Decreased receptor-mediated LDL catabolism in casein-fed rabbits precedes the increase in plasma cholesterol levels

Pramod Khosla,* Samir Samman,† and Kenneth K. Carroll

Department of Biochemistry, University of Western Ontario, London, Ontario, Canada

The effect of dietary protein on, in vivo, *receptor-dependent and receptor-independent low density lipoprotein (LDL) catabolism was assessed in rabbits fed semipurified cholesterol-free diets containing casein or soy protein for 300 days. Plasma cholesterol (7.37 ± 0.59 versus 1.84 ± 0.28 mmol/L; P < 0.001), LDL cholesterol (2.66 ± 0.41 versus 0.67 ± 0.10 mmol/L; P < 0.001) and LDL apo B concentrations (1.39 ± 0.40 versus 0.54 ± 0.12 g/L plasma; P < 0.001) were significantly higher in rabbits fed casein. Kinetic parameters calculated following the simultaneous injection of radiolabeled native and methylated LDL showed that in casein-fed rabbits, total LDL apo B fractional catabolic rate (FCR) was significantly lower (0.64 ± 0.11 versus 1.42 ± 0.20 pools/d; P < 0.02), and this was due exclusively to a four-fold reduction in receptor-dependent LDL apo B FCR (0.21 ± 0.07 versus 0.86 ± 0.10 pools/d; P < 0.002), since receptor-independent LDL apo B FCR was similar between groups (0.44 ± 0.05 versus 0.56 ± 0.12 pools/d). In groups of rabbits transferred from a soy protein diet to a casein diet, total LDL apo B FCR was decreased significantly in rabbits fed casein diet, 5 days after being switched from the soy protein diet (1.03 ± 0.08 versus 1.83 ± 0.13 pools/d; P < 0.05). This decrease in total LDL apo B FCR was due to decreases in receptor-mediated catabolism (0.56 ± 0.06 versus 1.03 ± 0.14 pools/d; P < 0.001) and receptor-independent catabolism (0.47 ± 0.09 versus 0.80 ± 0.10 pools/d; P < 0.05). However, the plasma cholesterol in these casein-fed rabbits was not significantly different from that observed in soy protein-fed rabbits (2.33 ± 0.52 versus 2.02 ± 0.39 mmol/L). Compared to soy protein-fed rabbits, the plasma cholesterol was significantly higher when the casein had been fed for 10 to 25 days, and in these rabbits total LDL apo B FCR was also significantly reduced, due exclusively to decreased receptor-mediated catabolism. There was a significant inverse correlation in all rabbits between plasma cholesterol and both total LDL apo B FCR (R = 0.66; P < 0.001) and receptor-dependent LDL apo B FCR (R = 0.72; P < 0.001). These studies show that down-regulation of LDL receptors* in vivo, *precedes the subsequent elevation in plasma cholesterol levels induced by a casein diet.*

Keywords: casein; soy protein; developing hypercholesterolemia; LDL catabolism; FCR

Introduction

Rabbits fed low-fat, cholesterol-free, semipurified diets containing casein become hypercholesterolemic, whereas normal levels of cholesterol are maintained if the casein is replaced by isolated soy protein.[1,2] The excess cholesterol in casein-fed rabbits accumulates in the atherogenic LDL fraction.[3-5] The mechanism of casein-induced hypercholesterolemia has yet to be resolved.

In vitro studies by Chao et al.[6] showed that hepatic LDL receptor activity was reduced in rabbits fed a wheat-starch casein diet, in comparison to the activity

Address reprint requests to Kenneth K. Carroll, Department of Biochemistry, University of Western Ontario, London, Ontario, Canada N6A 5C1.
Supported by the Heart and Stroke Foundation of Ontario and by Protein Technologies Inc., St. Louis, MO. Presented in part at the Federation of American Society for Experimental Biology meeting, New Orleans, LA, March 1989. Abstract published in *FASEB. J.* **3**, (1989), A957.
*Current address: Foster Biomedical Research Laboratory, Brandeis University, Waltham, MA 02254, USA.
†Current address: Department of Biochemistry, University of Sydney, NSW 2006, Australia.
Received June 25, 1990; accepted September 24, 1990.

seen in Chow-fed rabbits.[7] However, because a semipurified casein diet was compared to a laboratory Chow diet, the observed effects on LDL receptor activity reported in vitro cannot be attributed to a specific component of the semipurified diet.

Recent studies from this laboratory, designed specifically to look at the protein component of the diet, have shown that the elevated LDL apo B pool size in casein-fed rabbits, as opposed to rabbits fed soy protein, is associated with a decreased LDL apo B FCR and, although the production rates of LDL apo B were similar between dietary groups, there was evidence for increased synthesis of LDL apo B from very low density lipoprotein (VLDL)-independent pathways.[8] These differences in LDL apo B FCR could not be attributed to any functional abnormalities in the LDL particles.[9] Also, when rabbits were maintained on both diets for a longer time period (several months), the casein-fed rabbits showed an increase in their intermediate density lipoprotein (IDL) apo B pool, resulting exclusively from a decrease in the IDL apo B FCR with no differences between IDL apo B production rates.[10] Although these results are consistent with down-regulation of LDL receptors in casein-fed rabbits, the fact that LDL can be catabolized via either a high affinity receptor-mediated process[11] or a low affinity receptor-independent pathway,[12] therefore necessitates measurement of LDL catabolism via each of these processes in vivo. Furthermore, the above studies,[8-10] were carried out with the rabbits in a steady state with respect to intravascular lipoprotein concentrations and, therefore, it is not known if the observed results are a consequence of the hypercholesterolemia or vice-versa.

Therefore, we investigated the metabolism of LDL in casein and soy protein-fed rabbits, via both the receptor-mediated and receptor-independent pathways using radiolabeled native and reductively methylated LDL. (The former is catabolized via both the receptor-mediated and the receptor-independent routes, whereas the latter is metabolized exclusively via the receptor independent route. Thus the difference is a measure of LDL receptor activity in vivo. Additionally, we evaluated LDL apo B FCR, progressively, as rabbits were switched from a soy protein diet to a similar diet containing casein (i.e., during developing hypercholesterolemia). The FCR is a true measure of the catabolic efficiency of an animal and its usefulness as a metabolic indicator has been discussed previously.[13] Furthermore, possible alterations in pool size during a turnover study do not affect the FCR.[13]

Materials and methods

Animals and diets

Male New Zealand white rabbits were obtained from Reimen's Fur Ranches (Guelph, Ontario) at a weight of 1.5 kg, and maintained on semipurified diets containing casein or soy protein for approximately 300 days (long-term study). In the second study (short-term study), 16 rabbits were maintained on Purina Rabbit Chow (Ralston Purina, St. Louis, MO) for 1 week upon arrival after which they were transferred to a mixture of Chow and semipurified soy protein diet as detailed previously.[3] All rabbits were then fed soy protein diet for an additional 2 weeks. Following this, 10 rabbits were switched to the semipurified casein diet, which was fed for 5 days (4 rabbits), 10 days (3 rabbits), or 25 days (3 rabbits). The remaining 6 rabbits continued on the soy protein diet. The feeding period was staggered such that the kinetic studies were carried out in all the rabbits at the same time. The composition of the semipurified diets (*Table 1*) was identical to those used in previous studies.[8-10,14]

TABLE 1 Composition of the semipurified diets (g/kg)

Ingredients[a]	Casein diet	Soy protein diet
Dextrose	600	600
Celluflour	50	50
Casein (vitamin-free)	270[b]	0
Soy protein isolate	0	275
Salt mix	40	40
Molasses (50% v/v)	30	30
Vitamin mix[c]	2	2
Corn oil	10	10

[a] The vitamins, salt mixture (Philips and Hart salt mixture IV), and "vitamin-free" casein were obtained from ICN Life Sciences Group, Nutritional Biochemicals Division, Cleveland, OH. Dextrose was obtained from Teklad test Diets, ARS/Sprague-Dawley Division of the Mogul Corporation, Madison, WI. Celluflour was from Chicago Dietetic Supply House, Chicago, IL. Molasses was obtained from a local feed mill, and corn oil from a local supermarket. The soy protein (Supro 910) was from Ralston Purina Co., St. Louis, MO.
[b] Casein and soy protein were added in these amounts to provide 25% (wt/wt) protein in the diet.
[c] The composition of the vitamin mixture has been published previously.[14]

Isolation, iodination and methylation of LDL

LDL ($1.019 < d < 1.063$ g/mL) was isolated from the plasma of Chow-fed rabbits, by sequential ultracentrifugation[15] in a Beckman 70 Ti rotor (Beckman, Palo Alto, CA), and washed and concentrated by recentrifugation through an equal volume of $d = 1.063$ g/mL solution in a Ti 80 rotor. The isolated LDL was dialyzed against 0.15M NaCl/1 mM EDTA, pH 7.4, at 4° C for 24 hr.

The LDL was divided into two aliquots. One aliquot was labeled with $Na^{125}I$ and the other with $Na^{131}I$ (Amersham, Oakville, Ontario), by a modification[16] of the McFarlane method[17] as detailed previously.[8] The ^{125}I-LDL was now reductively methylated with $NaBH_4$ and formaldehyde by the procedure of Weisgraber et al.,[18] exactly as described.[19] Both tracers were sterilized as detailed previously.[8] Both tracers contained 93% of their total radioactivity in apo B as determined by isopropanol precipitation[20] and less than 1% as free iodine.[16]

Protocol for kinetic studies

Thyroidal uptake of radioiodine was minimized by the addition of KI (0.1 g/mL)[21] to the drinking water of each rabbit, 2 days before injection of the tracers, and throughout the duration of the kinetic study.

Each rabbit was injected simultaneously with 2-10 μCi of each tracer via the marginal ear vein. Eight blood samples were collected from the opposite ear, into EDTA-coated vacutainers (Becton-Dickinson, Mississauga, Ontario), from 3 min to 50 h-post-injection. Plasma apo B radioactivity was determined.[22] Additionally, for the rabbits fed casein or soy protein for 300 days, aliquots of plasma from each of the time points for each rabbit were pooled and LDL isolated from 4-6 mL of pooled plasma by sequential utlracentrifugation.[15] The isolated LDL was washed by respinning once. Following dialysis, LDL protein,[23] LDL apo B concentrations,[22] and LDL cholesterol concentrations were determined.

Analysis of results

The plasma apo B radioactivity decay curves were biexponential, and were resolved by curve peeling into two components and analyzed by the procedure of Matthews.[24] The FCR for the native ^{131}I-LDL apo B and the methylated ^{125}I-LDL apo B were calculated. The former represents the sum of both receptor-dependent and receptor-independent catabolism, whereas the latter represents receptor-independent catabolism. The difference, therefore, is a measure of receptor-dependent catabolism. In studies where LDL apo B was also isolated, the LDL apo B FCR was multiplied by the plasma concentration of LDL apo B to calculate the absolute catabolic rate (ACR) of LDL apo B, expressed as g/L plasma/d.

Other analyses

Plasma and LDL cholesterol concentrations were determined using the CHOD-PAP enzymatic kit (Boehringer-Mannhein, Montreal, Quebec). Statistical analyses were carried out using Student's *t* test (long-term study), or by a one factorial ANOVA (short-term study).

Results

Long-term study

Rabbits maintained on casein or soy protein diets for 300 days had comparable body weights (3.6 ± 0.04 versus 3.8 ± 0.05 kg). The casein-fed rabbits had a four-fold elevation in both plasma cholesterol concentrations (7.37 ± 0.59 versus 1.84 ± 0.28 mmol/L; $P < 0.001$) and LDL cholesterol concentrations (2.66 ± 0.41 versus 0.67 ± 0.10 mmol/L; $P < 0.001$) compared to rabbits fed soy protein. LDL apo B concentrations were more than two-fold higher in the casein-fed rabbits (1.39 ± 0.40 versus 0.54 ± 0.12 g/L plasma; $P < 0.001$) [*Figure 1*]. The decline in plasma apo B radioactivity for the native ^{131}I-LDL and the methylated ^{125}I-LDL tracers is shown in *Figure 2*. For both groups of rabbits, the decline in radioactivity for the methylated tracer was slower than that seen with the native tracer. For the rabbits fed soy protein, the decline in ^{131}I-LDL apo B radioactivity was considerably faster than the decline in ^{131}I-LDL apo B radioactivity observed in the casein-fed rabbits.

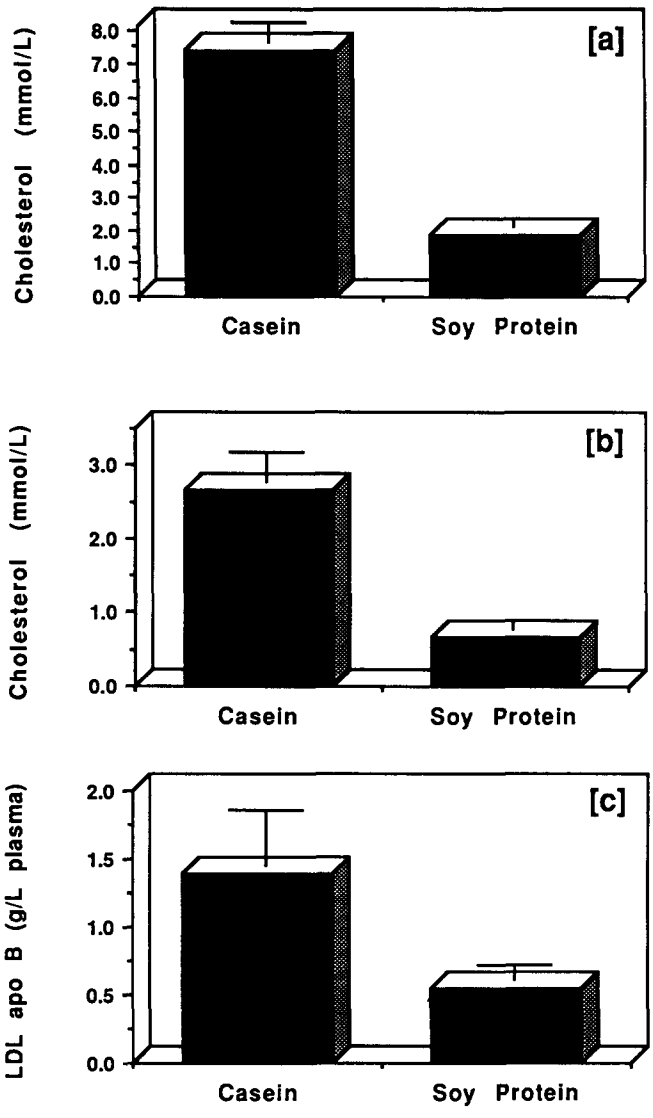
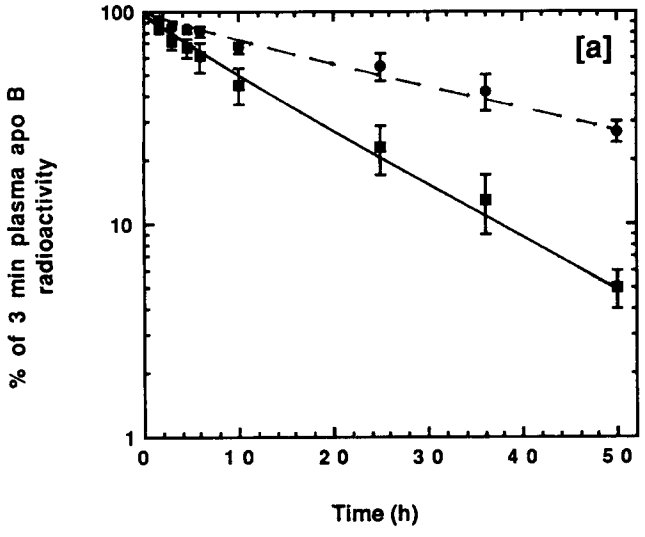


Figure 1 Long-term effects of dietary casein and soy protein on (a) plasma cholesterol; (b) LDL cholesterol; and (c) LDL apo B concentrations in rabbits. Values are the means ± SEM of 4 rabbits per dietary group. Each value was significantly different between dietary groups.

Table 2 shows the LDL kinetic parameters calculated from the radioactivity data. Both total and receptor-dependent LDL apo B ACR were similar in the two dietary groups. Although receptor-independent ACR was two-fold higher in the casein-fed rabbits (0.66 ± 0.27 versus 0.33 ± 0.09 g/L plasma/d), these values did not reach statistical significance. The total LDL apo B FCR was significantly lower in rabbits fed casein as opposed to those fed soy protein (0.64 ± 0.11 versus 1.42 ± 0.20 pools/d; $P <$



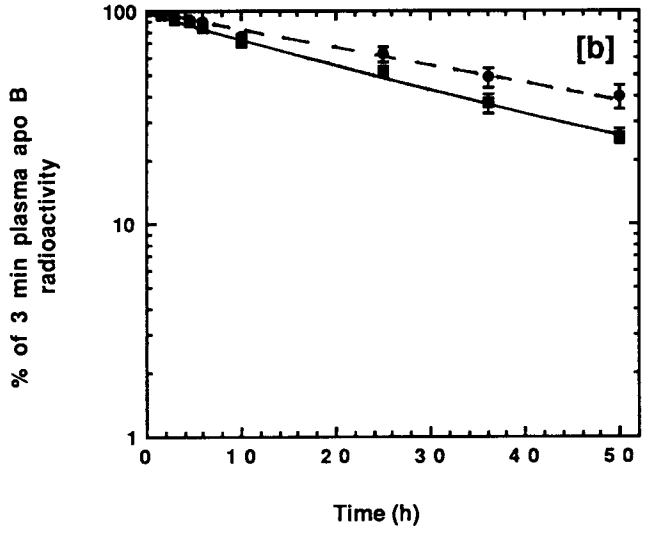


Figure 2 The decline in plasma apo B radioactivity following the simultaneous injection of ^{131}I-native LDL (■—■) and ^{125}I-methylated LDL (●—●) into rabbits fed a) soy protein or b) casein for 300 days. Each point represents the means ± SD of 4 rabbits.

TABLE 2 LDL apo B kinetic parameters in rabbits fed casein or soy protein containing diets for 300 days (long-term study)

	Casein diet	Soy protein diet
LDL apo B ACR[a]		
Total	0.98 ± 0.44	0.72 ± 0.10
RI	0.66 ± 0.27	0.33 ± 0.09
RD	0.34 ± 0.21	0.43 ± 0.08
LDL apo B FCR[b]		
Total	0.64 ± 0.11*	1.42 ± 0.20*
RI	0.44 ± 0.05	0.56 ± 0.12
RD	0.21 ± 0.07†	0.86 ± 0.10†

Values given are means ± SEM of 4 rabbits per dietary group.
[a] The LDL apo B ACR (g/L plasma/d) was calculated by multiplying the LDL apo B FCR[b] (pools/d) by the LDL apo B concentration (g/L plasma—*Figure 1*).
RI = Receptor-independent; RD = Receptor-dependent.
Mean values sharing a common superscript were significantly different ($P < 0.02$) as determined using Student's unpaired *t* test.

0.02). This was attributable to a four-fold decrease in the receptor-dependent FCR of LDL apo B (0.21 ± 0.07 versus 0.86 ± 0.10 pools/d; $P < 0.002$), since the receptor-independent FCR of LDL apo B was similar between dietary groups (0.44 ± 0.05 versus 0.56 ± 0.12 pools/d).

Short-term study

Table 3 shows the body weights, plasma cholesterol concentrations, and LDL apo B FCR in rabbits fed the soy protein diet or the casein diet for differing time periods (5 to 25 days). Casein-fed rabbits had comparable body weights to control rabbits fed soy protein, with the exception of the rabbits fed casein for 25 days. The latter were significantly heavier (2.7 ± 0.2 versus 2.4 ± 0.1 kg). Additionally, the rabbits fed casein for 25 days were significantly heavier than those fed casein for 10 days. With the exception of the rabbits fed casein for 5 days, all other groups of rabbits fed the casein diet were significantly hypercholesterolemic in comparison to the rabbits fed soy protein (*P*

TABLE 3 Body weights, plasma cholesterol concentrations, and LDL apo B fractional catabolic rates (total, receptor-independent, and receptor-dependent) in rabbits fed soy protein or casein for different time periods (short-term study)

Diet	Body weight (kg)	Plasma cholesterol (mmol/L)	LDL apo B FCR (pools/d)		
			Total	RI	RD
Soy protein[6]	2.4 ± 0.1[a]	2.02 ± 0.39[cd]	1.83 ± 0.13[ghi]	0.80 ± 0.10[j]	1.03 ± 0.14[klm]
Casein 5d[4]	2.6 ± 0.1	2.33 ± 0.26[ef]	1.03 ± 0.08[g]	0.47 ± 0.09[j]	0.56 ± 0.06[k]
Casein 10d[3]	2.3 ± 0.2[b]	4.73 ± 0.03[ce]	0.95 ± 0.24[h]	0.54 ± 0.20	0.41 ± 0.05[l]
Casein 25d[3]	2.7 ± 0.1[ab]	5.17 ± 0.80[df]	1.19 ± 0.09[i]	0.62 ± 0.04	0.58 ± 0.09[m]

RI = receptor-independent; RD = receptor-dependent. Superscript numerals refer to the number of rabbits in a given dietary group. Casein 5 d, 10 d, & 25 d refer to rabbits fed casein for 5, 10, & 25 days, respectively. Values are mean ± SEM.
The data for all 16 rabbits were analyzed with a one-way analysis of variance test. Statistical significance was assessed using Fisher's Protected Least Squares Difference test. Values sharing a common superscript were significantly different ($P < 0.05$).

< 0.05). Amongst the rabbits fed casein, those fed for 10 or 25 days were significantly hypercholesterolemic compared to those fed only for 5 days. Both total and receptor-dependent LDL apo B FCR in casein-fed rabbits were significantly lower than the values observed in rabbits fed soy protein ($P < 0.05$). The values obtained were not affected by the time for which the casein diet was fed. No significant difference was observed in receptor-independent LDL apo B FCR between casein- and soy protein-fed rabbits with the exception of the value obtained for the rabbits fed casein for 5 days. This value was significantly lower than the value obtained for soy protein-fed rabbits.

Among the rabbits fed soy protein, the time for which the diet was fed (long-term versus short-term) had no influence on plasma cholesterol concentrations (1.84 ± 0.28 versus 2.02 ± 0.39 mmol/L), total LDL apo B FCR (1.42 ± 0.20 versus 1.83 ± 0.13 pools/d), receptor-dependent LDL apo B FCR (0.86 ± 0.10 versus 1.03 ± 0.14 pools/d) or receptor-independent LDL apo B FCR (0.56 ± 0.12 versus 0.80 ± 0.10 pools/d).

For the 24 rabbits used in both studies, plasma cholesterol concentrations showed a strong inverse correlation with both total LDL apo B FCR ($R = 0.66$; $P < 0.001$) and receptor-dependent LDL apo B FCR ($R = 0.72$; $P < 0.001$). There was no significant correlation ($R = 0.20$; $P > 0.10$) between plasma cholesterol levels and receptor-independent LDL apo B FCR (*Figure 3, a-c*).

Discussion

In the study presented in this report, we have investigated the effects of dietary protein on LDL metabolism. To our knowledge, this is the first in vivo study in rabbits, which directly compares the effects of the dietary protein component (casein or soy protein) on both receptor-dependent and receptor-independent catabolism of LDL. Additionally, this is the first study to investigate the time course of the decrease in LDL receptor activity induced by a casein diet.

Recent studies from this laboratory, utilizing radiolabelled lipoproteins, showed that the elevated pool size of LDL in casein-fed rabbits (compared to rabbits fed soy protein) was governed by 1) an increase in the direct synthesis of LDL apo B (i.e., synthesis from sources other than VLDL apo B catabolism) and 2) a decrease in the FCR of LDL apo B.[8] These effects were not due to the LDL particles per se.[9] The current studies show that the decreased LDL apo B FCR in casein-fed rabbits in a steady state (i.e., with an established hypercholesterolemia) is specifically due to the receptor-mediated pathway, since metabolism via the receptor-independent route was similar between the two dietary groups (*Table 2*). This decrease in LDL receptor activity in casein-fed rabbits can be attributed to the high affinity, low capacity EDTA-sensitive binding site. The latter's ability to bind LDL in vitro has been shown to be greatly reduced or totally abolished in liver membranes from hypercholesterolemic rabbits

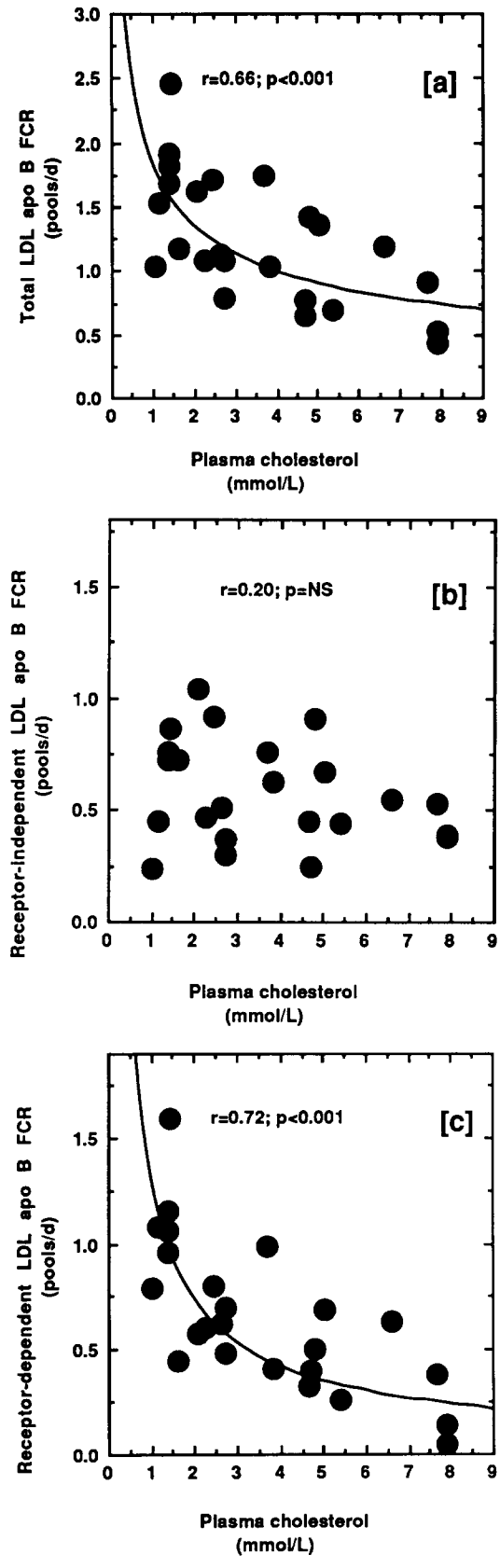


Figure 3 Correlation between plasma cholesterol concentrations and a) total LDL apo B FCR, b) receptor-independent LDL apo B FCR, and c) receptor-dependent LDL apo B FCR. The data are from all 24 rabbits used in both the long-term and short-term studies. Each point represents the value from an individual animal.

as opposed to liver membranes of normocholesterolemic rabbits.[6,7,25,26]

Our studies also show that this decrease in receptor-mediated LDL apo B FCR is apparent within 5 days of switching rabbits from a soy protein to a casein diet, and precedes the subsequent increase in plasma cholesterol concentrations (*Table 3*). Our results, therefore, suggest that casein-induced hypercholesterolemia is a consequence of decreased LDL receptor activity. The rapidity of this effect suggests that casein (or some component of casein) has the ability to directly or indirectly inhibit LDL receptor status. Conversely, soy protein has the ability to stimulate LDL receptor activity. In support of the latter hypothesis, it was shown in hypercholesterolemic human subjects consuming either an animal protein or a soy protein containing diet, that there was a significant increase in the LDL receptor activity in mononuclear cells isolated from subjects consuming the soy protein diet.[27]

Additionally, we found that receptor-independent LDL apo B FCR was also reduced in rabbits 5 days after being switched from the soy protein to the casein diet. The reason for this initial decrease in receptor-independent LDL apo B FCR is not clear from the present studies. It may be a consequence of the effects of the casein diet on general intracellular processes. However, from both our long- and short-term studies, it is apparent that once the hypercholesterolemia is established by feeding casein, the receptor-independent pathway has little impact on the LDL pool, for the latter is then principally governed by the receptor-dependent pathway.

The inverse correlation observed between plasma cholesterol concentrations and both total and receptor-dependent LDL apo B FCR (*Figure 3*) is similar to our previously observed inverse correlation between LDL apo B concentrations and total LDL apo B FCR.[9] The decrease in FCR with increasing plasma cholesterol (or LDL apo B) concentrations is consistent with saturation of the removal mechanism. The non-correlation between plasma cholesterol concentrations and receptor-independent LDL apo B FCR is further evidence that the receptor-independent pathway is of minor importance in the metabolic regulation of plasma cholesterol levels in casein-fed rabbits.

Recently, it has been proposed that casein-induced hypercholesterolemia occurs in two stages.[28,29] Upon replacing soy protein by casein, there is increased absorption of cholesterol and bile acid from the gut, which following transport to the liver, increases the latter's content of cholesterol.[30] The liver then down-regulates LDL receptors and/or increases cholesterol exodus from the liver (by increasing lipoprotein secretion) and inhibits de novo cholesterol synthesis.[30-32] We have shown now that rapid down-regulation of LDL receptor activity precedes the increase in plasma cholesterol levels (*Table 3*). This is also consistent with the observation that after 3 to 4 days on diet, casein-fed rabbits excrete less fecal bile acids than their counterparts fed soy protein.[33] Although a tendency for increased LDL apo B production (with a significant increase in direct LDL apo B secretion) was seen in rabbits fed casein for 6 to 9 weeks,[8] as opposed to rabbits fed soy protein, no significant effects on LDL apo B production were discernible when the rabbits had been fed the diets for 14 to 16 weeks[9] or 300 days (current long-term study). With regard to VLDL apo B, similar production rates were observed in casein- or soy protein-fed rabbits[8] and in rats fed either casein plus cholesterol or those fed soy protein plus cholesterol.[34] Collectively, these results suggest that in response to the increased hepatic intracellular cholesterol concentration induced by casein-feeding, the liver's primary response is to decrease the number of LDL receptors (and not to increase lipoprotein secretion). The decrease in LDL receptors increases the plasma cholesterol concentration. Simultaneously or at a later stage, the cholesterol synthetic pathway would be depressed,[30-32] thereby delivering less cholesterol into the intracellular hepatic cholesterol pool. This regulatory mechanism would protect the animal against further development of hypercholesterolemia (i.e., produce a steady-state). Hence the down-regulation of LDL receptors and diminished de novo cholesterol biosynthesis compensates for the increased intestinal absorption of cholesterol and bile acids in casein-fed rabbits.

It is, however, still not clear how dietary proteins exert their effects on LDL receptor status. The ability of dietary protein to rapidly affect LDL receptors may be dependent on some amino acid(s) or peptide(s) which are under hormonal control.[35-38] Additionally, the role of micronutrients in the diet cannot as yet be excluded.[39] Further studies are necessary to elucidate the exact mechanism.

References

1 Carroll, K.K. (1982). Hypercholesterolemia and atherosclerosis: Effects of dietary protein. *Fed. Proc.* **41**, 2792–2796.

2 Terpstra, A.H.M., Hermus, R.J.J., and West, C.E. (1983). The role of dietary protein in cholesterol metabolism. *World Rev. Nutr. Diet.* **42**, 1–55.

3 Hrabek-Smith, J.M. and Carroll, K.K. (1987). A comparative study of serum lipoproteins in rabbits fed a natural ingredient or low-fat, cholesterol-free, semipurified diets containg casein or isolated soy protein. *Biochem. Cell Biol.*, **65**, 610–616.

4 Hrabek-Smith, J.M., Kurowska, E.M., and Carroll, K.K. (1989). Effects of cholesterol-free, semipurified diets containing different levels of casein or soy protein on distribution of cholesterol and protein among serum lipoproteins of rabbits. *Atherosclerosis* **76**, 125–130.

5 Kurowska, E.M., Hrabek-Smith, J.M., and Carroll, K.K. (1989). Compositional changes in serum lipoproteins during developing hypercholesterolemia induced in rabbits by cholesterol-free, semipurified diets. *Atherosclerosis* **78**, 159–165.

6 Chao, Y.S., Yamin, T-T., and Alberts, A.W. (1982). Effect of cholestyramine on LDL binding sites to liver membranes from rabbits with endogenous hypercholesterolemia induced by a wheat-starch casein diet. *J. Biol. Chem.* **257**, 3623–3627.

7 Kita, T., Brown, M.S., Watanabe, Y., and Goldstein, J.L. (1981). Deficiency of low density lipoprotein receptors on liver and adrenal gland of the WHHL rabbit, an animal model of familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA* **78**, 2268–2272.

8 Khosla, P., Samman, S., Carroll, K.K., and Huff, M.W. (1989). Turnover of ^{125}I-VLDL and ^{131}I-LDL apolipoprotein B in rabbits fed diets containing casein or soy protein. *Biochim. Biophys. Acta.* **1002,** 157–163.

9 Samman, S., Khosla, P., and Carroll, K.K. (1989). Effects of dietary casein and soy protein on metabolism of radiolabelled low density apolipoprotein B in rabbits. *Lipids,* **24,** 169–172.

10 Samman, S., Khosla, P., and Carroll, K.K. (1990) . IDL apolipoprotein B turnover in rabbits fed semipurified diets containing casein or soy protein. *Ann. Nutr. Metab.* **34,** 98–103.

11 Goldstein, J.L. and Brown, M.S. (1977). The low density lipoprotein pathway and its relation to atherosclerosis. *Annu. Rev. Biochem.* **46,** 897–930.

12 Miller, N.E., Weinstein, D.B., and Steinberg, D. (1978). Uptake and degradation of high density lipoprotein: comparison of fibroblasts from normal subjects and from homozygous familial hypercholesterolemic subjects. *J. Lipid Res.* **19,** 644–653.

13 Shephard, J. and Packard, C.J. (1986). Receptor-independent low-density lipoprotein catabolism. *Methods Enzymol.* **129,** 566–590.

14 Roberts, D.C.K., Stalmach, M.E., Khalil, M.W., Hutchinson, J.C., and Carroll, K.K. (1981). Effects of dietary proteins on composition and turnover of apoproteins in plasma lipoproteins of rabbits. *Can. J. Biochem.* **59,** 642–647.

15 Havel, R.J., Eder, H.A., and Bragdon, J.H. (1955). The distribution and chemical composition of ultracentrifugally isolated lipoproteins in human serum. *J. Clin. Invest.* **34,** 1345–1353.

16 Fidge, N.H. and Poulis, P. (1974). Studies on the radioiodination of very low density lipoprotein obtained from different mammalian species. *Clin. Chim. Acta.* **52,** 15–26.

17 McFarlane, A.S. (1958). Efficient trace-labelling of proteins with iodine. *Nature* **182,** 53–56.

18 Weisgraber, K.H., Innerarity, T.L., and Mahley, R.W. (1978). Role of the lysine residues in high affinity binding to cell surface receptors on human fibroblasts. *J. Biol. Chem.* **253,** 9053–9062.

19 Gross, G., Sykes, M., Arellano, R., Fong, R., and Angel, A. (1987). HDL clearance and receptor-mediated catabolism of LDL are reduced in hypothyroid rats. *Atherosclerosis* **66,** 269–274.

20 Egusa, G., Brady, D.W., Grundy, S.M., and Howard, B.V. (1983). Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. *J. Lipid Res.* **24,** 1261–1267.

21 Roth, R.I., Gaubatz, J.W., Gotto, A.M., Jr., and Patsch, J.R. (1983). Effect of cholesterol feeding on the distribution of plasma lipoproteins and on the metabolism of apolipoprotein E in the rabbit. *J. Lipid Res.* **24,** 1–11.

22 Yamada, N., Shames, D.M., Stoudemire, J.B., and Havel, R.J. (1986). Metabolism of lipoproteins containing apolipoprotein B-100 in blood plasma of rabbits: heterogeneity related to the presence of apolipoprotein E. *Proc. Natl. Acad. Sci. USA* **83,** 3479–3483.

23 Markwell, M.A., Haas, S.M., Bieber, L.L., and Tolbert, N.E. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87,** 206–210.

24 Matthews, C.M.E. (1957). The theory of tracer experiments with ^{131}I-labeled plasma proteins. *Phys. Med. Biol.* **2,** 36–53.

25 Chao, Y.S., Kroon, P., Yamin, T-T., Thompson, G.M., and Alberts, A.W. (1983). Regulation of hepatic receptor-dependent degradation of LDL by mevinolin in rabbits with hypercholesterolemia induced by a wheat-starch casein diet. *Biochim. Biophys. Acta.* **754,** 134–141.

26 Stoudemire, J.B., Renaud, G., Shames, D.M., and Havel, R.J. (1984). Impaired receptor-mediated catabolism of low density lipoproteins in fasted rabbits. *J. Lipid Res.* **25,** 33–39.

27 Lovati, M.R., Manzoni, C., Canavesi, A., Sirtori, M., Vaccarino, V., Marchi, M., Gaddi, G., and Sirtori, C.R. (1987). Soybean protein diet increases low density lipoprotein receptor activity in mononuclear cells from hypercholesterolemic patients. *J. Clin. Invest.* **80,** 1498–1502.

28 Beynen, A.C., Van der Meer, R., and West, C.E. (1986). Mechanism of casein-induced hypercholesterolemia: Primary and secondary features. *Atherosclerosis* **60,** 291–293.

29 Beynen, A.C., Van der Meer, R., West, C.E., Sugano, M., and Kritchevsky, D. (1986). Possible mechanisms underlying the differential cholesterolemic effects of dietary casein and soy protein. In *Nutritional effects on cholesterol metabolism,* (A.C. Beynen, ed.), pp. 29–45, Transmondial, Voorthuizen, Netherlands.

30 Huff, M.W. and Carroll, K.K. (1980). Effects of dietary protein on turnover, oxidation and absorption of cholesterol and on steroid excretion in rabbits. *J. Lipid Res.* **21,** 546–558.

31 Nagata, Y., Ishiwaki, N., and Sugano, M. (1982). Studies on the mechanism of antihypercholesterolemic action of soy protein and soy protein-type amino acid mixtures in relation to the casein counterparts in rats. *J. Nutr.* **112,** 1614–1625.

32 Sirtori, C.R., Galli, G., Lovati, M.R., Carrara, P., Bosisio, E., and Galli-Kienle, M. (1984). Effects of dietary proteins on the regulation of liver lipoprotein receptors in rats. *J. Nutr.* **114,** 1493–1500.

33 Kuyvenhoven, M.W., West, C.E., Van der Meer, R., and Beynen, A.C. (1986). Fecal steroid excretion in relation to the development of casein-induced hypercholesterolemia in rabbits. *J. Nutr.* **116,** 1395–1404.

34 Cohn, J.S., Kimpton, W.G., and Nestel, P.J. (1984). The effect of dietary casein and soy protein on cholesterol and very low density lipoprotein metabolism in the rat. *Atherosclerosis* **52,** 219–231.

35 Redgrave, T.G. (1984). Dietary proteins and atherosclerosis. *Atherosclerosis* **52,** 349–351.

36 Sugano, M., Yamada, Y., Yoshida, K., Hashimoto, T., Natsuo, T., and Kimoto, M. (1988). The hypocholesterolemic action of the undigested fraction of soybean protein in rats. *Atherosclerosis* **72,** 115–122.

37 Kurowska, E.M. and Carroll, K.K. (1990). Essential amino acids in relation to hypercholesterolemia induced in rabbits by dietary casein. *J. Nutr.* **120,** 831–836.

38 Hubbard, R., Kosch, C.L., Sanchez, A., Sabate, J., Berk, L., and Shavlik, G. (1989). Effect of dietary protein on serum insulin and glucagon levels in hyper and normocholesterolemic men. *Atherosclerosis* **76,** 55–61.

39 Samman, S., Khosla, P., and Carroll, K.K. (1990). Influence of dietary minerals on apolipoprotein B metabolism in rabbits fed semipurified diets containing casein. *Atherosclerosis* **82,** 69–74.