The role of dietary protein source in the development of cholesterol metabolism in rabbits

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Twenty-one male 4-week-old New Zealand white rabbits were assigned to one of three dietary treatments (seven rabbits/treatment) in which protein source was derived from varying casein to whey ratios (100:0, 80:20, 40:60). Blood was collected at 10, 14, 18, and 20 weeks and animals were killed at 24 weeks and blood, livers, and aortas were removed. From weeks 20 to 24, a 0.4% cholesterol diet was fed. Results showed that animals fed the 40:60 diet had highest blood cholesterol concentrations prior to the cholesterol challenge ($P < 0.05$). After the challenge, differences disappeared. Low density lipoprotein and high density lipoprotein cholesterol concentrations in 40:60 animals were also higher than in 80:20 animals until 20 weeks of age ($P < 0.05$). Total triglycerides were highest in 100:0 fed animals at 10 and 14 weeks of age ($P < 0.05$) and stayed highest in this group after the challenge ($P < 0.05$). Rabbits fed 40:60 diet had higher 3-hydroxy-3-methylglutaryl coenzyme A reductase activities than the 100:0 rabbits ($P < 0.05$). Total liver lipids were also higher in the 40:60 than 100:0 group ($P < 0.01$), and fecal cholesterol content was higher in the 80:20 than 100:0 group ($P < 0.05$). Bile acid excretion and atherosclerotic plaque formation were not affected by dietary treatment. These results indicate that plasma lipids are influenced by dietary protein source during the postweanling period, but the mechanism for this response remains to be defined. (J. Nutr. Biochem. 5:232–237, 1994.)

Keywords: cholesterol; protein; rabbits; lipoproteins; HMG CoA reductase

Introduction

Available evidence suggests a causal relationship between diet, blood lipid concentrations early in life, and subsequent coronary heart disease in adulthood.[1,2] Reiser and Sidelman[3] stimulated interest in this area of study with their "cholesterol challenge" hypothesis. This hypothesis is based on their findings that lower blood cholesterol concentrations were found in adult rats that had been fed a high cholesterol diet during the suckling period. Although it is generally believed that a critical period exists during which the adjustment of cholesterol levels in the body can have a permanent effect on adult cholesterol metabolism, neither this period nor the causative dietary factors have been defined.

The suckling and weanling stages of development have received much attention. At weaning, abrupt dietary changes occur, from a diet composed of only milk to one of varied solid foods. Likewise, drastic metabolic changes occur. Dietary modifications during both the suckling and weanling stages have been shown to have a lasting impact on lipid metabolism.[4–6] Unfortunately, results from studies to date are difficult to interpret due to the use of different animal models, the potential for interaction of multiple dietary factors, and the inconsistent assessment of long-term effects on cholesterol metabolism and atherosclerosis in adulthood.

Although many investigations have focused mainly on the lipid components of milk, other dietary components, such as protein source, cannot be overlooked as playing a role in the development of cholesterol metabolism. Proprietary infant formulas generally contain varying ratios of the proteins casein and whey, and their effects on cholesterol metabolism in human infants are not completely understood. While casein is described as a hypercholesterolemic protein capable of producing atherosclerosis without added cholesterol in rabbits,[7] the effects of whey protein on cholesterol metabolism have received less attention. Therefore, the present study was designed to examine the effect of varying dietary casein to whey ratios during the postweanling period

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on the development of plasma lipid profiles, cholesterol metabolism, and atherosclerosis in rabbits.

Methods and materials

Animals and diets

Twenty-one male weanling New Zealand white rabbits (LSR Industries, Union Grove, WI USA; mean body weight 805 g ± 117 g, 4 weeks of age) were used. Upon arrival, rabbits were individually housed in stainless-steel cages at 21° C on a 12-hour light-dark cycle. All animals were fed nonpurified rabbit chow (alfalfa base) initially and then randomly assigned to one of three treatment groups. Groups 1 to 3 (n = 7/group) were then fed diets similar in all respects except for dietary protein source *(Table 1)*. Group 1 received 100% casein (casein:whey = 100:0); Group 2 received 80% casein and 20% whey (casein:whey = 80:20); and Group 3 received 40% casein and 60% whey (casein:whey = 40:60). Amino acid compositions of these diets are given in *Table 2*. Rabbits were adapted to the experimental diets through gradual incorporation of the pelleted experimental diet into nonpurified diet until each rabbit was consuming 100% pelleted experimental diet. This process took 4 days. All animals received the experimental diets until 20 weeks of age. From 20 to 24 weeks of age, animals received the same experimental diets with 0.4% cholesterol added to provide a modified cholesterol challenge. For a more exact measure of the lasting effects of protein on cholesterol metabolism, the challenge would need to be given in the nonpurified basal diet as opposed to the experimental diets. Rabbits were pair-fed by group for the duration of the experiment with 40:60 animals provided with food ad libitum, and the other two groups fed the amount this group consumed. Water was continuously available.

Plasma lipids

Blood samples were collected after a 12-hr fast into tubes containing EDTA (2 g/L blood collected) from the marginal ear vein at 10, 14, 18, 20, and 24 weeks of age. Plasma was collected after centrifugation for 30 minutes at 1200g, 4° C. High density lipoproteins (HDL) were separated using affinity chromatographic columns (Isolab, Inc., Akron, OH USA).[8] Very low density lipoproteins (VLDL) were separated by density gradient ultracentrifugation (d = 1.006 kg/L) for 24 hr at 105,000g, 10° C.[9] Cholesterol concentrations of plasma, HDL, and VLDL fractions and total triglyceride concentrations of plasma were analyzed enzymatically[10,11] (Sigma Diagnostics, St. Louis, MO USA). Low density lipoprotein (LDL) cholesterol concentration was determined by subtracting VLDL and HDL cholesterol from total cholesterol.

Atherosclerotic lesion quantification

At 24 weeks of age, all rabbits were killed by sedation with ketaset/rompun (1.5 mL/kg body weight), followed by CO_2 asphyxiation. Aortas were removed from the aortic arch to below the last point of bifurcation, flushed with normal saline, and fixed in 4% formalin solution. Aortas were transferred to the Wistar Institute of Anatomy and Biology (Philadelphia, PA USA) for examination by an individual blinded to the treatments. Aortas were stained with 0.5% Sudan IV (Sigma Chemical Company, St. Louis, MO, USA) in 70% isopropanol and intimal lesions quantified using grid point counting technique.[12]

HMG CoA reductase activity

Livers were removed and portions from all lobes were minced. From this, 4 g were homogenized and microsomes were prepared as described by Shapiro et al.[13] and Roitelman and Shecter.[14] Briefly, liver was homogenized in 15 mL buffer (30 mmol/L EDTA, 250 mmol/L sodium chloride, 1 mmol/L dithiothreitol, 50 mmol/L potassium phosphate, pH 7.4). The homogenate was centrifuged twice at 12,000g for 15 minutes at 4° C. The supernatant was collected and then centrifuged at 105,000g for 90 minutes, 4° C. Microsomal pellets were collected and stored under liquid nitrogen until analyses. 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity was assessed in a dual-labeled system using ^{14}C HMG CoA and ^{3}H-mevalonate (New England Nuclear, Boston, MA USA).[15] The reaction was carried out at 37° C for 20 minutes, stopped by the addition of 10 mol/L HCl, and allowed to lactonize for 60 minutes at 37° C. ^{14}C-mevalonate produced was separated by thin-layer chromatography (Silica Gel G, Alltech, Deerfield, IL USA), counted, and quantified. Protein content of microsomes was quantified using the bicinchoninic acid (BCA) copper reagent method for use in the presence of sulfhydryl reagents.[16]

Table 1 Experimental diet composition

Ingredients (g/100 g)	Casein:whey ratios		
	100:0	80:20	40:60
Casein*	23.0	18.4	9.2
Whey†	0.0	5.3	16.0
Cellulose‡	15.0	15.0	15.0
Corn oil	5.0	5.0	5.0
Molasses	5.0	5.0	5.0
Sucrose	20.0	20.0	20.0
Dextrin	5.0	5.0	5.0
Choline-Cl§	0.1	0.1	0.1
Mineral mix‖	6.0	6.0	6.0
Vitamin mix¶	1.0	1.0	1.0
Cornstarch	19.9	19.2	17.7

*Casein: Alacid 710 (lactic casein, 87% protein dry weight basis), New Zealand Milk Products, Inc., Petaluma, CA USA.
†Whey: Calpro 75 (whey protein concentrate, 75% protein dry weight basis), Calpro Ingredients, Corona, CA USA.
‡Cellulose: Solka Floc Grade BW-200FCC, James River Corporation, Berlin, NH USA.
§Choline-Cl: Sigma Chemical Company, St. Louis, MO USA.
‖Mineral mix: (g/kg) $DiKPO_4$ 176.85, K Bicarbonate 141.475, NaCl 88.425, Ca Carbonate 221.075, Dicalcium PO_4 176.85, Mg Sulfate 176.86, CoCl $6H_2O$ 0.0619, $CuSo_4$ $5H_2O$ 0.6119, $MgSo_4$ H_2O 1.43425, $ZnSO_4$ $7H_2O$ 2.988775, K Iodide 0.17685, Ferric Citrate 12.490925, Ammonium Molybdate 0.40145), 960094 ICN, Biochemicals, Cleveland, OH USA.
¶Vitamin mix (g/kg) (thiamine HCl 2.5, riboflavin 1.6, D-Ca pantothenate 2.0, pyridoxine HCl 0.6, biotin 0.06, folic acid 0.5, menadione 0.5, vitamin B_{12} (0.1% trit) 25.0, ascorbic acid 25.0, niacin 15.0, vitamin A acetate 2.0, vitamin D_3 0.15, vitamin E acetate 24.0) 960094 ICN, Biochemicals, Cleveland, OH USA.

Table 2 Amino acid composition of experimental diets

Amino acid	Casein:whey ratios (g/kg)		
	100:0	80:20	40:60
Ala	0.598	0.703	0.917
Arg	0.782	0.744	0.671
Asp	1.541	1.715	2.082
Cys	0.069	0.152	0.320
Glu	4.715	4.627	4.467
Gly	0.345	0.369	0.418
His	0.621	0.571	0.472
Ile	1.150	1.170	1.226
Leu	1.909	2.010	2.221
Lys	1.771	1.814	1.907
Met	0.690	0.641	0.543
Phe	1.173	1.064	0.848
Pro	2.116	1.987	1.736
Ser	1.380	1.337	1.256
Thr	0.966	1.091	1.346
Trp	0.368	0.339	0.282
Tyr	1.311	1.160	0.860
Val	1.495	1.440	1.336

Composition of the diet was determined by the manufacturer.

Liver lipid analyses

The remaining liver portions were frozen at −20° C for subsequent analysis of lipid content. Briefly, total lipids were extracted with chloroform:methanol (2:1) and quantified gravimetrically.[17]

Fecal cholesterol content

Total fecal lipid was extracted with chloroform:methanol (2:1)[17] and aliquots quantified enzymatically for cholesterol (Sigma Diagnostics).[10,11]

Fecal bile acid excretion

Feces were collected on wire screens placed over every cage tray for the final 4 days of the experiment, pooled, and dried. Total bile acids were extracted with butanol:water (1:1)[18] and quantified enzymatically (Sigma Diagnostics).[19]

Statistics

A repeated measures analysis of variance was used to analyze plasma lipids, and a one-way analysis of variance was used for analyses of atherosclerotic lesion development, HMG CoA reductase activity, liver lipid content, and bile acid excretion.[20] Mean comparisons were made using Tukey's Honestly Significant Difference (HSD) test. A significance level of $P < 0.05$ was used to define statistical significance. Values in text are means ± SEM.

Results

Weight gain from 4 to 24 weeks of age in 40:60 fed rabbits was significantly lower than the other two groups ($P < 0.05$), as was feed efficiency ($P < 0.01$). Mean weight gain over the experimental period for animals fed the 100:0, 80:20, and 40:60 formulations was: 2646 ± 126 g, 2729 ± 90 g, and 1940 ± 250 g, respectively, with feed efficiencies of 225 ± 12, 234 ± 27, and 155 ± 42 g weight gain per kg feed intake.

Mean plasma total, LDL, HDL, and VLDL cholesterol concentrations are given in *Table 3*. Prior to the cholesterol challenge, rabbits fed the 40:60 diet had highest total cholesterol concentrations at all timepoints compared with the other two groups ($P < 0.05$). Following the cholesterol challenge, concentrations between groups were similar, but elevated compared with the previous timepoint ($P < 0.05$).

LDL cholesterol concentrations until 20 weeks of age were higher in animals fed the 40:60 compared with the 80:20 formulation ($P < 0.05$). In rabbits fed 100% casein, concentrations were similar to the 40:60 group at 10 and 20 weeks, but at 14 and 18 weeks, concentrations were lower in the 100:0 group ($P < 0.05$).

HDL cholesterol concentrations at all timepoints prior to the cholesterol challenge were higher in rabbits consuming the 40:60 diet compared with those who ate the 80:20 diet ($P < 0.05$). At 14 weeks 100:0 rabbits had similar HDL values as 40:60 rabbits, but at 20 weeks 40:60 rabbits had higher values than 100% casein-fed rabbits ($P < 0.05$). LDL to HDL ratios were highest at 10 weeks of age for all animals and fell throughout feeding, but remained highest in the 40:60 fed rabbits even following cholesterol dose ($P < 0.05$).

In 40:60 fed rabbits, VLDL cholesterol concentrations were increased only at 10 weeks of age compared with animals fed 100:0 and 80:20 diets. Following the cholesterol challenge, concentrations of LDL, HDL, and VLDL cholesterol were elevated in all animals ($P < 0.05$), but no differences between dietary treatments were observed.

Mean total triglyceride concentrations are reported in *Table 4*. Rabbits fed 100% casein diets had higher triglyceride concentrations at 10 and 14 weeks of age compared with rabbits consuming either whey-containing diet ($P < 0.05$), but levels were similar between groups at 18 and 20 weeks of age. Following the cholesterol challenge, total triglycerides were highest in the 100% casein-fed rabbits ($P < 0.05$).

Mean HMG CoA reductase activities and total liver lipid values are given in *Table 5*. Rabbits fed the whey-dominant diet (40:60) had higher reductase activities compared with those fed 100% casein ($P < 0.05$). Total liver lipids were also increased in rabbits fed the 40:60 diet compared with 100% casein ($P < 0.01$). Bile acid excretion and fecal choles-

Table 3 Plasma cholesterol concentrations of postweanling rabbits fed casein:whey diets*

Casein:whey	Weeks of age†				
	10	14	18	20	24
			(mmol/L)		
Total					
100:0	6.4±0.7[b]	6.6±1.1[b]	6.2±1.3[b]	5.5±1.1[b]	23.6±4.0
80:20	5.1±0.3[b]	5.4±0.7[b]	5.3±0.9[b]	4.3±0.6[b]	21.9±2.5
40:60	8.5±0.8[a]	8.7±0.8[a]	12.0±1.9[a]	9.8±2.0[a]	26.5±2.6
LDL					
100:0	4.6±0.7[ab]	4.9±1.0[b]	4.5±0.9[b]	6.4±4.0	12.5±3.3
80:20	4.0±0.3[b]	4.4±0.5[b]	3.4±0.6[b]	2.7±0.4	14.9±1.3
40:60	6.1±0.6[a]	7.4±0.4[a]	7.8±1.5[a]	6.2±1.3	18.7±2.3
HDL					
100:0	0.8±0.1[ab]	0.9±0.1[a]	1.2±0.2[ab]	1.3±0.2[b]	3.5±0.9
80:20	0.6±0.0[b]	0.7±0.1[b]	1.0±0.2[b]	0.9±0.1[b]	4.1±1.1
40:60	0.9±0.1[a]	1.1±0.1[a]	1.8±0.3[a]	2.2±0.3[a]	4.4±0.9
VLDL					
100:0	1.0±0.1[b]	0.7±0.2	1.0±0.3	1.5±0.4	3.1±0.4
80:20	0.7±0.1[b]	0.9±0.2	3.3±1.7	1.0±0.2	4.5±0.8
40:60	1.5±0.3[a]	0.9±0.2	2.4±0.5	1.4±0.4	3.3±0.3

*Values represent means ± SEM, n = 7/treatment. Means in the same column for each lipoprotein fraction with different superscripts are significantly different ($P < 0.05$).
†Cholesterol challenge (0.4%) is between weeks 20 and 24.

Table 4 Plasma triglyceride concentrations of postweanling rabbits fed casein:whey diets*

Casein:whey	Weeks of age†				
	10	14	18	20	24
			(mmol/L)		
Total					
100:0	0.6±0.1[a]	0.6±0.1[a]	0.4±0.1[a]	0.4±0.1[a]	0.5±0.1[a]
80:20	0.4±0.0[b]	0.5±0.1[b]	0.3±0.0[a]	0.4±0.0[a]	0.1±0.1[b]
40:60	0.3±0.1[b]	0.3±0.1[b]	0.3±0.1[a]	0.5±0.1[a]	0.2±0.1[b]

*Values represent means ± SEM, n = 7/treatment. Means in the same column with different superscripts are significantly different ($P < 0.05$).
†Cholesterol challenge (0.04%) is between weeks 20 and 24.

Table 5 HMG CoA reductase activity and total liver lipids in rabbits fed varying casein:whey ratios and 0.4% cholesterol diets*

Casein:whey	HMG CoA reductase activity† (mevalonate/mg microsomal protein/min)	Total lipids (mg lipid/g liver)
100:0	67.0 ± 6.3[b]	66.7 ± 4.8[b]
80:20	87.3 ± 14.1[ab]	75.1 ± 5.3[ab]
40:60	99.0 ± 12.3[a]	84.0 ± 9.7[a]

*Values represent means ± SEM, n = 7/treatment. Means in same column with different superscripts are significantly different ($P \leq 0.05$).

terol content are given in *Table 6*. Bile acids were unaffected by dietary treatment, but fecal cholesterol was significantly higher in 80:20-fed rabbits compared with 100% casein-fed animals ($P < 0.05$). Atherosclerotic plaque formation was not affected by dietary treatments *(Table 7)*.

Discussion

The present study showed that dietary protein source manipulation was effective in altering cholesterol metabolism during the postweanling developmental stage. We found that rabbits fed a diet with whey as the predominant protein source had elevated plasma cholesterol concentrations compared with those fed a diet that differed only in that it contained 100% casein as protein. Following the cholesterol challenge, however, blood cholesterol concentrations were not different, but HMG CoA reductase activity and hepatic lipid concentration were elevated in rabbits fed the whey-predominant diet.

The relationship between growth and plasma lipids cannot be ignored. Rabbits fed the 40:60 diet exhibited lower feed efficiency compared with the other two groups. Both total and LDL cholesterol followed a similar trend with highest concentrations occurring in animals fed the 40:60 diet until

Table 6 Fecal cholesterol content and bile acid excretion in rabbits fed varying casein:whey ratios and 0.4% cholesterol diets*

Casein:whey	Fecal cholesterol (mg cholesterol/ 100 mg feces)	Bile acid excretion (mg/d)
100:0	83.5 ± 35.6[b]	29.8 ± 5.0
80:20	155.8 ± 33.5[a]	43.0 ± 14.8
40:60	127.9 ± 53.3[ab]	34.9 ± 4.9

*Values represent means ± SEM, n = 7/treatment. Means in same column with different superscripts are significantly different ($P \leq 0.05$).

Table 7 Atherosclerotic lesions in rabbits fed varying casein:whey ratios and 0.4% cholesterol diets*

Casein:whey	Score† (% plaque in aorta)
100:0	3.2 ± 1.1
80:20	4.7 ± 2.3
40:60	7.0 ± 2.7

*Values are means ± SEM, n = 7/treatment.
†Score is the percentage of aorta containing plaques visualized by staining.

the cholesterol challenge. However, HDL cholesterol and total triglyceride concentrations were different between rabbits fed 100:0 and 80:20 diets at certain timepoints prior to the challenge, and triglycerides were highest in rabbits fed the 100:0 diet after the cholesterol challenge. In work with growing rabbits fed different protein sources, Carroll[21] reported that though overall growth differs depending on protein fed, this was not related to cholesterolemia. In the present study, rabbits were pair-fed and the amino acid content of the diet was calculated to be adequate to promote growth.[22] The resultant depression in growth in the group fed the whey-predominant diet may be due to differences in digestibility between the diets.

In contrast to our findings, Lovati et al.[23] reported that blood cholesterol was elevated with both casein and whey in 15-week-old rabbits fed cholesterol-containing diets for 4 weeks. Carroll and Hamilton[24] also found that both casein and lactalbumin elevated cholesterol levels when fed a 1% corn oil and 60% dextrose diet, while Sautier et al.[25] reported a hypocholesterolemic effect of whey protein in rats fed very low fat diets (1 to 3% fat) without added cholesterol.

These conflicting reports represent the importance of interaction of multiple dietary components and species differences in the regulation of cholesterol metabolism. It is difficult to compare experiments conducted with rats with those with rabbits. Rats are known to respond differently to dietary manipulation, as they possess different lipoprotein and tissue distribution of cholesterol.[26] Previous data in rabbits also are not directly comparable to this study, as it is known that level and type of dietary fat, amount of cholesterol, and carbohydrate source influence blood cholesterol levels and development of atherosclerosis in rabbits.[27]

The mechanism whereby protein may be causing its effect has several hypotheses, many of which center around the amino acid composition.[28] A general argument for the noted hypercholesterolemic effect of casein in comparison to soy protein is its high content of branch chain amino acids (leucine, isoleucine, and valine).[29] In the present study, the branch chain amino acid content of the predominantly whey diet is 6.7 g/kg diet compared with 6.3 g/kg of 100% casein diet. Furthermore, a high ratio of lysine to arginine has been hypothesized to play a role in hypercholesterolemia and atherogenic plaque formation.[27] The predominantly whey diet has a higher lysine to arginine ratio (2.8) compared with the 100% casein diet (2.3). The rabbits in our study fed the predominantly whey diet with a higher ratio of lysine to arginine displayed hypercholesterolemia, as this theory would suggest, and also had increased (though not statistically significant) amounts of atherosclerotic plaque formation. Because differences in amino acid composition are not large, lipids may be further influenced by minor differences in the non-protein components of protein mixtures fed. The non-protein components of the 75% whey protein include 11% lactose and 7% fat compared with the 1.2% lactose and 0.1% fat of the 87% casein. Though once again these differences are small, lactose and fat (both found to be related to atherogenesis) may be contributing to the lipid effects noted here.

In the current study, changes in blood cholesterol may be reflective of alterations in different lipoprotein fractions. Feeding casein results in increased cholesterol in the LDL fraction and in the VLDL cholesterol fraction.[23] Although our blood collection schedule missed measurement of the potential immediate response in the LDL fraction, our findings confirmed that VLDL cholesterol in the 100% casein-fed rabbits was significantly higher than in the predominantly whey-fed rabbits after 5 weeks on the diets. Feeding whey protein, on the other hand, resulted in little effect on VLDL cholesterol levels and mainly increased HDL and LDL cholesterol. It has been suggested that when a large amount of plasma cholesterol is carried by the triglyceride-rich lipoproteins, hypercholesterolemia may be secondary to hypertriglyceridemia.[5] Hypertriglyceridemia was also noted in the 100% casein-fed rabbits of this study. These findings suggest that casein and whey proteins may elicit their cholesterolemic responses via different mechanisms.

In the present study, the absence of differences in bile acid excretion, while HMG CoA reductase activity and liver lipids varied, suggests that protein source did not have an impact on enterohepatic circulation but may have affected hepatic lipid metabolism directly. This is in agreement with the theory that dietary protein-induced changes in lipid metabolism are a result of effects at the hepatic versus intestinal level, though differences in fecal cholesterol excretion indicate further alterations in cholesterol excretion.[30]

In addition to examining the impact of dietary proteins on lipid metabolism, this experiment was designed to test the cholesterol challenge hypothesis, which suggests that dietary manipulation of cholesterol and fatty acids early

in life can have persistent, even permanent, effects.[3] The developmental stages when dietary manipulation has been hypothesized to be effective include the prenatal, suckling, weanling, and immediate postweanling phases.[31] As represented by the lack of a difference between dietary treatments following the cholesterol challenge from 20 to 24 weeks of age, it is apparent that the different protein sources fed postweaning did not have persistent effects on plasma cholesterol in the timepoint studied.

This lack of a persistent effect may involve the age of the animal used. Rabbits are naturally weaned at 8 to 10 weeks of age, whereas the rabbits in the present study were weaned prior to 4 weeks of age. There is much evidence that premature weaning in animals causes high blood cholesterol levels and a relative inability to handle a cholesterol challenge in adult life.[1] The effects are suggested to involve a decrease in cholesterol 7-α-hydroxylase[31] and an increase in HMG CoA reductase activities subsequent to the removal of mother's milk.[4] Perhaps the premature weaning removed any potential effect of protein manipulation on adult cholesterol levels. The dramatic increase in plasma cholesterol levels in all the animals of this study following a 0.4% cholesterol challenge may indicate that their ability to handle exogenous cholesterol challenge was hampered, though it most likely is a result of the excessive (0.4%) cholesterol dose. It is possible that using such a high dose removes any potential protein effect seen earlier. Another possibility is that regardless of the age of weaning, the postweanling phase of rabbit development may not be the critical period in which permanent changes in cholesterol metabolism develop.

In conclusion, results from this study show plasma lipids are altered by dietary protein source during the postweanling period. Although plasma cholesterol concentrations were not affected by protein source following the cholesterol challenge, both HMG CoA reductase and total liver lipids were elevated in animals fed the whey-predominant diet. This suggests that dietary protein source alters cholesterol metabolism in the postweanling period, and that these effects may be lasting.

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