Diet-Induced Type IV-Like Hyperlipidemia and Increased Body Weight Are Associated with Cholesterol Gallstones in Hamsters[1]

K.C. Hayes*, Pramod Khosla and **Andrzej Pronczuk**
Foster Biomedical Research Laboratory, Brandeis University, Waltham, Massachusetts 02254

Male Syrian hamsters (60–70 g) were fed purified diets containing 5% fat (American Fat Blend) and 15% fiber with or without 0.3% cholesterol (0.86 mg/kcal), for 12 weeks. Hamsters fed the cholesterol-supplemented challenge diet revealed a major increase in plasma triglyceride between 9 and 12 weeks, whereas plasma cholesterol (which reflected body weight dynamics) increased threefold up to nine weeks and plateaued (342 ± 22 *vs.* 122 ± 5 mg/dL). The greatest increases in cholesterol occurred in the very low density lipoprotein (VLDL) and high density lipoprotein (HDL_2) fractions. Gallstone incidence was similar (69% *vs.* 78%) for cholesterol-supplemented *vs.* control hamsters, but the type of stones differed. Of the cholesterol-supplemented hamsters with gallstones, 45% had cholesterol stones and 55% had pigment stones. Only pigment stones were seen in control hamsters. Hamsters with cholesterol stones were 25% heavier and transported most cholesterol in VLDL (33 ± 5%), approximately double that in VLDL of cholesterol-supplemented hamsters with no stones (19 ± 3%) or cholesterol-supplemented hamsters with pigment stones (21 ± 3%). Hamsters with pigment stones or no stones (regardless of diet fed) transported the majority of their cholesterol in HDL_2 (44%), whereas this figure was only 27% in hamsters that developed cholesterol stones. Thus pigment stones develop routinely in hamsters fed casein-based purified diets. Adding dietary cholesterol resulted in cholesterol gallstones only in those hamsters that gained the most weight and whose terminal VLDL/HDL cholesterol ratio exceeded 1.0, not unlike the lipoprotein profile of obese humans who develop cholesterol gallstones.
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Syrian hamsters have been used extensively as an animal model for the study of gallstones because they are one of the few species in which gallstones can be readily induced by dietary manipulation (1-4). Their gallbladder bile seems to contain the requisite mucus, and biliary stasis with "sludging" appears to enhance their gallstone formation once lithogenic bile develops (5,6). Although both pigment stones and cholesterol stones occur (3-6), the dietary factors and the mechanisms responsible for these two types of stones are unresolved.

Diets with defined composition, as opposed to crude diet formulations or laboratory chow diets, allow study and identification of the specific dietary factor(s) causing lithogenesis. Recent work from this (5,6) and other investigators (3,4) describe purified diets that induce either pigment (3,5) or cholesterol gallstones (4,6).

Although it is well appreciated that cholesterol gallstones in humans are associated with abnormal cholesterol metabolism (7) and elevated plasma triglycerides (8), and that cholesterol metabolism itself is linked to the metabolism of plasma lipoproteins, minimal information exists concerning the relationship between lipoprotein metabolism and gallstone development in any species (9-12). In a recent study (6) we identified an association between an elevated very low density lipoprotein (VLDL) cholesterol pool, bile lithogenecity and cholesterol gallstones in Charles River (Lakeview, MA) hamsters fed butter fat and cholesterol.

To follow the time-course of the changes in circulating lipoproteins and gallstone development (including the type of gallstones induced), we have fed hamsters purified diets differing solely in their cholesterol content. The Sasco strain of male golden Syrian hamsters was studied because a recent report suggested that this hamster was especially sensitive to cholesterol gallstone induction (4).

MATERIALS AND METHODS

One hundred and ten male golden Syrian hamsters (*Mesocricetus auratus*) were obtained from Sasco Inc. (Omaha, NE; Sasco, golden Syrian outbred hamster derived from a closed colony from the University of Nebraska Medical Center, Eppley Cancer Institute) at a starting weight of 60–70 g. Hamsters were housed 4 per cage in a controlled environment with a 12-hr light-dark cycle (lights on at 1800 hr). All hamsters were fed a purified, cholesterol-free control diet with 5% fat (w/w). After 5 days of feeding the control diet, and just prior to feeding of the cholesterol-supplemented challenge diet (designated t=0 weeks), plasma lipids and lipoproteins were evaluated in 16 hamsters selected at random. Of the 16 hamsters, 7 were sacrificed for gallstone evaluation (no visible lesions or gallstones were observed). The data from these 16 animals constitutes the basal (t=0 week) values. The hamsters were then randomly divided into two groups. Group 1 (n=25) was continued on the control diet, whereas Group 2 (n=78) was fed the control diet supplemented with 0.3% (w/w) cholesterol (0.86 mg/kcal) for the remainder of the study. Hamsters had access to fresh food and water (provided daily) *ad libitum*. The composition of the diets (Table 1) reveals a polyunsaturated to saturated fatty acid ratio (P/S) of 0.54. The experimental design and procedures were approved by the Brandeis University Animal Use Committee.

In addition to the analysis at t=0 weeks, lipoprotein changes were also assessed at t=2, 5, 9 and 12 weeks in the cholesterol-supplemented hamsters. For this purpose, 16 hamsters were randomly selected from the pool of 78 animals. Individual hamsters were fasted overnight in wire-bottomed cages prior to being anesthetized lightly

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*To whom correspondence should be addressed.
Abbreviations: ANOVA, analysis of variance; EDTA, ethylenediaminetetraacetic acid; HDL, high density lipoprotein; HPLC, high-performance liquid chromatography; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; PLSD, protected least significant difference; P/S, ratio of polyunsaturated to saturated fatty acids; VLDL, very low density lipoprotein.

TABLE 1

Composition of Purified Diets Fed to Hamsters[a]

	Control diet (cholesterol-free)	Challenge diet (cholesterol-supplemented)
	g/100g	
Casein	20	20
Glucose	18.9	18.9
Corn starch	35	34.7
Fat[b]	5	5
Cellulose	10	10
Wheat bran	5	5
Mineral mix[c]	4.6	4.6
Vitamin mix[d]	1.2	1.2
Choline chloride	0.3	0.3
Cholesterol	—	0.3

[a]Diets were fed as gel blocks, prepared by withholding 60 g corn starch (per Kg mix) from the formulation and premixing it with 800 mL of simmering water to form a gel to which the remaining ingredients were added.

[b]American fat blend (2.6 butter, 1.6 canola oil, 0.8 corn oil) had a P/S ratio of 0.54 and a P/M/S ratio of 1.0:1.0:1.9 (P, polyunsaturated; M, monounsaturated; S, saturated).

[c]Ausman-Hayes Salt Mix (BioServ Inc., Frenchtown, NJ).

[d]Hayes-Cathcart Vitamin Mix; the composition of the vitamin mix has been detailed previously (5).

for blood sample collection (2–3 mL) by cardiac puncture, into ethylenediaminetetraacetic acid (EDTA)-wetted syringes using a 25-gauge needle. (Unless sacrificed for gallstone evaluation, all hamsters recovered from the blood sampling). Lipoproteins were also analyzed at t=12 weeks in 16 animals from the control group. To allow for gallstone evaluation (at t=12 weeks), all 16 hamsters fed the cholesterol-supplemented diet (and 9 of the 16 animals fed the control diet) were exsanguinated by cardiac puncture. Gallbladders from the 9 control and 16 test hamsters were opened under an Olympus M100 dissecting microscope and all gallstones present were examined under regular and polarized light. Spherical white cholesterol gallstones (0.1–1.2 mm) and dark green-to-black pleomorphic pigment gallstones (<0.1–0.6 mm) were identified. Upon drying, representative gallstones of both cholesterol and pigment varieties were analyzed by high-performance liquid chromatography (HPLC) for their cholesterol content (13). The cholesterol content of cholesterol gallstones was routinely over 90%, whereas this figure was less than 2% for pigment gallstones. A detailed description of the two types of gallstones has been reported elsewhere (6).

Sodium azide (10%, w/v) was added to the plasma samples (10 μL/mL plasma). Throughout the study, lipoproteins were isolated from plasma pooled from 3–4 hamsters, with the exception of the analysis at t=12 weeks for the cholesterol-supplemented animals when lipoproteins were isolated from the plasma of individual hamsters. In all cases lipoproteins were isolated using stock density solutions of d=1.006 g/mL and d=1.38 g/mL by sequential ultracentrifugation (14) in Quick seal tubes®™ (Beckman, Palo Alto, CA) using a Ti 70.1 rotor at 12°C. Stock density solutions contained EDTA, gentamycin sulfate, sodium azide (15) and benzamidine (16). Five lipoprotein fractions were isolated, VLDL (d<1.006 g/mL), intermediate density lipoprotein (IDL) (1.006<d<1.019 g/mL), low density lipoprotein (LDL) (1.019< d<1.055 g/mL), HDL_2 (1.055<d<1.125 g/mL) and HDL_3 (1.125<d<1.21 g/mL). These density cuts for hamsters were determined in an initial study in which lipoproteins were isolated by discontinuous density-gradient ultracentrifugation (17) (data not shown). With the exception of VLDL and IDL, all lipoprotein fractions were dialyzed against 0.15M NaCl/1 mM EDTA, pH 7.4, at 4°C, for 24–36 hr. Lipoprotein cholesterol and triglyceride content were measured by enzymatic methods. Lipoprotein protein was measured using a modification (18) of the procedure of Lowry *et al.* (19). The sum of the cholesterol measured in each lipoprotein fraction was divided by the total plasma cholesterol to determine the percent recovery (20). The percent recovery for the lipoprotein isolations carried out at 12 weeks (n=21; 5 controls and 16 experimental) was 81% ± 2%. The final concentration of cholesterol, triglyceride and protein in each lipoprotein fraction was calculated by applying the same percent recovery.

To assess whether gallstone formation might be associated with differences in cholesterol esterification and/or changes in essential fatty acid availability, VLDL free cholesterol and individual cholesteryl esters were determined by HPLC (13). Cholesterol (free and esterified) was extracted by a double-solvent extraction procedure (isopropanol/0.75 M NaOH, 33:17, v/v and n-octane). The individual cholesteryl esters were separated on a Waters Radial-Pak Resolve™ C18 column by isocratic elution with acetonitrile/isopropanol (45:55, v/v) at 2 mL/min with a Beckman 110B solvent delivery module. The absorbance of the eluate was measured at 210 nm using a Waters model 480 LC spectrophotometer (Waters Associates, Wilmington, MA).

Plasma and lipoprotein cholesterol and triglyceride concentrations were determined enzymatically using kits (#336 and #352, respectively) obtained from Sigma Diagnostics (St. Louis, MO). Statistical analyses were carried out on a Macintosh Plus®™ computer (Apple Computer Inc., Cupertino, CA) using the Statview 512®™ software package (Brain Power, Inc., Calabasca, CA). Statistical differences between mean values for the control and cholesterol-supplemented dietary groups was assessed using Student's unpaired *t*-test. For subgroup analysis of the cholesterol-supplemented hamsters, the various parameters related to the gallstone response were analyzed by a one-factorial analysis of variance (ANOVA), and the Fisher protected least significant difference (PLSD) was used to test for statistical significance.

RESULTS

Time course of the changes in body weights, plasma lipids and lipoproteins. The time course of the changes in body weights and plasma lipid concentrations in hamsters fed the cholesterol-supplemented challenge diet over the 12 weeks of the study is shown in Figure 1. The final body weights represented a two-fold increase (p<0.001) in comparison to the body weights observed at the start of the study (68 ± 5 g). The plasma lipid values reflected body weight dynamics in that plasma cholesterol increased three-fold up to nine weeks (p<0.001), and then plateaued. By contrast, the plasma triglyceride concentration

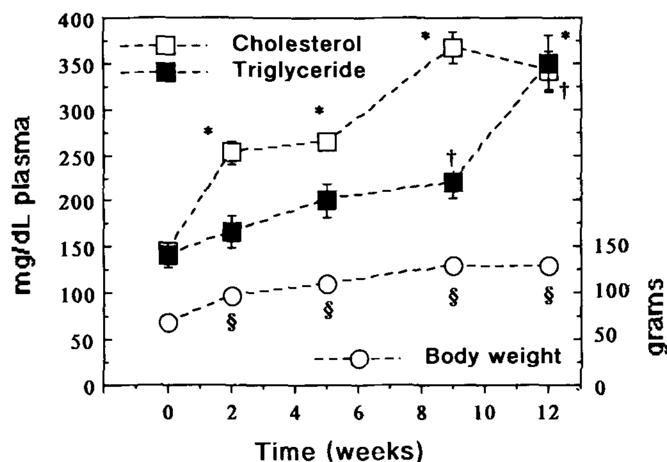

FIG. 1. Time-course of the change in body weight, plasma cholesterol and plasma triglyceride concentrations in hamsters fed the cholesterol-supplemented diet. Each point is the mean ± SEM of 16 hamsters. The data was analyzed with a one-way ANOVA followed by Fisher's PLSD to test for statistical significance ($p<0.05$). Symbols indicate values that were significantly different from the zero-time value.

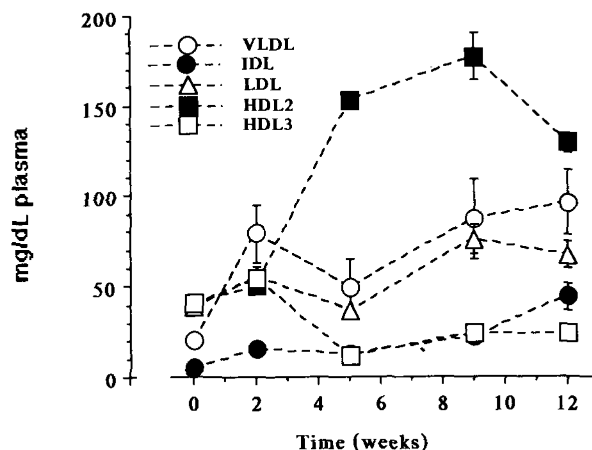

FIG. 2. Time-course of the change in lipoprotein cholesterol concentrations in hamsters fed the cholesterol-supplemented diet. Values are means ± SEM of four isolations using the plasma pooled from 3–5 animals, with the exception of the analysis at 12 weeks, which is the mean ± SEM of 16 individual hamsters. The data was analyzed with a one-way ANOVA followed by Fisher's PLSD to test for statistical significance ($p<0.05$). For VLDL and IDL, all values were significantly higher than the zero-time values. For HDL_2 the 5, 9 and 12 week values were significantly higher than the zero-time values. For LDL, the 9 and 12 week values were significantly higher than the zero-time values.

increased throughout the study, but significant increases were noted at 9 and 12 weeks ($p<0.001$).

The time-course for changes in lipoprotein cholesterol concentrations in the cholesterol-supplemented hamsters over the 12 weeks of the study is depicted in Figure 2. Fluctuating cholesterol concentrations in most of the lipoprotein fractions were apparent during the first 5 weeks, with the greatest relative increase in VLDL and HDL_2 cholesterol. After 5 weeks the cholesterol increased in all the lipoprotein fractions except HDL_3, but the most striking increase in mass occurred in HDL_2 until 9 weeks. By 12 weeks, HDL_2 cholesterol had decreased while only VLDL and IDL cholesterol revealed further increases.

Terminal weights and plasma lipid responses. Body weights, liver weights and plasma lipid levels after 12 weeks of feeding the two diets are presented in Table 2. Although body weights were comparable in the two dietary groups (125 ± 4 *vs.* 129 ± 4 g), the liver weights (as a percentage of the body weight) were significantly higher in hamsters fed the cholesterol-supplemented challenge diet ($p<0.001$). The terminal plasma cholesterol (361 ± 28 *vs.* 122 ± 5 mg/dL; $p<0.001$) and plasma triglyceride concentrations (405 ± 63 *vs.* 141 ± 13 mg/dL; $p<0.001$) were almost threefold higher in the challenge group. The final plasma cholesterol concentration in the hamsters fed the control diet was modestly, but significantly, lower than the value obtained at the start of the study (122 ± 5 *vs.* 144 ± 5 mg/dL; $p<0.003$), whereas the concentration of plasma triglyceride was similar (142 ± 12 *vs.* 141 ± 13 mg/dL).

The 12-week lipoprotein data revealed a greater lipoprotein mass in hamsters fed the challenge diet (Table 3). For both dietary groups the HDL_2 fraction transported the greatest percentage of cholesterol. The hypercholesterolemia in the experimental group was characterized by a four-fold increase in VLDL cholesterol, a seven-fold increase in IDL cholesterol, a three-fold increase in LDL cholesterol and a two-fold increase in HDL_2 cholesterol.

TABLE 2

Body Weights, Liver Weights and Plasma Lipid Concentrations in Hamsters Fed the Control and Challenge Diets for 12 Weeks[a]

	Control diet (cholesterol-free)	Challenge diet (cholesterol supplemented)
Body weight (g)	125 ± 4	129 ± 4
Liver weight (% body weight)	3.5 ± 0.5	5.6 ± 0.5
Plasma cholesterol (mg/dL)	122 ± 5	361 ± 28
Plasma triglyceride (mg/dL)	141 ± 13	405 ± 63

[a]Of the 16 hamsters fed the control diet, 9 hamsters were sacrificed for gallstone evaluation. Therefore, with the exception of the liver weights for the hamsters fed the control diet (n=9), all values are the means ± SEM of 16 animals. All parameters were significantly different between groups as determined by Student's unpaired *t*-test ($p<0.001$) with the exception of body weights.

These changes in the terminal cholesterol distribution represented a relative decrease in HDL_2 cholesterol, but relative and absolute increases in VLDL cholesterol as compared to control hamsters. The hypertriglyceridemia in the supplemented group was reflected principally in the VLDL fraction. In terms of cholesterol distribution, cholesterol-feeding almost doubled the cholesterol load in the VLDL/IDL pool (38% *vs.* 22%) while decreasing the cholesterol in HDL by 30% and leaving the LDL pool unaltered.

Gallstone incidence. No gallstones were detected in hamsters fed the cholesterol-supplemented challenge diet over the first five weeks of the study (Table 4). At nine weeks, three of five hamsters killed had early pigment stone formation only (represented by soft, mucus-rich clusters of greenish, sawdust-like material which was typically amorphic and less than 0.1 mm long). By 12

TABLE 3

Lipoprotein Lipid and Protein Concentrations in Hamsters Fed the Control or Challenge Diets for 12 Weeks[a]

	Control diet (cholesterol-free)	Challenge diet (cholesterol-supplemented)
VLDL (d < 1.006 g/mL)		
Cholesterol	22 ± 1 (17.4)[b]	96 ± 18[c] (26.2)
Triglyceride	85 ± 6	302 ± 61[d]
Protein	16 ± 1	22 ± 5
IDL (1.006 < d < 1.019 g/mL)		
Cholesterol	6 ± 0.1 (4.6)	44 ± 7[c] (12.4)
Triglyceride	13 ± 0.2	38 ± 3[d]
Protein	5 ± 0.2	16 ± 2[c]
LDL (1.019 < d < 1.055 g/mL)		
Cholesterol	23 ± 0.9 (18.3)	67 ± 7[e] (18.4)
Triglyceride	10 ± 0.3	24 ± 1[d]
Protein	14 ± 0.3	44 ± 5[e]
HDL_2 (1.055 < d < 1.125 g/mL)		
Cholesterol	57 ± 2 (45.9)	129 ± 5[e] (36.2)
Triglyceride	9 ± 0.6	30 ± 2[e]
Protein	114 ± 6	257 ± 9[e]
HDL_3 (1.125 < d < 1.21 g/mL)		
Cholesterol	17 ± 0.9 (13.8)	24 ± 2 (6.7)
Triglyceride	3 ± 0.3	11 ± 1[c]
Protein	50 ± 5	77 ± 4[c]
VLDL-C/HDL_2-C	0.4 ± 0.04	0.8 ± 0.2

[a]mg/dL Plasma. Values are the means ± SEM; n=5 for the control group and n=16 for the experimental group. For the control group lipoproteins were isolated from plasma pooled from 3-4 hamsters, whereas for the experimental group, lipoproteins were isolated from the plasma of individual animals.

[b]Values in parentheses represent percent distribution.

[c,d,e]Statistical significance between mean values for the two dietary groups was calculated using Student's unpaired *t*-test. p Values: [c]p<0.02; [d]p<0.05; [e]p<0.001.

TABLE 4

Gallstone Incidence in Hamsters Fed the Control and Challenge Diets

Time (weeks)	Criterion	Control diet (cholesterol-free)	Challenge diet (cholesterol supplemented)
0	Gallstone incidence[a]	0/7 [0%]	
2	Gallstone incidence		0/16 [0%]
5	Gallstone incidence		0/11 [0%]
9	Gallstone incidence		3/5 [60%][b]
12	Gallstone incidence	7/9 [78%]	11/16 [69%]
	Cholesterol stones[c]	0/7 [0%]	5/11 [45%]
	Pigment stones[c]	7/7 [100%]	6/11 [55%]
	Gallstone severity[d]		
	Cholesterol stones	0	5
	Pigment stones	3.6 ± 0.5	3.3 ± 1.5

[a]The number of hamsters with gallstones/number of hamsters examined.

[b]Only early pigment stone formation observed. Additional hamsters were not killed.

[c]The number of hamsters with cholesterol or pigment stones/number of hamsters with gallstones.

[d]Both cholesterol and pigment stones were scored microscopically on a scale of 0–5 to characterize relative size and number.

TABLE 5

Body Weights, Liver Weights, Plasma Lipid and Lipoprotein Concentrations in Subgroups of Hamsters Fed the Challenge Diet for 12 Weeks (Based on Gallstone Response)[a]

		No stones	Cholesterol stones	Pigment stones
Number of hamsters		5	5	6
Body weight (g)		120 ± 8[b]	149 ± 4[b,c]	121 ± 4[c]
Liver weight (% body wt.)		4.6 ± 1.0[d,e]	6.6 ± 1.5[d]	6.7 ± 1.0[e]
Plasma cholesterol (mg/dL)		319 ± 35	421 ± 70	346 ± 34
Plasma triglyceride (mg/dL)		338 ± 64	562 ± 173	329 ± 54
VLDL[f]	Cholesterol	64 ± 17[g] (19.8)[h]	152 ± 46[g,i] (35.3)	77 ± 19[i] (21.4)
	Triglyceride	231 ± 60	462 ± 166	228 ± 55
	Protein	25 ± 6	49 ± 13	27 ± 6
IDL	Cholesterol	39 ± 9 (13.2)	60 ± 15 (14.1)	36 ± 10 (10.5)
	Triglyceride	36 ± 4	43 ± 6	35 ± 2
	Protein	14 ± 3	21 ± 5	14 ± 4
LDL	Cholesterol	53 ± 8 (16.3)	78 ± 18 (18.4)	69 ± 4 (20.3)
	Triglyceride	23 ± 3	25 ± 3	23 ± 1
	Protein	43 ± 10	49 ± 8	41 ± 5
HDL_2	Cholesterol	136 ± 4[j] (42.4)	110 ± 6[j,k] (27.0)	139 ± 7[k] (40.6)
	Triglyceride	34 ± 3	22 ± 4	33 ± 5
	Protein	260 ± 17[l]	224 ± 12[l,m]	281 ± 12[m]
HDL_3	Cholesterol	26 ± 2 (8.1)	22 ± 5 (5.2)	25 ± 2 (7.1)
	Triglyceride	14 ± 2	10 ± 1	11 ± 1
	Protein	75 ± 7	74 ± 9	82 ± 6
$\frac{VLDL\text{-}C}{HDL_2\text{-}C}$		0.47 ± 0.12[n]	1.43 ± 0.45[n,o]	0.53 ± 0.11[o]

[a]Mean ± SEM.
[f]mg/dL Plasma.
[h]Values in parentheses represent the percent distribution of cholesterol among the lipoprotein fractions. Data from the 16 hamsters fed the Challenge diet (cholesterol-supplemented) are expressed in terms of the gallstone response. The data was analyzed by a one factorial ANOVA followed by Fishers PLSD test for significance between the subgroups. Mean values, sharing a common superscript are significantly different.

p Values: [b,c]$p<0.02$; [j,k]$p<0.03$; [d,e,n,o]$p<0.05$; [g,i,l,m]$0.05<p<0.10$.

weeks, the incidence of gallstones in the two dietary groups was comparable (78% *vs.* 69%). When the control hamsters were sacrificed, the livers in the first nine hamsters were dark red-brown and smaller than test hamster livers, and since none of these had cholesterol crystals in their bile and the only gallstones present were pigment stones, the other seven animals in this group were not killed. By contrast, hamsters fed the cholesterol-supplemented challenge diet were found to have enlarged, pale-yellow livers. Among the 69% with gallstones, either cholesterol stones (45%) or pigment stones (55% incidence) were observed. Both number and size of pigment stones in the control dietary group were similar to those in the cholesterol-supplemented group.

Lipoprotein distribution in relation to gallstone response. Since the cholesterol-supplemented group revealed three responses in terms of gallstones (no stones, cholesterol stones, or pigment stones), the data from this dietary group was re-analyzed according to gallstone subgroup (Table 5). Although all three subgroups experience elevated total plasma cholesterol and triglyceride concentrations by comparison to controls, the cholesterol-stone formers tended to have higher lipid values than the other two subgroups, and their lipoprotein profile was characterized by 20% less HDL_2 cholesterol ($p<0.03$). The HDL protein tended to be lower as well ($0.05<p<0.10$). Their VLDL cholesterol, however, tended to be higher ($0.05<p<0.10$). The combined effect of the lipoprotein differences resulted in the ratio of VLDL to HDL_2 cholesterol being essentially three-fold higher ($p<0.05$) in hamsters with cholesterol stones as compared to cholesterol-supplemented hamsters having no stones or pigment stones. The percentage of plasma cholesterol in the VLDL fraction of hamsters with cholesterol stones (35%) was almost double that of the other two subgroups. All other lipoprotein parameters between the three subgroups of hamsters were similar. Furthermore, no differences were noted in any of the measured lipoprotein parameters between hamsters with no stones and those with pigment stones. In essence, the two types of gallstones were associated with distinct VLDL and HDL_2 cholesterol distributions such that pigment stone bearers transported the majority of their lipoprotein cholesterol in the HDL_2 fraction (41%), whereas hamsters bearing cholesterol gallstones transported the majority of cholesterol in the VLDL fraction (35%).

A striking finding was that the mean body weight of cholesterol-supplemented hamsters developing cholesterol stones was 25% greater ($p<0.02$) than either of the other two subgroups having no stones or pigment stones.

VLDL cholesterol. The two-fold increase in total VLDL cholesterol observed in the subgroup of hamsters with

TABLE 6

VLDL Cholesteryl Ester Distribution in Hamsters Fed the Challenge Diet for 12 Weeks[a]

Criterion	No stones	Cholesterol stones	Pigment stones
		mg/dL plasma	
Total VLDL cholesterol	64 ± 17[b]	152 ± 46[b,c]	77 ± 19[c]
VLDL free cholesterol	15 ± 3 (23.5)[d]	44 ± 12 (28.7)	24 ± 7 (31.6)
Cholesteryl esters	49 ± 11[e] (76.5)	108 ± 20[e,f] (71.2)	53 ± 7[f] (68.4)
Arachidonate (20:4)	1.1 ± 0.3 (4.0)	2.1 ± 0.4 (2.0)	1.3 ± 0.2 (2.6)
Linoleate (18:2)	11.1 ± 2.7[g] (22.8)	27.3 ± 5.7[g,h] (25.1)	11.3 ± 1.8[h] (21.5)
Palmitate (16:0)	9.1 ± 1.8 (18.7)	18.6 ± 3.3 (17.1)	10.0 ± 2.2 (19.0)
Oleate (18:1)	27.7 ± 6.3[i] (56.4)	60.0 ± 11.3[i,j] (55.2)	30.4 ± 3.6[j] (57.9)

[a]Values are the mean ± SEM for five hamsters with no stones, five with cholesterol stones and six with pigment stones. Mean values sharing a common superscript differ significantly between groups using a one factorial ANOVA.

[d]Parentheses represents percent distribution of the total cholesterol fraction or the fatty acid cholesteryl esters, respectively.

p values: [e,f]$p<0.05$; [b,c,g-j]$0.05<p<0.10$.

cholesterol stones was reflected in the mass of cholesterol esters (Table 6), although the relative distribution between free and esterified cholesterol was similar for the three subgroups. Furthermore, the cholesteryl ester fatty acid profile was similar for the three subgroups with no difference in the 18:1/18:2 ratio.

DISCUSSION

The objective of this investigation was to define the progressive impact of dietary cholesterol on hamster plasma lipoproteins and to determine the relationship of the lipoprotein changes to the induction of cholesterol gallstones observed previously (6). In addition, we wanted to establish the time-frame of the associated lipoprotein alterations. Upon termination of our previous study we noted an elevated VLDL/HDL cholesterol ratio in hamsters with cholesterol gallstones, but the diet in that study was more severe, containing 5% butter, more glucose, and more cholesterol (0.4% or 1.14 mg/kcal). Evidence of mild essential fatty acid depletion also was deduced from plasma fatty acid profiles. Our previous hamsters (Charles River) were allegedly more resistant to gallstones than the Sasco strain used in the present experiment (4), yet with the previous cholesterol challenge diet and hamster strain we found a much higher overall incidence of cholesterol gallstones (93% *vs.* 45%) over the same experimental period (6).

In the present study an American Fat Blend (P/S ratio 0.54) that provided 2.6% dietary energy as 18:2 was fed to improve essential fatty acid status, and dietary cholesterol was reduced by 25%. Furthermore, the sequential changes in plasma lipids, including the lipoprotein profile, were measured during gallstone induction to document the time-course in lipoprotein alterations. This identified HDL_2 as the predominant lipoprotein in the normal hamster. Feeding a 0.86 mg/kcal cholesterol supplement (0.3%, w/w) initiated a rapid rise in VLDL and HDL_2 cholesterol, but the latter pool of cholesterol then declined somewhat by 12 weeks, while VLDL and IDL cholesterol continued to increase. This distribution of cholesterol was most pronounced in the subset of hamsters that developed cholesterol gallstones, a process that appeared to occur between 9 and 12 weeks in our study. By contrast, the hamsters fed the same diet that managed to resist the VLDL-cholesterol increase and maintain their HDL_2, remained free of cholesterol gallstones and were either free of all gallstones or developed only pigment stones. The animals that developed cholesterol stones also gained 25% more body weight than controls fed no cholesterol or the other two subgroups (no stones, pigment stones) also fed cholesterol. Essential fatty acid status did not appear compromised in any group. In fact, the cholesterol-stone formers had the highest percentage of 18:2 among their cholesteryl esters.

At least three implications can be inferred from these data. First, cholesterol gallstone induction in response to cholesterol feeding in hamsters appeared to be associated with an expansion of the VLDL cholesterol pool and relative decrease in HDL_2, the principal lipoprotein cholesterol pool found in normal hamsters. Previously in hamsters fed a comparable diet with a similar shift in their lipoprotein profile (6), we found that the gallbladder bile was markedly supersaturated with cholesterol (lithogenic index of 1.72 with bile acids, phospholipids and cholesterol of 79, 13 and 8 mol%, respectively) and that the liver was enlarged and cholesterol laden (2 mg *vs.* > 20 mg cholesterol/g liver) as it was herein. In the present study the plasma cholesterol (and triglyceride) concentrations tended to be higher in hamsters with cholesterol stones than in the two other subgroups that were fed cholesterol without developing cholesterol stones, supporting our previous observation that increased cholesterol transport was associated with cholesterol gallstone induction (6). However, both the plasma cholesterol and triglyceride values in all three cholesterol-fed subgroups were higher than the control values. This suggests that 0.3% cholesterol (0.86 mg/kcal) approximates the minimal threshold for initiating a cholesterol "overload", causing approximately one-third of the hamsters to exceed their ability to metabolize the increased rate of cholesterol transport. Similar to the situation in gallstone patients where

triglyceridemia could not be separated from decreased HDL levels (7), we are not able to ascertain why HDL_2 should protect against, or VLDL-C should be associated with, cholesterol supersaturation of bile in hamsters. One possibility might be that VLDL-C (as VLDL remnants) has more direct access to the biliary cholesterol pool or that HDL_2 cholesterol has preferential access to bile acid synthesis, since we previously noted an increase in biliary cholesterol (8 *vs.* 2 mol%) and a decrease in the relative concentration of bile acids (79 *vs.* 90 mol%) in hamsters with cholesterol-gallstones under similar circumstances (6). It is interesting that Syrian hamsters, as a species, like women whose gender seems to predispose to gallstones (21), have a predominant HDL_2 profile.

The second point relates to the greater body weight of hamsters with cholesterol gallstones. It is well accepted that obesity is the single most important risk factor for cholesterol stones in humans (7,8,21,22). Obese persons typically experience increased cholesterol transport rates and a lipoprotein profile (expanded VLDL and LDL pools) reflective of hypercholesterolemia and hypertriglyceridemia (7,23,24). Even in non-obese women with increased gallstone incidence, the factor most associated with gallstones was excessive caloric intake (22). If the propensity for increased body weight in hamsters bearing cholesterol gallstones eventually proves to be a reflection of increased energy flux through lipoproteins with its attendant cholesterol transport, it would suggest that at least one mechanism of lithogenesis in cholesterol-fed hamsters and humans may be comparable, *i.e.*, increased caloric consumption resulting in elevated cholesterol transport and increased flux and catabolism of VLDL cholesterol through the liver. If bile acid production is compromised, or the direct transport of free cholesterol into bile is enhanced, or phospholipid synthesis is curtailed in any way, lithogenic bile may ensue.

Although the adipose stores were not measured in these hamsters, subsequent experiments have revealed that weight increments above 100 g body weight in male hamsters are highly correlated (r=0.88) with the size of the adipose depots (unpublished observations). Thus, it would appear that the extra body weight in the cholesterol-stone formers represented fat and that this subgroup had assumed certain characteristics of the moderately obese, type-IV hyperlipidemic human characterized by elevated plasma cholesterol and triglyceride concentrations (7,8,23-26). It will be important to determine whether the propensity to gain weight by the subgroup of susceptible hamsters represents excessive food intake (behavioral) or whether this subpopulation is metabolically more energy efficient resulting in an aberrant shift in cholesterol transport and disposition (genetic).

Finally, as we noted previously (6), susceptibility and induction of pigment gallstones would appear to be independent of changes in cholesterolemia or the lipoprotein profile, as the incidence of pigment stones and the degree of pigment stone involvement (number, size, etc.) was similar in control and cholesterol-fed hamsters.

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