Serum Lipoproteins of Rabbits Fed Semi-Purified Diets Varying in Protein and Carbohydrate Source

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The effect of dietary protein and carbohydrate type on the composition and properties of serum lipoproteins in rabbits was studied. The animals were pair-fed either casein-sucrose and soy protein-sucrose or casein-dextrose and soy protein-dextrose-containing low fat, low cholesterol, semi-purified diets for 84 days. Main effects due to protein and carbohydrate type were observed to be altered lipoprotein class compositions. These effects were due primarily to the presence of casein which resulted in cholesterol-rich, triglyceride-poor lipoproteins among all isolated classes. Increases in serum low and high density lipoprotein cholesterol and protein concentrations, as well as lipoprotein total mass, were also observed with casein feeding. Carbohydrate effects due to dietary sucrose were elevated high density lipoprotein phospholipid and protein concentrations, independent of protein source. When casein was included, sucrose-containing diets resulted in increased intermediate density lipoprotein cholesterol; when soy protein was used, low density lipoprotein cholesterol elevations were observed. Dietary interactions were also found especially between casein and sucrose, resulting in increased intermediate lipoprotein mass and total lipoprotein concentrations. Comparisons of lipoprotein class particle size among rabbits indicated similar particle diameters among animals in all dietary groups. Evidence that casein feeding caused increased lipoprotein particle number in addition to compositional differences was obtained. It was concluded that lipoprotein structure within a given class is maintained in spite of compositional variations due to dietary protein or carbohydrate source.

That hypercholesterolemia and atherosclerosis can be produced in rabbits by feeding cholesterol-free, semi-purified diets has been known for some time (1-3). The hypercholesterolemia observed is enhanced by adding saturated fats, largely prevented by the addition of unsaturated fats and is analogous to similar effects seen in human studies (4). These changes do not seem to occur, however, in rabbits fed commercially available chow-type diets even when large amounts of saturated fats are added (5). The nonlipid portion of semi-purified diets has thus been implicated in the hypercholesterolemic response when low fat, low cholesterol diets are fed. While type of fat should not be absolved of guilt in the complex etiology of atherogenesis, the effects of the nonlipid dietary components must also be known and understood in order to fully explain their role in

TABLE 1

Composition of Diets (%)

Ingredient	Diet			
	CD	SD	CS	SS
Casein (vitamin-free)[a]	25.0	–	25.0	–
Soy protein isolate[b]	–	25.0	–	25.0
Dextrose[c]	51.5	51.5	–	–
Sucrose[d]	–	–	51.5	51.5
Corn oil	2.5	2.5	2.5	2.5
Cellulose (alphacel)[e]	15.0	15.0	15.0	15.0
Mineral mix[f]	5.0	5.0	5.0	5.0
Vitamin mix[g]	1.0	1.0	1.0	1.0

[a]Crude protein, 99.1%

[b]Crude protein, 98.8%; DL methionine (99%); DL methionine (99%) added at 2.2 mg/kg.

[c]Anhydrous, 99+%.

[d]Crystalline powder.

[e]Fiber length 30-35 microns.

[f]USP XIV salt mix, contains (in percent): $Al_2(SO_4)_3.24H_2O$, 0.0009; $CaHPO_4 4\ 2H_2O$, 11.28; $CaCO_3$, 6.86; $Ca_2\ (C_6H_5O_7)_2\ 4H_2O$, 30.83; $CuSO_4$, 0.008; $Fe(NH_4)(C_6H_5O_7)_2$, 1.526; $MgCO_3$, 3.520; $MgSO_4$, 3.83; $MnSO_4$, 0.02; KCl, 12.47; KI, 0.004; KH_2PO_4, 21.88; NaCl, 7.71; NaF, 0.05. Supplemented with $ZnCO_3$, 0.05 and $Cr(C_2H_3O_2)$, 0.002.

[g]Vitamin mix (in g/kg diet): Vitamin A acetate (200,000 units/g), 0.099; vitamin D_3 (400,000 units/g), 0.0055; α–tocopheryl acetate, 0.11; ascorbic acid, 0.99; inositol, 0.11; choline chloride, 1.65; menadione, 0.050; p-aminobenzoic acid, 0.11; niacin, 0.099; riboflavin, 0.022; pyridoxine hydrochloride, 0.022; thiamin hydrochloride, 0.022; calcium pantothenate, 0.066; biotin, 0.0044; folic acid, 0.002; vitamin B_{12}, 0.00003.

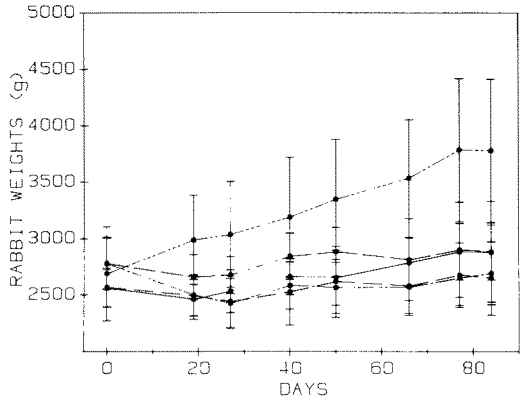

FIG. 1. Mean body weight of rabbits fed the various diets during the experimental period: (●— - - —●), C; (●— · —●), CS; (●— · —●), SS; (●———●), CD, and (●— —●), SD. For abbreviations used see text.

this disease process in the rabbit model.

The atherogenic effect of casein versus soy protein in rabbits and other animal species is well established and has recently been reviewed (6,7). Additionally, dietary carbohydrate type can alter lipid metabolism and possible susceptibility to atherosclerosis (7). Epidemiological observations, animal studies, and experiments in humans have indicated changes in atherosclerosis mortality, experimental atherosclerosis, or risk factors associated with atherosclerotic heart disease when dietary sucrose is compared with glucose or starch (8-12). These observations are inconsistent, however, and mechanisms which may involve triglyceride and cholesterol synthesis and/or bile acid turnover have yet to be specifically defined. More detailed studies are needed in order to better understand these processes.

A considerable amount of work has been done on the atherogenic potential of various semi-purified diets in the rabbit (6-9). However, unified experiments which investigate the effect of both dietary protein and carbohydrate type on the properties of serum lipoproteins in this species have not been reported. The objective of this study was to provide an initial characterization of the composition and properties of the serum lipoproteins, including the intermediate density lipoprotein (IDL, $1.006<d<1.019$ g/ml), in rabbits fed different protein and carbohydrate sources as components of low fat, low cholesterol, purified diets. To this end, compositional analysis of the lipoprotein subclasses, lipoprotein electrophoresis, electron microscopy of the very low density lipoprotein (VLDL), and estimated lipoprotein particle diameters are presented.

TABLE 2

Serum Cholesterol and Triglyceride Concentration of Rabbits[a]

Dietary group	Cholesterol	Triglyceride
	mg/dl	
CD	175 ± 30[b]	77 ± 9
SD	48 ± 10	116 ± 21
CS	258 ± 80[c]	111 ± 23
SS	42 ± 4	130 ± 11
C	82 ± 5	91 ± 17

[a]Mean ± SEM. For dietary group abbreviations see text.
[b]Significantly different from SD, $p<0.05$.
[c]Significantly different from SS, $p<0.05$.

MATERIALS AND METHODS

Animals and diets. In the experiment, 30 3-month old New Zealand white rabbits, matched for size (ca. 2 kg), were used. The rabbits (6 animals/dietary group) were pair-fed pelleted, semi-purified diets (ICN Nutritional Biochemicals, Cleveland, Ohio) containing either casein-sucrose (CS) and soy protein-sucrose (SS) or casein-dextrose (CD) and soy protein-dextrose (SD) during an experimental period of 84 days (Table 1). Pair feeding was begun after the first week of the experiment, during

TABLE 3

Composition of Very Low Density Lipoproteins of Rabbits[a]

Dietary group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Phospholipid (mg/dl)	Protein (mg/dl)
CD	12.0 ± 2.5 (36.3)	8.3 ± 2.0[b] (25.1)	9.3 ± 1.6 (28.1)	3.5 ± 0.9 (10.6)
SD	7.1 ± 1.3 (13.4)	22.9 ± 3.9 (43.1)	15.9 ± 4.6 (29.9)	7.2 ± 2.5 (13.6)
CS	19.1 ± 11.3 (36.6)	12.2 ± 4.4 (23.4)	14.9 ± 4.5 (28.5)	6.0 ± 2.0 (11.5)
SS	7.5 ± 1.7 (19.1)	19.0 ± 2.3 (48.3)	9.6 ± 1.1 (24.4)	3.2 ± 0.4 (8.1)
C	6.3 ± 3.1 (15.0)	23.0 ± 14.5 (54.6)	9.2 ± 5.1 (21.9)	3.6 ± 2.0 (8.6)

[a]Mean ± SEM. For dietary group abbreviations see text. Values in parentheses are relative percent composition.
[b]Significantly different from SD, $p<0.05$

which 100 g of each respective diet was offered daily to each rabbit in its appropriate group and actual consumption recorded. During this time it was noted that the rabbits fed the soy protein-containing diets consumed less per day than their casein-fed counterparts. As a result, when pair-feeding was started (day 8), the amount of pelleted diet actually consumed by each rabbit in either the SS or SD group on the previous day was fed to the paired rabbit in either the CS or CD group. Each animal in the SS group had its dietary pair in the CS group, and each rabbit in the SD group had its dietary pair in the CD group throughout the experiment. Food consumption was noted daily, and body weights were recorded at ca. 10-day intervals during the course of the study. A fifth group of rabbits (C) was fed a standard, chow-type, pelleted diet (Ralston Purina Co., St. Louis, Missouri) concurrently. Data obtained from these animals were for comparison purposes only and not subjected to statistical analysis.

Sample collection and lipoprotein isolation. At the end of the experiment, animals were killed without prior fasting by exsanguination. Blood samples, collected in the absence of anticoagulant, were allowed to clot 30 min at room temperature, and serum was isolated after centrifugation at 800 × g.

Known amounts of serum, to which 0.01% EDTA and

TABLE 4

Composition of Intermediate Density Lipoproteins of Rabbits[a]

Dietary group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Phospholipid[b] (mg/dl)	Protein[b] (mg/dl)
CD	7.6 ± 1.5[c] (48.1)	2.9 ± 0.9[e] (18.4)	2.4 ± 0.5 (15.2)	2.9 ± 0.3 (18.4)
SD	5.7 ± 1.0 (18.3)	10.6 ± 2.4 (34.0)	7.0 ± 0.4 (22.4)	7.9 ± 1.1 (25.3)
CS	33.0 ± 14.4[d] (36.9)	14.3 ± 4.5 (16.0)	20.3 ± 7.6 (22.7)	21.8 ± 5.2 (24.4)
SS	7.5 ± 0.7 (18.8)	14.9 ± 3.4 (37.3)	8.0 ± 1.1 (20.1)	9.5 ± 1.4 (23.8)
C	3.5 ± 0.8 (25.5)	5.2 ± 1.4 (37.9)	2.0 ± 0.7 (14.6)	3.0 ± 0.6 (21.9)

[a]Mean ± SEM. For dietary group abbreviations see text. Values in parentheses are relative percent composition.

[b]Significant interactions between protein and carbohydrate sources by 2 × 2 ANOVA, $p<0.05$.

[c]Significantly different from CS, $p<0.05$.

[d]Significantly different from SS, $p<0.05$.

[e]Significantly different from SD, $p<0.05$.

TABLE 5

Composition of Low Density Lipoprotein of Rabbits[a]

Dietary group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Phospholipid (mg/dl)	Protein (mg/dl)
CD	88.7 ± 25.6[b] (44.6)	21.7 ± 5.1 (10.9)	40.1 ± 11.0 (20.2)	48.3 ± 12.5[b] (24.3)
SD	15.2 ± 3.8 (24.4)	16.4 ± 2.3 (26.3)	12.0 ± 3.2 (19.3)	18.7 ± 2.1 (30.0)
CS	144.2 ± 47.5[c] (37.4)	47.1 ± 16.5 (12.2)	91.6 ± 31.9 (23.8)	102.4 ± 29.0[c] (26.6)
SS	15.1 ± 1.7 (21.4)	18.4 ± 2.4 (26.0)	15.2 ± 1.7 (21.5)	22.0 ± 3.1 (31.1)
C	17.2 ± 4.0 (28.5)	7.5 ± 2.5 (12.4)	14.1 ± 2.8 (23.8)	21.6 ± 2.8 (35.8)

[a]Mean ± SEM. For dietary group abbreviations see text. Values in parentheses are relative percent composition.

[b]Significantly different from SD, $p<0.05$.

[c]Significantly different from SS, $p<0.05$.

TABLE 6

Composition of High Density Lipoprotein of Rabbits[a]

Dietary group	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Phospholipid (mg/dl)	Protein (mg/dl)
CD	22.6 ± 3.2[b] (20.8)	6.1 ± 0.4 (5.6)	14.8 ± 4.6 (13.7)	64.7 ± 7.8[b] (59.8)
SD	12.7 ± 2.3 (14.2)	11.5 ± 2.3 (12.8)	17.6 ± 3.1 (19.7)	47.7 ± 4.1 (53.3)
CS	31.8 ± 7.6 (15.9)	11.3 ± 2.5 (5.7)	39.3 ± 8.2[c] (19.7)	117.5 ± 12.9[c] (58.8)
SS	16.9 ± 1.1 (11.4)	12.3 ± 1.9 (8.3)	31.5 ± 4.7[b] (21.2)	88.1 ± 11.7[b] (59.2)
C	27.5 ± 2.4 (14.1)	18.6 ± 4.2 (9.6)	42.2 ± 6.1 (21.6)	106.4 ± 21.1 (54.6)

[a]Mean ± SEM. For dietary group abbreviations see text. Values in parentheses are relative percent composition.

[b]Significantly different from SD, $p<0.05$.

[c]Significantly different from CD, $p<0.05$.

TABLE 7

Free Cholesterol Content of Rabbit Lipoproteins[a]

Dietary group	VLDL	IDL	LDL	HDL
CD	7.8 ± 0.6[b]	10.6 ± 0.5	10.7 ± 0.7[b]	4.7 ± 0.7[b]
SD	3.8 ± 0.5[c]	4.0 ± 0.3	4.1 ± 0.6	1.7 ± 0.2
CS	8.7 ± 1.1	9.0 ± 1.5[c]	8.7 ± 1.0[c]	3.5 ± 1.1
SS	6.7 ± 1.0	5.2 ± 0.4	3.8 ± 0.2	1.4 ± 0.2
C	4.8 ± 0.4	7.1 ± 1.0	6.0 ± 0.8	2.4 ± 0.4

[a]Mean ± SEM. For dietary group abbreviations see text. VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein. Lipoprotein mass determined by summing masses of lipid and protein in the various fractions. Values in parentheses are relative percent of total lipoprotein in each respective dietary group.

[b]Significantly different from SD, $p<0.05$.

[c]Significantly different from SS, $p<0.05$.

0.02% Na azide had been added, were used to isolate lipoprotein classes by sequential density ultracentrifugation as described by Lindgren et al. (13) and Meusing and Nishida (14). The serum was fractionated into four density classes, very low density lipoproteins (VLDL, $d<1.006$ g/ml); intermediate density lipoproteins (IDL, $1.006<d<1.019$ g/ml); low density lipoproteins (LDL, $1.019<d<1.063$ g/ml), and high density lipoproteins (HDL, $1.063<d<1.21$ g/ml). Purity of the lipoprotein fractions was assessed by agarose gel electrophoresis after dialysis against 0.15 M NaCl solution, pH 7.4 containing 0.01% EDTA. Recoveries of lipoprotein fractions averaged 93 ± 3% based on cholesterol recoveries.

Analyses. Electrophoresis of serum lipoproteins was performed using 1% agarose gels containing 5% sucrose and 0.035% disodium ethylenediamine tetraacetate (EDTA) in 65 mM barbital buffer, pH 8.6. Gels were oven-dried at 75 C and lipoproteins were stained with 0.02% fat red 7B, then sequentially cleared and rinsed with CH_3OH:H_2O (1:1, v/v) and 2% glycerol. The separated lipoprotein fractions were scanned densitometrically at 550 nm, and peak areas were integrated using a Gelman ACD-15 scanning densitometer (Gelman Instrument Co., Ann Arbor, Michigan).

Serum and lipoprotein total cholesterol and triglyceride concentrations were measured enzymatically using reagents supplied by Gilford Diagnostics (Gilford Instrument Co., Cleveland, Ohio). Lipid phosphorus concentrations were determined as described by Bartlett (15), and protein was estimated by the procedure of Lowry et al. (16) in the presence of 1% sodium dodecyl sulfate using bovine serum albumin as standard. Lipoprotein free cholesterol was determined as mentioned above after lipid extraction and fractionation on thin layer chromatography (17).

Electron microscopy of freshly isolated VLDL was performed after dilution to avoid artifactual aggregation of particles. Negative staining was accomplished at room temperature with 1% phosphotungstic acid and adjusted to pH 7.1 with KOH according to Stange et al. (18). A drop of the lipoprotein suspension was placed on a formvar and carbon-coated grid, drained onto filter paper after 10-15 seconds and immediately covered with the staining solution for about 30 seconds. Excess stain

TABLE 8

Lipoprotein Concentration of Rabbits[a]

Dietary group	VLDL	IDL[b]	LDL	HDL	Total mass
CD	33 ± 7 (10)	16 ± 3 (5)	164 ± 39[c] (50)	117 ± 4[e] (35)	327 ± 39
SD	53 ± 12 (22)	31 ± 6 (13)	62 ± 7 (26)	90 ± 11 (38)	233 ± 23
CS	55 ± 22 (9)	90 ± 30 (14)	277 ± 66[d] (43)	221 ± 25 (34)	649 ± 88[f]
SS	40 ± 4 (13)	40 ± 6 (13)	71 ± 8 (24)	148 ± 15[c] (49)	307 ± 10
C	42 ± 25 (14)	14 ± 3 (5)	64 ± 12 (21)	178 ± 38 (60)	292 ± 29

[a]Mean ± SEM. For abbreviations see Table 7.

[b]Significant interaction between protein and carbohydrate sources by 2 × 2 ANOVA, $p<0.05$.

[c]Significantly different from SD, $p<0.05$.

[d]Significantly different from SS, $p<0.05$.

[e]Significantly different from CS, $p<0.05$.

[f]Significant interaction between casein and sucrose by 2 × 2 ANOVA, $p<0.05$.

was then removed with filter paper. The grids were air-dried and examined in a Zeiss EM10C electron microscope. Pictures were taken at 112,000 × magnification. Diameters of the VLDL were estimated manually by measurement of a representative number (usually 100) of particles per photomicrograph.

Statistical significance among the various dietary groups was determined by 2 × 2 analysis of variance (ANOVA) to identify any dietary interaction among protein and carbohydrate sources at $p<0.05$. Analysis of main dietary effects as detected by the ANOVA was subsequently performed using a modified Student's t test.

RESULTS

Mean body weights of rabbits fed the paired, purified diets and the commercial chow diet are presented in Figure 1. All rabbits fed the semi-purified diets maintained their initial body weights during the feeding period. Those fed the chow diet tended to gain weight to some extent during the course of the study. Chow-fed animals were fed ad libitum, however; with pair feeding, consumption may have been restricted somewhat. Feed consumption among the rabbits fed the CS and SS diets averaged 55 g/day and, in rabbits fed the CD and SD diets, 50 g/day. Calculated average caloric intakes of rabbits fed the purified diets approximated those generally regarded as necessary for maintenance but not optimal growth (19).This finding is in agreement with the final weights observed in these animals. Data of rabbits fed the chow diet are intended only as a point of reference and were not used for statistical comparisons.

Table 2 shows the serum total cholesterol and triglyceride concentrations at the end of the feeding period. Rabbits fed both the CS and CD diets were moderately, yet significantly, hypercholesterolemic when compared to their respective soy protein-fed rabbit pairs. Changes in serum triglyceride concentrations were not apparent.

TABLE 9

Agarose Gel Electrophoresis of Rabbit Lipoproteins[a]

Dietary group	α	Pre-β	β
	%		
CD	53.1 ± 3.6	21.9 ± 4.3	29.7 ± 1.7
SD	36.4 ± 6.4	37.5 ± 4.1	25.7 ± 3.1
CS	65.8 ± 2.4[b]	17.0 ± 1.8[b]	17.1 ± 1.4[c]
SS	32.5 ± 3.0	39.9 ± 2.8	27.7 ± 0.8
C	37.5 ± 3.8	16.8 ± 3.6	44.8 ± 2.5

[a]Relative percent composition ± SEM. For dietary group abbreviations see text.

[b]Significantly different from SS, $p<0.05$.

[c]Significant interaction between both casein and sucrose by 2 × 2 ANOVA, $p<0.05$.

Compositional differences among the VLDL and IDL fractions of rabbits fed the purified diets were variable. Serum VLDL of animals fed the casein diets tended to be triglyceride poor and cholesterol rich (Table 3). Variability of this response, however, precluded statistical significance by ANOVA in most cases. Only the triglyceride concentrations of CD diet-fed rabbits were statistically different from the SD diet-fed animals in this lipoprotein class. In the case of serum IDL (Table 4), CS diet-fed animals were found to have elevated cholesterol concentrations compared to the SS diet-fed group. A carbohydrate effect was also noted resulting in increased IDL cholesterol in those rabbits fed casein when sucrose was present. Significant dietary interactions between protein and carbohydrate sources occurred in both the phospholipid and protein concentrations

of the IDL. Pronounced increases in these components were seen in the CS diet-fed group compared with the other purified diet-fed animals. Serum LDL (Table 5) and HDL (Table 6) of rabbits fed either casein-containing diet showed increased cholesterol and protein concentrations compared to their soy protein pairs. Additionally, carbohydrate effects were noted in HDL phospholipid fractions. When sucrose was fed, regardless of protein source, significant increases in HDL phospholipid content resulted. Similar findings were seen regarding HDL protein concentrations. Significantly increased HDL protein was also noted when casein was fed compared to soy protein in the dextrose-containing diets.

The free cholesterol content of rabbit lipoproteins can be seen in Table 7. Casein feeding resulted in increased lipoprotein-free cholesterol in all fractions analyzed compared to the soy protein-fed animals. In the CS diet-fed case, these increases were statistically significant only in the IDL and LDL fractions, however. In addition, a significant carbohydrate effect was seen in the VLDL of rabbits fed the SS diet with elevated free cholesterol concentrations observed compared to the SD case.

Increases in total lipoprotein mass were observed when casein-containing diets were fed compared to their respective soy protein diet pairs (Table 8). Elevations in HDL mass were seen with casein feeding, however, only when sucrose was also present (CS vs SS groups). A carbohydrate effect resulting in increased total HDL was also found when sucrose diets were fed. Lastly, interactions between carbohydrate and protein were observed to affect IDL concentrations in the CS diet-fed case, resulting in a marked elevation in this lipoprotein class. A similar interaction was seen when total lipoprotein mass was analyzed by the 2 × 2 ANOVA used in this study.

Densitometric evaluations of the lipoproteins after agarose gel electrophoresis are indicated in Figure 2 and Table 9. Figure 2 depicts individual scans of serum lipoproteins of representative animals from each dietary group, whereas Table 9 shows the combined means ± SEM of the relative percentage lipoprotein composition of all animals fed their respective diets. Qualitatively, casein feeding appeared to cause a relative shift in the β-lipoprotein band with a concomitant decrease in the α band. Soy protein, on the other hand, apparently resulted in an increased pre-β fraction. Statistically, an increase in β and decrease in pre-β lipoprotein fractions were observed when the CS diet was fed, compared with the SS case. In addition, changes in the relative amount of α lipoprotein content in the CS diet-fed group were observed as a result of dietary interactions between casein and sucrose as determined by ANOVA.

Lipoprotein size (Tables 10 and 11) was estimated from mean compositional analysis and, in the case of VLDL, also by electron microscopy (Fig. 3). Due to the hetereogeneity (18) of VLDL molecular weights, actual measurements of particle diameters were made on this fraction in addition to their calculation from compositional data. Calculated mean VLDL particle diameters were similar overall to those obtained by electron microscopy (375 Å vs 333 Å). Furthermore, no differences among any of the lipoprotein fraction diameters were found as the result of dietary influences (Table 11).

DISCUSSION

Earlier, we reported that rabbits fed casein-containing semi-purified diets for 84 days became hypercholesterolemic (20). In that study, changes in the distribution

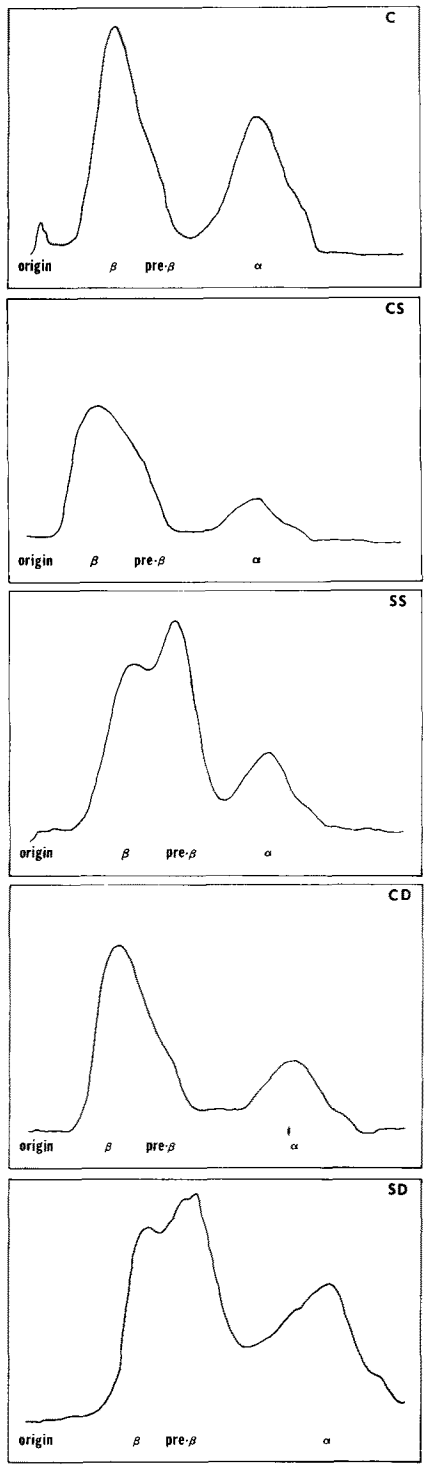

FIG. 2. Scanning densitometry of rabbit serum lipoproteins on 1% agarose gel electrophoresis. For abbreviations used see text.

TABLE 10

Molecules of Core Components per Particle Calculated From Compositional Data[a]

Dietary Group	Particles							
	VLDL		IDL		LDL		HDL	
	TG	CE	TG	CE	TG	CE	TG	CE
CD	5790	8590	650	1730	270	1100	20	70
SD	9940	2900	1200	660	650	660	40	50
CS	5400	8410	570	1290	300	930	20	50
SS	11140	3740	1320	630	640	570	30	40
C	12590	3080	1340	850	310	730	30	50

[a]For dietary group and lipoprotein abbreviations used see text. TG, triglyceride; CE, cholesteryl ester. Molecules per particle were calculated from molecular weight and mean weight percentage composition, assuming no structural water. Lipoprotein particle molecular weights used were as follows: VLDL, 19.6×10^6; IDL, 3.0×10^6; LDL, 2.1×10^6; HDL, 0.27×10^6, and are based on data of Lindgren (IDL, LDL) (32) and Shen (VLDL, HDL) (33). Other molecular weights used were: triglyceride, 850; cholesteryl ester, 650.

of cholesterol among lipoprotein fractions were observed when casein diets were fed. In the present work, we have observed that main effects of dietary protein and, to some extent, carbohydrate source can influence lipoprotein composition but not particle size.

While the effects of casein feeding on VLDL and IDL composition were variable, these lipoproteins were observed to be cholesterol rich and triglyceride poor in rabbits fed this protein source, as has been reported previously (6,21-25). Additionally, casein feeding was observed to increase LDL and HDL free and total cholesterol and protein concentrations independent of carbohydrate source used in this study. Terpstra et al. (22) observed similar changes in their studies on the time course of the hypercholesterolemia in rabbits fed similar casein-containing diets which were also higher in fat content. Main effects of dietary carbohydrate source seen in this study included a marked increase in IDL cholesterol content when the CS diet was fed, compared with CD diet feeding. Additionally, increased HDL phospholipid and protein concentrations were observed with sucrose feeding, especially when CS and SS diets were compared with the SD group. Changes in IDL cholesterol content may be the result of the more lipogenic nature of sucrose (26,27). Furthermore, when sucrose is fed, not only does lipoprotein cholesterol content increase (26) but increased VLDL production may also occur (8,27-29), thereby contributing to the overall lipoprotein flux in the circulation due to VLDL triglyceride hydrolysis and lipid and protein transfer mechanisms.

In a number of instances, dietary interactions between protein and carbohydrate sources also have been noted. The feeding of casein in conjunction with sucrose appeared to have the most marked effect on lipoprotein composition in this study. This interaction is especially evident as manifested by elevations in IDL phospholipid and protein concentrations and by increased total lipoprotein mass. It should be mentioned that decreased α lipoprotein content determined by agarose gel electrophoresis was also observed in serum of rabbits fed the CS diet, apparently as the result of a protein-carbohydrate interaction. This discrepancy with total lipoprotein mass data might best be explained, in part,

TABLE 11

Lipoprotein Particle Size[a]

Dietary group	Diameter (A)			
	VLDL	IDL	LDL	HDL
CD	367	218	186	98
SD	370	212	189	102
CS	362	204	182	95
SS	385	214	186	96
C	393	220	175	98

[a]Particle diameters (d) were calculated according to Shen (33) using the following equation: $d = 2\{[1556n_{tg} + 1068n_{ce}/(4/3)]1/3 + 20.5\}$, where n_{tg} = molecules of triglyceride/particle and n_{ce} = molecules of cholesteryl ester/particle from Table 10.

by the reported lower affinity of fat red 7B for cholesterol and its esters compared with other lipids (30). Consequently, lipoprotein triglyceride and phospholipid may be stained most intensely. A large amount of β migrating lipoprotein with an increased lipid mass (especially triglyceride and phospholipid, see Table 5) may therefore be relatively more intensely stained than the α (or HDL) lipoproteins. Also, in view of the finding that the composition of CS diet-fed rabbit HDL was not as markedly different in its relative concentrations of triglyceride and phospholipid from that of animals fed the other purified diets, relatively less staining intensity of α lipoproteins may have resulted. This explanation, however, is purely speculative. Direct comparisons of electrophoretic and mass lipoprotein data should most likely be avoided due to the limitations of uniform staining of lipoprotein particles.

In spite of compositional differences of lipoproteins of rabbits fed the purified diets, the sum of core molecules per particle was constant (Table 10). As a result, in those instances where cholesterol enrichment occurred, triglyceride depletion was also present. This correlation suggests that the physical forces which govern the structural organization of lipoproteins are cap-

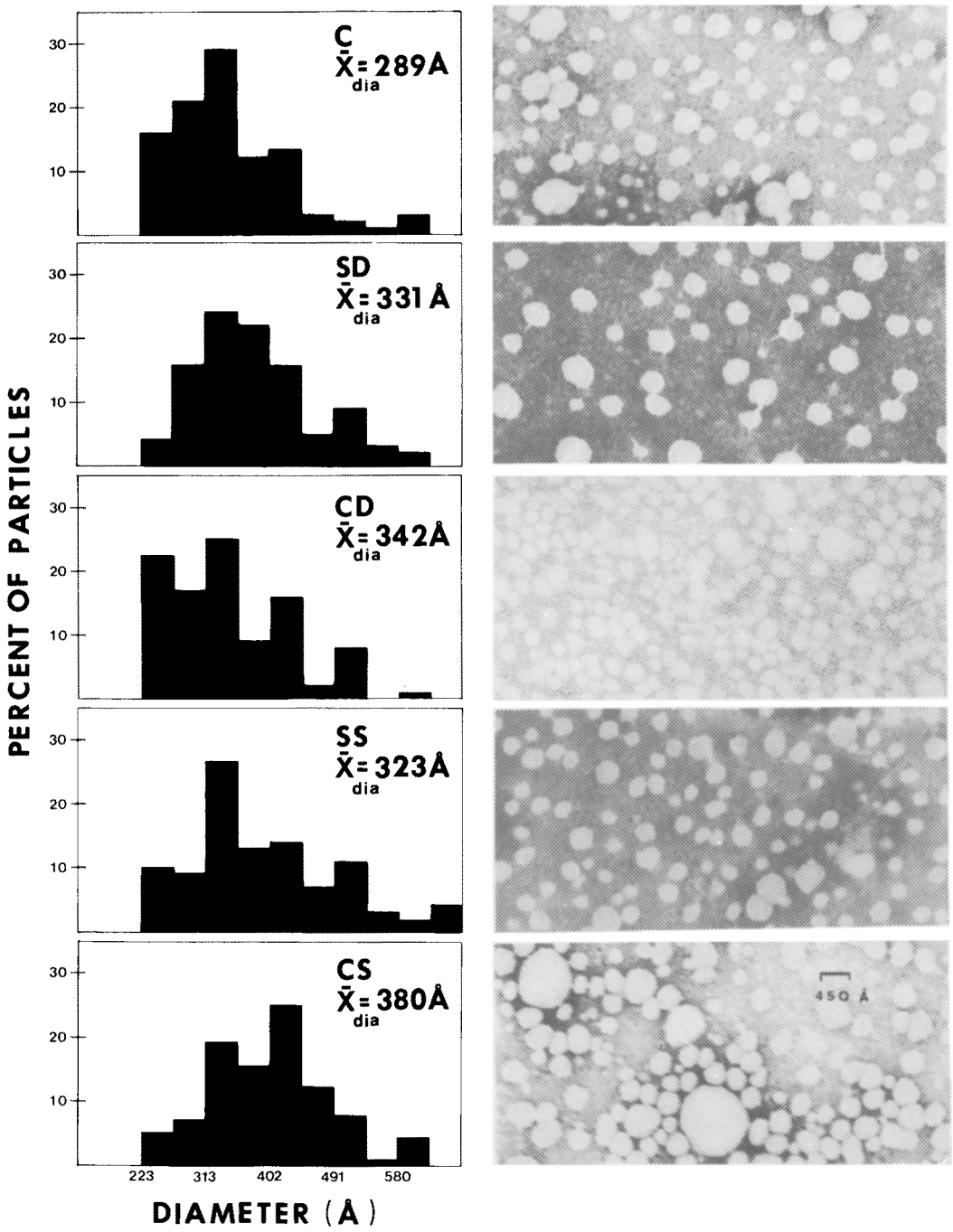

FIG. 3. Electron microscopy of serum VLDL of rabbits fed the diets for 84 days. 112,000 × magnification. For abbreviations used see text.

able of maintaining particle size in spite of compositional variations within a given class.

Comparisons of lipoprotein size among rabbits fed the various semi-purified diets in this study are also of interest. It was found that calculated lipoprotein particle diameters were similar for each lipoprotein class analyzed, independent of the diets used. These values are valid, however, only to the extent that the assumed molecular weights of the lipoprotein fractions used in the calculation are reasonable (31,32), and that lipoprotein structure is generally consistent with a model in which a hydrophobic core of triglycerides and cholesteryl esters is surrounded by a 20.5-Å thick monolayer of free cholesterol, phospholipid and protein closely packed on the surface of the core (33). Using this approach, calculated VLDL diameters would perhaps be subject to the greatest amount of variability due to the heterogenous molecular size within the d<1.006 g/ml class (33). It was found, however, that comparisons of actual measured VLDL diameters after electron microscopy with calculated values from mean compositional analysis revealed VLDL particles of similar size. There-

fore, comparisons of calculated lipoprotein diameters as a function of protein and carbohydrate type determined in this study appear valid. The increase in particle mass per volume is consistent with an increase in particle number.

Evidence that casein feeding causes increased lipoprotein particle number was thus obtained in this study. Calculated amounts of individual lipoprotein fractions indicated that the consumption of either the CS or CD diet significantly increased both IDL and LDL total mass and particle number as well as their cholesterol content. Total number of HDL particles were also increased in rabbits fed this diet. Furthermore, interactions between casein and sucrose were observed to affect IDL particle number as well as total serum lipoprotein number.

It is tempting to speculate about possible mechanisms involved in the production of cholesterol-rich low density lipoprotein with casein feeding. By comparison, when the rabbit is fed cholesterol, a large portion of it is carried in so-called β-VLDL particles (34,35). It has been suggested that the origin of these particles may be either secretion as such by the liver (34) or the result of lipoprotein lipase acting on chylomicrons produced in the intestinal mucosa (35). In the casein-fed rabbit, results of the present study and others (36) indicate that observed cholesterol-rich VLDL and/or IDL is similar in many respects to this β-VLDL. It is uncertain, based on the present work, whether the VLDL of casein feeding are due to intestinal or hepatic synthesis or whether they are metabolic remnants of hepatic or intestinal lipoproteins containing endogenous cholesterol. In any event, the subsequent action of a normal functioning triacylglycerol hydrolase system would result in the conversion of these VLDL to cholesterol-rich IDL and LDL particles (37). Increases in any of these serum fractions, especially if cholesterol enriched, may then contribute to the down regulation of hepatic receptors leading to the elevations among these lipoproteins in the circulation (38-40) and subsequent atherogenic risk.

In the present work, increases in VLDL cholesterol were not statistically significant when casein was fed compared with the soy protein diets. Nonetheless, a tendency toward cholesterol enrichment of VLDL was observed with casein feeding. Reasons for this finding might best be explained due to the existence of both hyper- and hyporesponder rabbits among those used in this study. Differences in susceptibility to hypercholesterolemia within the New Zealand white strain have been reported previously due to dietary modification including casein feeding (41,42). Indeed, within the CS diet-fed animals in the present experiment, lipoprotein cholesterol content was quite variable as evidenced by relatively large standard errors of means observed. While this variation has been reported previously (20,22,24,43)(29-32), reasons for it are unclear. It may be that interactions between any one of a number of dietary constituents might contribute to this effect. The presence of dextrose as carbohydrate source when casein-containing diets are used may thus help minimize these within-group differences. The exclusive use of either hyper- or hyporesponder animals after a preliminary screening may also be warranted in studies involving casein diet feeding.

The assessment of VLDL particle diameters based on both electron microscopy and compositional analysis revealed few differences among rabbits fed the various diets in this study. Shore et al. (34) have reported that VLDL particle size of cholesterol-fed rabbits were larger than normal VLDL of rabbits fed commercial chow diets. The possibility of cholesterol fed rabbit VLDL being a remnant of triglyceride-rich, intestinal lipoproteins containing dietary cholesterol may help reconcile this difference (36). More recently, however, Kroon et al. have shown that the β-migrating VLDL of cholesterol fed rabbit is of hepatic origin (42). A similar mechanism may exist with casein feeding, in which case the production of cholesterol-rich VLDL may be a primary source of serum cholesterol. The extent of this response, however, may not be as great. The feeding of 1% cholesterol as used by Shore et al. (34) resulted in elevations of serum total cholesterol of from 1300 to 2000 mg/dl after 10 days, while the endogenous hypercholesterolemia of casein feeding in the present study resulted in only a moderate hypercholesterolemia. Studies on the production and turnover of these lipoproteins and their apoprotein composition will provide evidence to better define actual mechanisms responsible for the changes observed in this experiment.

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