starch-rich diet was evaluated as a more defined complex carbohydrate diet for comparison with the fructose-rich diet. Therefore, starch was substituted isocalorically for fructose. A standard chow diet also was included in this study for comparison of current results with those of previous studies. To determine the effect of fructose and starch on the size, lipid, and apolipoprotein composition of lipoproteins, hamsters were fed purified diets containing 60% fructose or cornstarch.

Materials and Methods

Animals and diets. Six-week-old male golden Syrian hamsters (Sasco strain, Charles River, Wilmington, MA) were housed in pairs at 22 °C with a 12:12-h light:dark cycle and had free access to water and chow diet over a 2-wk adaptation period. Hamsters then were randomly assigned to 1 of 3 groups of 15 hamsters each. Hamsters were housed at 2/cage except for 1 hamster housed individually in each group (mean group body weight, 110 g). Hamsters consumed 1 of 2 nutritionally adequate purified diets or continued on chow (Harlan Teklad, Madison, WI). All diets provided 60% carbohydrate by weight. Chow carbohydrates were provided by mixed whole-feed sources.[25] Purified diets contained either 60% fructose (Dyets, Bethlehem, PA) or 60% cornstarch (Dyets, Bethlehem, PA) in combination with 22% casein, 6% corn oil, 7.1% cellulose, 1% vitamin mix, and 3.5% mineral mix (Dyets) . The animals had free access to the experimental diets for 2 wk prior to study. Feed intakes were measured daily. Body weights were measured twice weekly. Food was removed 16 h prior to study. The hamsters were anesthetized (4% isoflurane in 100% oxygen), and blood was collected individually by cardiocentesis by using EDTA-treated syringes and needles. Plasma was separated within 2 h of blood collection by centrifugation at 1200 × *g* for 20 min at 4 °C. All procedures involving animals had been approved by Texas A and M University Laboratory Animal Care and Use Committee.

Lipoprotein isolation. Plasma lipoproteins were subfractionated according to their hydrated density by sequential density-gradient ultracentrifugation.[6,66] Very low-density lipoprotein (VLDL; density of gradient, less than 1.006 g/ml) was isolated from 1 ml plasma by aspiration with a narrow-bore pipet after 18 h centrifugation at 14 °C and 100,000 × *g* (TFT 50.3 rotor and L8-70M ultracentrifuge, Beckman Coulter, Sunnyvale, CA). The density of the gradient was increased to 1.053 g/ml, and LDL were isolated by centrifugation for 18 h at 100,000 × *g*. HDL were isolated at a gradient density of 1.053 to 1.21 g/ml and centrifugation for 18 h at 100,000 × *g*.[6] The diameters of the particles in each lipoprotein fraction were determined immediately after their isolation from plasma. After diameter analysis, the fractions were stored at –70 °C prior to compositional analysis.

Metabolic and lipid analyses. Plasma glucose concentrations were determined by using spectrophotometry and a kit (Wako, Richmond, VA). Plasma insulin concentration was measured by using a rat insulin ELISA kit (Crystal Chem, Chicago, IL) that has 79% crossreactivity to hamster insulin. Protein concentration was measured by a modified Lowry method[38] by using bovine serum albumin as the standard.

Plasma cholesterol distribution was determined by analytical size-exclusion chromatography.[32] Typically, 20 µl of fresh plasma was injected onto a size-exclusion column (Superose 6 HR 10/30, Amersham Pharmacia Biotech, Piscataway, NJ) by using an automated sample injector (ASI-100, Dionex, Sunnyvale, CA) and eluted with saline containing 0.02% sodium azide at a flow rate of 0.5 ml/min (P580 pump, Dionex). Plasma cholesterol distribution was determined by using an inline photodiode array detector (PDA-100, Dionex) with primary chromophore detection at 505 nm. Triacylglycerol concentration was determined enzymatically by using a kit(Sigma Chemical, St Louis, MO). Total cholesterol, free cholesterol (Fch), nonesterified fatty acids (NEFA), and phospholipids (PL) were measured by use of enzymatic kits (Wako). Esterified cholesterol (CE) was calculated by multiplying the difference between total cholesterol and Fch by 1.67.[6]

Analysis of lipoprotein fractions particle size. Diameters of VLDL particles were measured by dynamic laser light-scattering analysis by using a ultrafine particle analyzer with a laser probe tip (UPA-250; Microtrac, Clearwater, FL) and appropriate software (Microtrac, Honeywell, Washington, PA).[26,64] After ultracentrifugation, sample tubes were removed from the rotor and uncapped. The laser probe was placed gently on the top layer of supernatant portion so as to prevent air bubbles at the probe–liquid interface. Because of the low lipoprotein content of some samples, a measurement time of 540 s was used for all samples. The results of primary data collection can be expressed as a distribution of particle number, particle area, or particle volume;[7] particle area distribution was used throughout these studies. Raw particle diameter distributions were converted to population percentiles to facilitate statistical comparisons.

Sizes of LDL and HDL particles were measured by using nondenaturing gradient gel electrophoresis.[41] Typically, a 15-µl portion of fresh LDL or HDL was applied onto a nondenaturing gradient gel (Alamo Gels, San Antonio, TX). Gels with a linear gradient range of 2% to 16% polyacrylamide were used to measure the diameter of LDL particles, and gels with a linear gradient range of 4% to 30% polyacrylamide were used to measure the diameter of HDL particles. A high-molecular–weight standard (Amersham) also applied to each gel. Electrophoresis was performed by using

a vertical electrophoresis apparatus (GE-2/4 LS, Pharmacia) at a constant voltage of 125 V for 24 h at 4 °C. Gels were stained with 0.15% Coomassie brilliant blue in ethanol:water:glacial acetic acid (3:8:1, v/v/v) for 15 min at 60 °C, and then destained at 60 °C in a destaining solution of ethanol:water:glacial acetic acid (3:8:1, v/v/v) until backgrounds were clear. Migration of lipoproteins in gel was measured by using an image analysis system and accompanying software (Odyssey, Li-Cor, Lincoln, NE). Calibration curves for LDL proteins were constructed by plotting the logarithm of particle diameter against the distance migrated relative to that of the migration distance of the front moving boundary (Rf) (abscissa). Particle sizes were calibrated by comparison with migration distances of standard proteins of known diameter.[52]

Quantitation of apolipoprotein B. The concentration of apolipoprotein B (ApoB, ApoB100) in VLDL was determined by analytical SDS-PAGE with Coomassie blue staining. [33] Typically, sample volumes containing 50 to 200 µg protein were combined with 60 µg apotransferritin carrier protein prior to delipidation to ensure quantitative apolipoprotein recovery. [33] Samples were delipidated overnight at –20 °C in an ethanol–diethyl ether (3:1, v/v) system in which a 400- to 1200-µl sample was mixed with 10 volumes of ice-cold ethanol–diethyl ether. The delipidated mixture was centrifuged for 25 min at 600 × *g* to remove the organic phase. The pellet was extracted twice with 4 ml cold anhydrous diethyl ether. The resultant whitish pellet was dissolved in 50 to 100 µl sample buffer containing 10% glycerol, 3% SDS, 1.5% dithiothreitol, 1% mercaptoacetic acid, and 0.02% bromophenol blue in 1 M Tris-HCl (pH 6.8). Excess ether was removed under nitrogen, and the dissolved apolipoproteins were denatured at 100 °C for 3 min prior to application to the SDS–PAGE gel.

Individual ApoB isoforms were separated by using a previously described SDS-PAGE gradient gel[33] with some modifications. The 3% to 12% gradient polyacrylamide gel was cast by using a 2-chamber gradient mixer (Bio-Rad Laboratories, Hercules, CA). Apolipoprotein separation was achieved by using a vertical gel apparatus (Mini-Protein II, Bio-Rad).[36] After loading of 50 µl delipidated VLDL or standard in each well, the gels were run at 96 V until the dye front was 1 mm from the bottom of the gel. Narrow-cut human LDL served as the source for the ApoB100 used as the reference protein for the standard curves.

The procedures for staining and destaining of SDS-PAGE gels were similar to those for nondenaturing gradient gel electrophoresis gels. After destaining, gels were placed between 2 sheets of cellophane gel wrap (BioDesign of New York, Carmel, NY) and dried in a fume hood overnight. The dry gels were scanned by using a laser scanner (Fluor-STM MultiImage, Bio-Rad) equipped with ImageQuant software (Bio-Rad) to automatically integrate the volume of stained apolipoprotein band. The standard curve for ApoB100 was constructed by relating the intensity of dye uptake of each band to its known mass. The line of fit was evaluated[33] at least-squares analysis for the power function $y = ax^b$ ($r^2 = 0.9852$, n = 44). ApoB concentrations in samples were calculated from the regression equation.

Statistical analysis The results are presented as mean ± SE unless otherwise noted. Comparisons among 3 groups were performed by using 1-way analysis of variance. Difference in mean values was tested by a least-squares means procedure. *P* values less than 0.05 were considered statistically significant. All analyses were performed by using SAS software (SAS Institute, Cary, NC).

Results

Physiological and metabolic changes. Final body weight, body weight gain, and food intake after 2 wk of consuming the diets were similar among groups, but the livers of the hamsters fed fructose were about 10% heavier ($P < 0.05$) than those of animals fed starch or chow (Table 1). Plasma glucose was not significantly different among the 3 dietary groups, whereas fasting insulin was nearly 2-fold higher in the fructose-fed group than in the starch and chow groups ($P < 0.05$). Plasma TG concentration in fructose-fed hamsters was 59% higher ($P < 0.001$) than that of chow-fed animals and 72.2% higher than that of starch-fed hamsters ($P < 0.001$). Similarly, fructose-fed animals had significantly higher fasting plasma NEFA concentrations than did either starch-fed animals (32%) or chow-fed animals (14%).

Plasma total and lipoprotein cholesterol. Plasma lipoprotein cholesterol profiles were obtained by size-exclusion HPLC. Plasma cholesterol distribution in lipoproteins in hamsters fed chow, fructose, or starch diets for 2 wk are shown in Figure 1. Fructose-fed hamsters had higher concentrations of total plasma cholesterol ($P < 0.05$) and VLDL-C ($P < 0.01$) than did chow-fed and starch-fed hamsters. HDL-C was significantly higher in fructose-fed than chow-fed hamsters ($P < 0.05$), whereas that of starch-fed hamsters was not different from that of either chow- or fructose-fed animals. When measured in ultracentrifugally separated fractions, HDL free and esterified cholesterol concentrations showed step-wise reductions from fructose-fed to starch-fed to chow-fed animals (Table 6). Plasma LDL-C concentrations were similar in all dietary groups (Figure 1). The area under the curve was calculated for individual lipoprotein classes (VLDL, LDL, HDL) and divided by total area under the curve to indicate fractional plasma cholesterol distribution. Animals fed either purified carbohydrate diet had consistently and significantly ($P < 0.05$) higher fractional VLDL-C, with values of 6.2% in chow-fed hamsters, 8.7% in starch-fed hamsters, and 9.3% in fructose-fed hamsters. The LDL-C fraction accounted for 21.9% of total cholesterol in the chow-fed group. In contrast, LDL-C accounted for significantly ($P < 0.05$) lower proportions of total cholesterol in the fructose-fed (18.6%) and starch-fed (18.7%) groups. Averaging 72%, the percentage of HDL-C did not differ among the 3 groups. The retention time of VLDL particles from hamsters fed the fructose diet was shorter than those of animals fed either the starch or chow diet ($P < 0.05$; Table 2). Reduced elution times indicate a larger particle diameter, and LDL particles from the fructose-fed group eluted more slowly than did those from either chow-fed or starch-fed animals ($P < 0.05$). HDL eluted more quickly ($P < 0.05$) in animals fed either purified carbohydrate when compared with those fed chow.

Size distributions of lipoprotein fractions. The changes in VLDL particle diameter suggested by size-exclusion HPLC were confirmed by dynamic laser light particle sizing. The raw density function plot for VLDL particle area distribution is given in Figure 2. Particle diameter of VLDL from plasma showed marked heterogeneity among the 3 groups of hamsters. Diameter distributions were asymmetric and tailed toward small-diameter particles. The median particle diameter of plasma VLDL particles from fructose-fed hamsters was significantly ($P < 0.05$) larger than those of chow- or starch-fed hamsters. Although the largest and smallest VLDL particles were similar in diameter regardless of dietary carbohydrate source, the bulk of VLDL particles (that is, those at the 20th through 60th population percentiles) were significantly ($P < 0.05$) larger in fructose-fed hamsters.

Table 1. Plasma physiologic and metabolic changes in hamsters fed chow, fructose-enriched, and starch-enriched diets for 2 wk

	Chow	Fructose-enriched	Starch-enriched
Body weight (g)	122.9 ± 4.5	126.2 ± 3.2	124.0 ± 2.4
Weight gain (g)	9.44 ± 1.59	11.06 ± 1.16	9.46 ± 0.90
Food intake (g)	9.77 ± 0.46	9.47 ± 0.34	9.92 ± 0.21
Liver weight (g)	21.50 ± 1.50^b	25.03 ± 0.82^a	22.42 ± 0.54^b
Glucose (mmol/l)	7.71 ± 0.41	6.98 ± 0.26	7.25 ± 0.30
Insulin (pmol/l)	110.2 ± 13.8^b	189.3 ± 37.9^a	117.0 ± 36.1^b
Triacylglycerol (mmol/l)	0.90 ± 0.06^b	1.43 ± 0.09^a	0.83 ± 0.04^b
Free fatty acids (mmol/l)	0.58 ± 0.04^b	0.66 ± 0.09^a	0.50 ± 0.06^b
Cholesterol (mmol/l)	3.13 ± 0.14^c	4.22 ± 0.15^a	3.59 ± 0.17^b

Values are presented as mean ± SE (n = 15 per treatment group). Analysis of variance was used to compare the 3 feeding groups. For each row, means with different superscripts are significantly different ($P < 0.05$).

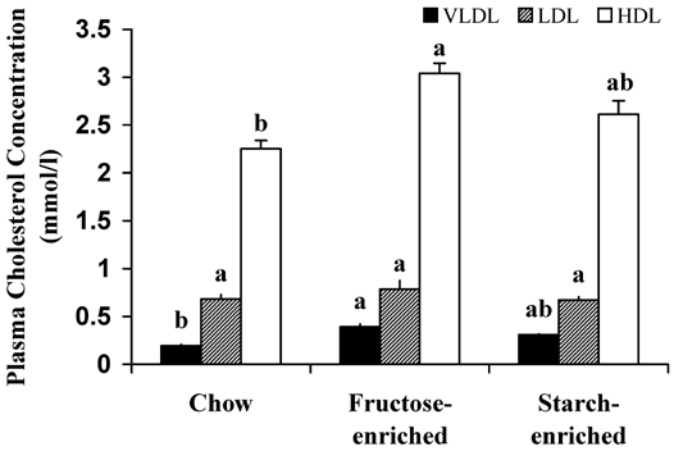

Figure 1. Distribution of cholesterol in plasma lipoproteins from hamsters fed chow, fructose-enriched, and starch-enriched diets for 2 wk. Values are presented as mean ± SE of 15 hamsters per treatment group. Values indicated with different superscript letters differ significantly ($P < 0.05$).

The effect of different dietary carbohydrate sources on the diameters of LDL and HDL particles was analyzed by using nondenaturing gradient gel electrophoresis. All of the lipoproteins bands exhibited a polydisperse diameter profile, indicating particle diameter heterogeneity (Figure 3). A gradient gel with 2% to 16% polyacrylamide was used to evaluate the size of LDL particles. LDL particles from fructose-fed hamsters were significantly smaller than those in either the starch-fed or chow-fed group (Table 3). Electrophoresis in 4% to 30% gradient gels showed that HDL diameters in hamsters fed the high-fructose diet were slightly larger as than those of hamsters fed either high-starch diet ($P = 0.07$) or chow diet ($P < 0.05$). There was a wide range in HDL particle diameter and overlap in diameter distributions from hamsters fed the different diets. Within HDL gels, a large band averaging 21 nm was noted for a number of animals. The appearance of this LDL-size band was 3 times and 4 times more frequent in fructose-fed hamsters than in chow-fed and starch-fed

Table 2. Retention time (min) of plasma lipoprotein particles from hamsters fed chow, fructose-enriched, and starch-enriched diets for 2 wk

	VLDL	LDL	HDL
Chow	19.66 ± 0.01^b	25.63 ± 0.04^b	33.89 ± 0.06^a
Fructose-enriched	19.53 ± 0.02^a	25.90 ± 0.05^a	33.36 ± 0.07^c
Starch-enriched	19.61 ± 0.02^b	25.55 ± 0.15^b	33.54 ± 0.03^b

Values are presented as mean ± SE (n = 15 per treatment group). Analysis of variance was used to compare the data from the 3 feeding groups. Within each column, means having different superscripts are significantly different ($P < 0.05$).

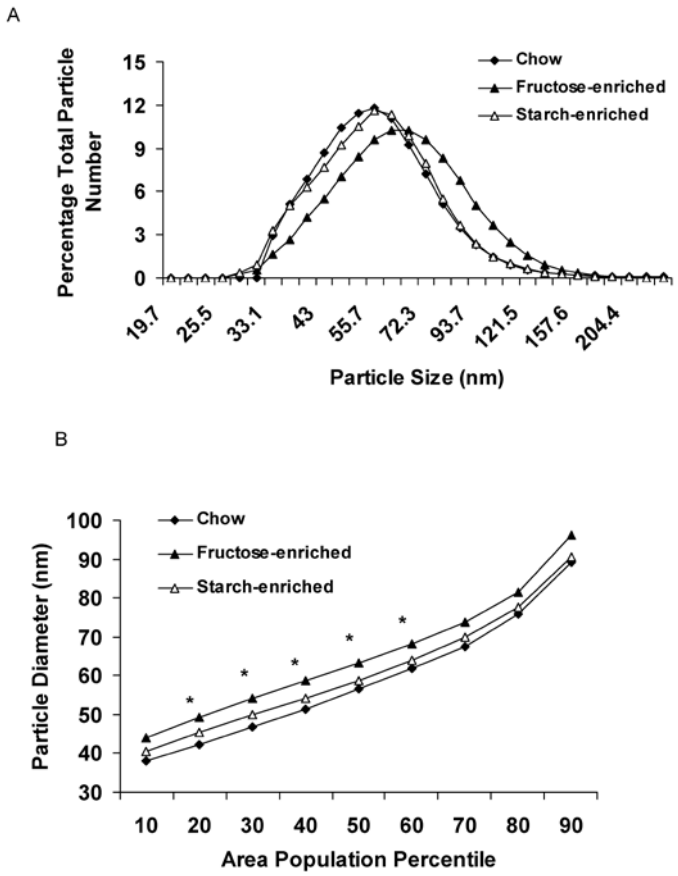

Figure 2. Dynamic light scattering-determined diameter distribution of very-low–density lipoprotein (VLDL) isolated from hamsters fed a chow, fructose-enriched, or starch-enriched diet for 2 wk. (A) Diameter distribution, (B) percentile distribution of plasma VLDL particle density function (area). Within a percentile class and density function, values indicated with * are significantly different from each other.

hamsters, respectively. The protein composition of this particle was not determined.

Concentration and chemical composition of plasma lipoprotein fractions. Both the concentration and chemical composition of lipoprotein VLDL fractions varied among the groups of hamsters (Table 4). In general, fructose-fed hamsters had the highest concentrations of all VLDL components, with progressive decreases in those values seen in starch-fed hamsters and the chow-fed hamsters. Differences in the amount of each chemical component between fructose-fed and chow-fed hamsters were significant

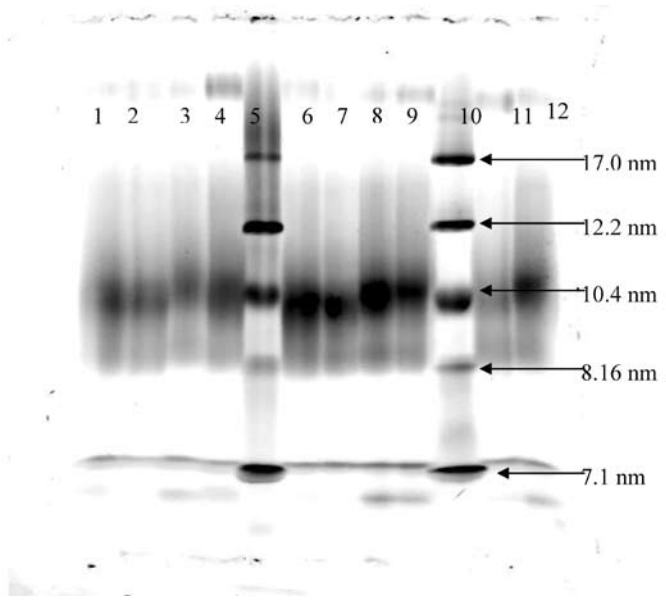

Figure 3. 4% to 30% gradient nondenaturing polyacrylamide gel electrophoresis of hamster HDL (1.053 < d < 1.21 g/ml) particles. Lanes 1, 2, 6, and 7: HDL from starch-fed hamsters; Lanes 3, 4, 8, 9, and 12: HDL from fructose-fed hamsters; Lane 11, HDL from chow-fed hamsters; Lanes 5 and 10, molecular weight standard.

Table 3. Plasma LDL and HDL particle diameters from hamsters fed chow, fructose-enriched, and starch-enriched diets for 2 wk

	LDL (nm)	HDL (nm)	Frequency of LDL band on HDL gels
Chow	23.76 ± 0.07^{b}	10.31 ± 0.08^{a}	$4/15^{b}$
Fructose-enriched	23.61 ± 0.05^{c}	10.53 ± 0.04^{b}	$12/15^{a}$
Starch-enriched	24.40 ± 0.08^{a}	10.45 ± 0.05^{b}	$3/15^{b}$

Values are presented as mean ± SE (n = 15 per treatment group). Data were compared by complete weighted least-square means analysis. Within each column, values with different superscripts are significantly different ($P < 0.05$).

($P < 0.001$). The concentrations of all components except VLDL protein were significantly higher ($P < 0.01$) in fructose-fed hamsters than those in starch-fed hamsters. ApoB100 in VLDL was quantified using a 3% to 12% gradient SDS-PAGE (Figure 4). The amount of ApoB100 in VLDL from fructose-fed hamsters was higher than that in VLDL from either starch-fed (20.6% higher, $P = 0.04$) or chow-fed (68.9% higher, $P = 0.02$). Surprisingly, there was no significant difference in the amount of apolipoprotein in LDL among the 3 dietary groups. There was similar amount of total ApoB in ApoB-LP in the plasma from fructose-fed and starch-fed animals, which were significantly higher than that from chow-fed animals (Figure 4).

The fractional contribution of TG in the fructose-fed group, was significantly higher than that of starch-fed group. VLDL from the fructose-fed group was poorer in CE ($P < 0.05$) than that of the starch-fed group. Starch feeding selectively increased the fractional concentration of VLDL cholesterol (Fch + CE), while not stimulating that of TG. Both purified carbohydrates elevated VLDL fractional content of cholesterol. VLDL from fructose-fed

Table 4. Concentration and chemical composition (%) of lipoproteins from the <1.006 g/ml fraction (VLDL) from the plasma of hamsters fed chow, fructose-enriched, and starch-enriched diets

	Concentration (μg/ml)		
	Chow	Fructose-enriched	Starch-enriched
TG[2]	600.0 ± 24.19^{b}	1084.8 ± 64.8^{a}	596.8 ± 22.8^{b}
CE	55.6 ± 4.0^{c}	104.5 ± 8.6^{a}	84.5 ± 3.8^{b}
Fch	30.3 ± 2.4^{c}	74.2 ± 8.1^{a}	44.0 ± 3.2^{b}
PL	97.5 ± 11.2^{b}	181.7 ± 21.9^{a}	103.2 ± 8.1^{b}
Apo	67.8 ± 4.1^{b}	91.2 ± 6.2^{a}	83.4 ± 4.9^{a}
	Composition (%)		
TG	70.5 ± 1.4^{a}	70.5 ± 1.6^{a}	65.4 ± 6.0^{b}
CE	6.5 ± 0.5^{b}	6.8 ± 0.7^{b}	9.3 ± 0.7^{a}
Fch	3.6 ± 0.3^{b}	4.8 ± 0.5^{a}	4.8 ± 0.5^{a}
PL	14.3 ± 1.2^{a}	11.8 ± 0.6^{b}	11.3 ± 4.0^{b}
Apo	7.9 ± 0.8^{a}	5.9 ± 0.6^{b}	9.2 ± 1.5^{a}

Apo, apolipoprotein; CE, cholesterol ester; Fch, free cholesterol; PL, phospholipid; TG, triacylglycerol

Values are presented as mean ± SE (n = 15 per treatment group). Data were compared by complete weighted least-square means analysis. For each row, values with different superscripts differ significantly ($P < 0.05$).

hamsters was significantly poorer in total protein than that from chow-fed and starch-fed animals. Purified carbohydrate feeding significantly increased the concentration of LDL-Fch and LDL-PL in hamsters (Table 5). Fructose significantly increased LDL-TG concentration, producing a 42.5% increase at the expense of a 20% reduction in LDL-CE concentration. The concentration of LDL-CE was significantly higher in starch-fed hamsters than in either fructose-fed or chow-fed hamsters. However, the amount of LDL-Apo in fructose-fed animals was slightly lower than that in the starch-fed and chow-fed groups ($P = 0.08$). The fractional contribution of TG in the fructose-fed hamsters was higher ($P < 0.01$) than those of the chow-fed and starch-fed hamsters. Again, purified carbohydrates significantly increased the fractional content and concentration of Fch. LDL from starch-fed group was CE enriched. However, LDL from fructose-fed and starch-fed animals were significantly poorer in PRO than were those of chow-fed animals.

The concentration and chemical composition of lipoproteins in the HDL fraction of plasma from hamsters fed chow, fructose and starch diets are shown in Table 6. Fructose-fed animals had the highest concentrations, starch-fed the next highest and chow-fed hamsters the lowest concentrations of HDL-Fch, HDL-CE, HDL-PL and HDL–protein. Differences between fructose-fed and chow-fed hamsters were significant ($P < 0.001$). However, neither absolute plasma concentration nor plasma fractional content of HDL was significantly different in fructose-fed hamsters when compared with starch-fed and chow-fed hamsters. HDL from the fructose-fed group was significantly richer in CE and PL, and significantly poorer in total protein than that of chow-fed ($P < 0.01$) and starch-fed animals ($P = 0.04$). The fractional contribution of TG in the fructose-fed group was lower than that of chow-fed ($P = 0.03$) and starch-fed ($P = 0.08$) groups. The lower fractional HDL-TG was due to the higher fractional HDL-CE in the fructose-fed group.

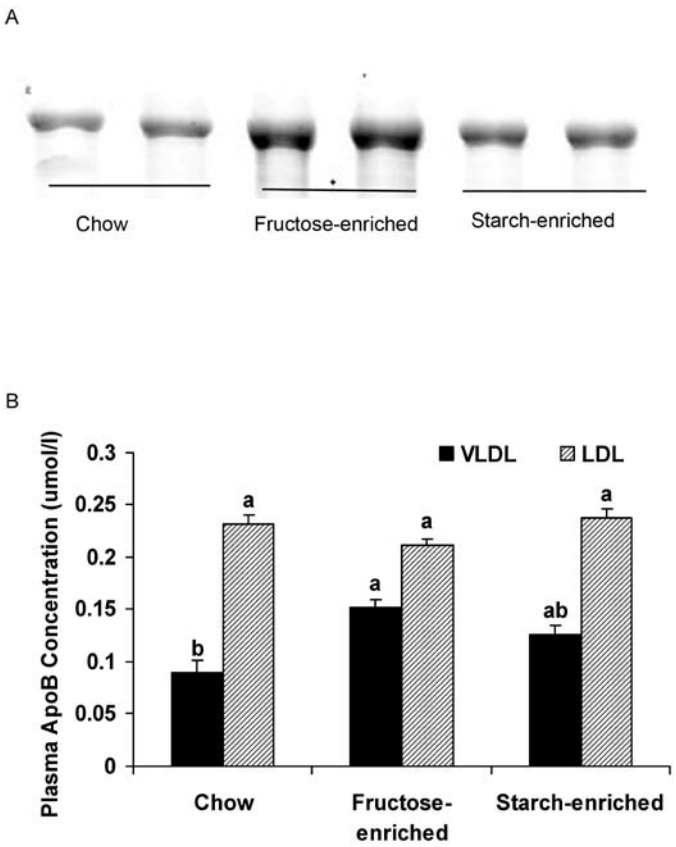

Figure 4. Quantification of plasma VLDL-ApoB from hamsters fed chow, fructose-enriched, and starch-enriched diets. (A) 3% to 12% gradient SDS-PAGE. (B) ApoB concentration in VLDL and LDL particles. Values are presented as mean ± SE (n = 15 per treatment group). Complete weighted least-squares means analysis was used to compare chow, fructose-enriched, and starch-enriched diets. Values indicated with different superscript letters differ significantly ($P < 0.05$).

Discussion

Research relative to fructose-enriched diets, insulin resistance and abnormalities in lipoprotein metabolism has been carried out using the hamster as an animal model.[31,46,58] These studies have shown that hypertriglyceridemia and insulin resistance develop when hamsters consume fructose-rich diets, however, none of these studies reported detailed lipoprotein profiles of all lipoprotein species. The objective of the present study was to systemically characterize all plasma lipoproteins in hamsters fed high-carbohydrate diets of widely differing complexity. The effect of the purified carbohydrates fructose and cornstarch on the physical and biochemical properties of lipoproteins was determined.

In vivo Triton WR-1339 injection and in vitro experiments with primary hamster hepatocytes have been used to demonstrate that both VLDL-TG and VLDL-ApoB are overproduced in hepatocytes from fructose-fed hamsters as compared with chow-fed animals.[58] Our data regarding changes in lipoproteins support these previous results. Fructose slightly increased the plasma concentration of VLDL-Apo but significantly decreased the fractional VLDL-Apo. In addition, VLDL particle number tended to be slightly increased in fructose-fed hamsters, and each VLDL particle contained a greater fraction of neutral lipid in the form of triacylglycerol and was larger than those from chow-fed or starch-fed hamsters. The results from the retention time and diameter determinations were consistent with this conclusion. Circulating particle diameter is determined by particle diameter at assembly and subsequent metabolic effects. For example, particle

Table 5. Concentration and chemical composition of lipoproteins from the 1.006 < d < 1.053 g/ml density fraction (LDL) separated from the plasma of hamsters fed chow, fructose-enriched, and starch-enriched diets

	Concentration (µg/ml)		
	Chow	Fructose-enriched	Starch-enriched
TG	73.7 ± 3.5^{c}	119.0 ± 8.3^{a}	88.0± 6.1^{b}
CE	140.8 ± 3.0^{b}	151.3 ± 9.8^{b}	187.8 ± 8.1^{a}
Fch	40.7 ± 7.5^{a}	63.4 ± 7.3^{a}	60.4 ± 4.2^{a}
PL	107.7 ± 1.8^{b}	135.4 ± 13.5^{a}	126.38 ± 8.8^{a}
Apo	134.6 ± 2.78	121.0 ± 7.44	125. 6 ± 6.7
	% Composition		
TG	14.8 ± 0.8^{b}	20.2 ± 1.3^{a}	15.8 ± 0.7^{b}
CE	28.2 ± 1.2^{b}	25.6 ± 0.7^{b}	33.6 ± 0.6^{a}
Fch	8.2 ± 0.7^{b}	10.7 ± 0.6^{a}	10.8 ± 2.3^{a}
PL	21.6 ± 0.8	22.9 ± 0.9	22.6 ± 0.5
Apo	27.1 ± 3.2^{a}	20.5 ± 2.8^{b}	22.5 ± 1.1^{b}

Apo, apolipoprotein; CE, cholesterol ester; Fch, free cholesterol; PL, phospholipid; TG, triacylglycerol
Values are presented as mean ± SE (n = 15 per treatment group). Data were compared by complete weighted least-square means analysis. For each row, values with different superscripts differ significantly ($P < 0.05$).

Table 6. Concentration and chemical composition of lipoproteins from the 1.053 < d < 1.21 g/ml density fraction (HDL) separated from the plasma of hamsters fed chow, fructose-enriched, and starch-enriched diets

	Concentration (µg/ml)		
	Chow	Fructose-enriched	Starch-enriched
TG	68.5 ± 10.2^{b}	78. 9 ± 3.4^{a}	66.6 ± 4.3^{b}
Fch	103.0 ± 8.9^{c}	215.8 ± 9.5^{a}	144.2 ± 9.9^{b}
CE	778.4 ± 48.6^{c}	1399.8 ± 28.8^{a}	990.5 ± 33.2^{b}
PL	933.7 ± 73.4^{b}	1562.5 ± 50.1^{a}	1089.2 ± 70.1^{b}
Apo	1089.2 ± 67.5^{b}	1363.6 ± 53.4^{a}	1082.0 ± 46.6^{b}
	% Composition		
TG	2.3 ± 0.3^{a}	1.7 ± 0.1^{b}	1.9 ± 0.1^{b}
CE	26.2 ± 0.6^{b}	30.3 ± 0.2^{a}	28.7 ± 0.3^{b}
Fch	3.5 ± 0.1^{b}	4.7 ± 0.1^{a}	4.2 ± 0.1^{a}
PL	31.4 ± 0.5^{b}	33.8 ± 0.1^{a}	31.6 ± 0.8^{b}
Apo	36.6 ± 0.8^{a}	29.5 ± 0.6^{c}	33.6 ± 0.7^{b}

Apo, apolipoprotein; CE, cholesterol ester; Fch, free cholesterol; PL, phospholipid; TG, triacylglycerol
Values are presented as mean ± SE (n = 15 per treatment group). Data were compared by complete weighted least-square means analysis. For each row, values with different superscripts differ significantly ($P < 0.05$).

diameter might decrease due to TG lipolysis mediated by lipoprotein lipase.

The 2 primary factors responsible for maintenance of the concentrations of VLDL-TG and VLDL-PL are synthesis and secretion of TG and PL by liver and removal of TG by extrahepatic tissues. VLDL-TG synthesis is derived from 3 sources:[30] plasma NEFA, the main substrate for hepatic TG synthesis; hepatic uptake of partly lipolyzed chylomicron and VLDL remnants; and de novo synthesis of TG from carbohydrate. In the present study, fructose significantly elevated plasma NEFA and insulin concentrations, indicating that fructose feeding caused insulin resistance. In insulin resistance, increased efflux of NEFA from adipose tissue and impaired insulin-mediated skeletal muscle uptake of NEFA increase hepatic NEFA concentrations.[21] NEFA are the principal substrate for hepatic TG, PL synthesis, and subsequently, increased production of TG and PL. Presumably, TG and PL overproduction result in abnormal assembly into VLDL particles. Therefore, both de novo lipogenesis and increased serum NEFA (presumably due to insulin resistance and increased lipolysis) may have contributed to the increase in VLDL in our fructose-fed hamsters.

Feeding a high-carbohydrate diet induces the synthesis of glycolytic and lipogenic enzymes.[24,42,57] The coordinated induction of these enzymes is due to sterol regulatory element binding protein (SREBP)-stimulated gene transcription.[27,47,49,51] The induction of lipogenic gene transcription increases fatty acid and TG concentrations in the liver. Excess TG is incorporated and secreted by the liver as VLDL-TG.

Targeted disruption of SREBP showed that SREBP1 is crucial for the carbohydrate-mediated stimulation of lipogenic genes in mice.[49] SREBP2 is relatively selective for transcriptional activation of cholesterol biosynthesis.[48] The relative expression of SREBP1a, 1c, and 2 in vivo is complex and can be affected by the nutritional and hormonal status.[17,18] Studies link lipid and carbohydrate metabolism through the activities of both insulin and SREBPs. The content of SREBPs in livers from obese (ob/ob) mice and transgenic (aP2-SREBP1c) mice that overexpress nuclear SREBP1c only in adipose tissue was investigated.[50] Both lines of mice develop hyperinsulinemia, hyperglycemia, and hepatic steatosis, but aP2-SREBP1c mice also exhibit a syndrome that resembles congenital generalized lipodystrophy in humans. Nuclear SREBP1c protein levels were elevated significantly in livers from both ob/ob and aP2-SREBP1c mice compared with wild-type mice. Increased nuclear SREBP1c protein was associated with elevated mRNA levels for known SREBP target genes involved in fatty acid biosynthesis, which led to significantly higher rates of hepatic fatty acid synthesis in vivo. Treatment of isolated hepatocytes with insulin induced the transcription of genes such as acetyl-CoA carboxylase, fatty acid synthase, glucokinase, and pyruvate kinase.[18,19] Addition of a dominant-negative form of SREBP1c blocked the stimulatory effect of insulin on these target genes. Expression of a dominant-positive SREBP1c stimulated the expression of these same insulin-activated target genes in the absence of insulin.[18] These results indicate that insulin stimulates the transcription of SREBP1c and that the mature protein, together with a signal derived from glucose, result in enhanced transcription of genes involved in both lipogenesis and glucose metabolism. In the present study, fructose feeding induced insulin resistance; subsequently elevated insulin levels might increase the transcription of SREBP1c and SREBP-1c-dependent genes, resulting in increased hepatic synthesis of fatty acids and TG. Such effects occurred in the intestines of other hamsters fed a similarly fructose-rich diet.[14] In the present study, starch increased the plasma cholesterol level rather than inducing insulin resistance and the elevating TG level, notably, serum NEFA was not increased in starch-fed hamsters. Therefore, feeding starch appears to induce a different response than does feeding fructose, and the difference may be due to the effect of starch on SREBP2. However, the precise mechanism for this action remains unknown.

Despite overproduction of ApoB in liver, most ApoB will be degraded through 3 mechanisms.[16] ApoB newly synthesized in the endoplasmic reticulum initially is complexed with small amounts of lipid that are shuttled by microsomal triglyceride transfer lipoprotein. The ApoB can be degraded via ER-associated degradation because of lipid deprivation or microsomal triglyceride transfer protein deficiency. Assembly of VLDL depends on the relative availability of all lipid-cholesterol, -PL, -TG, and -CE populations.[8] The relative availability of CE to TG has been suggested to determine the relative composition of hydrophobic lipid in the core of newly secreted VLDL.[9] In addition, the availability of free cholesterol may act as a regulator of the assembly of VLDL.[8] The present results show that VLDL assembly can be influenced by diet. The difference in circulating VLDL composition suggests that fructose feeding leads to overproduction of TG, PL and ApoB but not cholesterol resulting in formation of TG-rich large diameter VLDL particles instead of overproduction of smaller VLDL particles.

In the present study, the LDL-Apo mass in plasma did not differ among fructose-fed, starch-fed, and chow-fed animals. This result suggested that high-carbohydrate diets did not increase LDL particle number. When estrogen was used to treat postmenopausal women, the amount of large-diameter VLDL increased in subjects with high-normal plasma TG concentration.[65] Large VLDL are unlikely to form noteworthy quantities of LDL.[43,54] Perhaps similar, albeit estrogen-independent, changes in assembly processes resulted in the increased VLDL particle diameter and number in the absence of a change in the average LDL particle number of our fructose-fed hamsters. Characteristic abnormalities in the lipid profile in type 2 diabetes include increased concentrations of small, dense LDL.[15,56] The present results showed that fructose feeding also induced the formation of small, dense LDL, but these LDL were relatively rich in TG and poor in CE, suggesting that further lipolysis and decreases in particle diameter were possible. Indeed this situation may underlie the increased frequency of the very small (21 nm), dense LDL-sized particles in the nominal HDL fraction of fructose-fed hamsters subjected to nondenaturing gradient gel electrophoresis. Isopycnic studies[22] have documented an overlap in LDL and HDL particles in the 1.057 to 1.065 g/ml density range. In contrast, increased LDL lipoproteins in starch-fed hamsters were enriched in CE. In these short-term studies, it is not possible to determine the atherogenicity of the large CE-rich LDL associated with starch feeding relative to that of the small TG-rich LDL associated with fructose feeding.

In humans, insulin resistance with atherosclerosis is associated more often with elevated TG and low HDL-C than with elevated total and LDL-C concentrations. Functional defects in HDL also may contribute to atherosclerotic cardiovascular diseases. HDL does not prevent the oxidation of LDL as well as in diabetic patients as in nondiabetic patients.[23] However, in the fructose-fed hamster model used in the present study, several

HDL characteristics were different from those noted in human studies. First, hamsters fed fructose- or starch-rich diets had significantly higher, not lower, concentrations of plasma HDL-C than did chow-fed hamsters. Other scientists who fed fructose-rich diets to *Mesocricetus auratus* for as long as 5 wk have seen a similar response.[37] In addition, the concentration and proportion of HDL-C were significantly higher in our fructose-fed hamsters than in the starch-fed hamsters. Second, fructose feeding significantly increased all HDL lipid concentrations but reduced the relative amount of HDL-TG. Third, fructose tended to increase the plasma concentration of HDL-Apo, but the percentage of HDL-Apo was lower in fructose-fed hamsters than in either chow-fed or starch-fed animals, in accordance with the larger diameter of HDL-Apo particles in fructose-fed hamsters.

These characteristics suggest that feeding the fructose diet resulted in the formation of lipid-rich HDL. The increased HDL mass in both purified carbohydrate-fed groups suggests that hamsters respond differently to simple carbohydrates than other rodent species. The exact mechanism of this response is under investigation.

Insulin resistance and elevated HDL-C also occur in hamsters made hyperlipidemic by saturated-fat feeding.[67] The mechanisms underlying changes in hamster HDL-C with fat feeding are well studied[1,12,46,53,68] and include decreased scavenger receptor class B type I gene expression and increased ApoAII gene expression. In humans, ethanol increases HDL-C due to dose-dependent increases in plasma concentrations of ApoAI and ApoAII after increases in the HDL-Apo transport rates.[10] In saturated-fat–fed hamsters, increases in HDL-C concentration were not associated with changes in rates of reverse cholesterol transport.[53,68] Studies investigating the causes of insulin resistance in hamsters fed diets high in either fructose or beef tallow found that plasma concentrations of total cholesterol, TG, NEFA, and insulin as well as acyl-CoA:diacylglycerol acyltransferase (DGAT1-2) mRNA transcripts and TG in adipose and muscle showed similar increases in the 2 dietary groups, suggesting that similar mechanisms were operative in the 2 nutritional states.[5]

The following factors are thought to affect HDL-C concentration or particle number. First, apoAI is the major apolipoprotein component of HDL-C and therefore serves as a marker of HDL-C concentration or particle number. Our results showed that apoproteins were increased in fructose- and starch-fed hamsters. Plasma apoAI and hepatic apoA1 mRNA concentrations in young and aged rats fed either a 60% fructose diet or a 60% glucose diet were significantly higher than those in rats fed rat chow.[40] The investigators suggested that apoAI expression in rats reflects factors related to the type of dietary carbohydrate rather than an association with insulin resistance after a high-fructose diet. Second, overexpression of lecithin-cholesterol acyl-transferase (LCAT) in transgenic mice substantially increased HDL-C and even further increased the plasma concentration of CE-rich, large HDL.[20,63] ApoAI activates LCAT,[20] thus, more apoAI means greater LCAT activation. Third, transfer of CE from HDL to ApoB-LP in exchange for TG by cholesterol ester transfer protein is the main pathway by which HDL-C is returned to the liver in humans.[59] The concentration and activity of cholesterol ester transfer protein were increased in the plasma and adipose tissue of fructose-fed hamsters.[46] In hamsters, adipose tissue and muscle are important sources of cholesterol ester transfer protein,[29] and lipoprotein composition analysis by other investigators[22] supports our findings that the hamster is partially deficient in neutral lipid (CE, TG) transfer activity. Therefore, fructose-fed hamsters produce large CE-rich HDL, increasing HDL-C and particle number rather than lowering HDL-C. Third, both the cellular protein ABCA1, which facilitates the transport of cholesterol and PL between HDL particles and peripheral cells, and SR-BI, which facilitates the uptake of HDL-CE by liver, can affect HDL-C and particle number. Species differences or specific HDL metabolic pathways are also likely to contribute to increased HDL-C in hamsters.

Whether the increased HDL always plays a protective role against atherosclerotic cardiovascular diseases is unclear. HDL isolated from subjects with noninsulin-dependent diabetes mellitus exhibited a decreased capacity to induce cholesterol efflux.[23] The composition and protective effects of HDL_2, but not of HDL_3, differed significantly between control and diabetic subjects. HDL_2 from diabetics, which was TG-enriched and cholesterol-depleted compared with those from control subjects, were less able to protect LDL from oxidation and oxidation-induced ApoB100 fragmentation. In the present study, HDL species were not separated, nor was HDL functionality measured. It is unclear whether the hamsters fed a fructose-rich diet had HDL_2 that was enriched in TG and depleted of cholesterol but total HDL that was enriched with cholesterol and depleted of TG.

The metabolism of plasma lipoproteins is highly interrelated, and considering the complete lipoprotein and lipid profiles is crucial, rather than focusing on just 1 or 2 lipoprotein features. We have found that carbohydrate-rich diets cause significant changes to the entire lipoprotein profile of hamsters, as well as to the physical properties of plasma lipoprotein particles. The changes caused by dietary carbohydrate depend on the carbohydrate's degree of polymerization (complex or simple) and monomer identity. Further studies are needed to define the mechanism of induction of a high HDL concentration in plasma as well as clarifying HDL type and function. Using a starch-based diet clearly demonstrated the selective increase in TG-rich VLDL formation due to fructose feeding. Future investigations will provide new insight into lipoprotein metabolism and the effect of high-carbohydrate diets on atherosclerotic cardiovascular diseases in hamsters.

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