

Cholesterol Feeding Increases Low Density Lipoprotein Synthesis

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ABSTRACT This study examines the effects of increased dietary cholesterol (6 eggs/d) on the metabolism of low density lipoproteins in a group of seven healthy volunteers. Egg supplementation raised high density and low density lipoprotein cholesterol levels by 18 and 40%, respectively. The composition of the low density lipoprotein was unaltered and therefore the number of circulating particles must have increased. Kinetic studies indicated that this was due primarily to a 23% rise in the rate of synthesis of the lipoprotein. Catabolism was also affected. The fractional removal rate of native low density lipoprotein fell by 10% ($P < 0.05$). However, the clearance of the 1,2 cyclohexanedione-treated lipoprotein remained unchanged (control fractional clearance rate [FCR] = 0.188 pools/d; cholesterol feeding FCR = 0.183 pools/d). Therefore, the reduction in low density lipoprotein catabolism appeared to be due to a fall in receptor activity. Consequently, an increased sterol load (34.2 $\mu\text{mol/kg}$ per d vs. 27.7 $\mu\text{mol/kg}$ per d in the control phase, $P < 0.02$) was channelled into the receptor-independent route during egg feeding.

INTRODUCTION

Epidemiological studies have clearly shown that there is a relationship between diet, plasma cholesterol levels, and the incidence of ischemic heart disease (1-4). The dietary factors that feature prominently in this relationship are cholesterol and saturated fat intake. Metabolic studies (5-7) have shown that increasing consumption of either of these constituents raises the plasma concentration of cholesterol primarily by its effect on the major sterol transporting vehicle, low density lipoprotein (LDL). Therefore, much time and ef-

fort has been expended in an attempt to define the mechanisms whereby these dietary constituents affect plasma lipoprotein metabolism. Animal experiments have shown that, in addition to LDL, other lipoproteins, specifically cholesterol- and apolipoprotein E-enriched very low density lipoprotein (VLDL) and high density lipoprotein (HDL) accumulate in plasma in response to cholesterol feeding (8, 9). However, the implications of such changes, which also occur to a lesser extent in man (9, 10), remain obscure.

In a previous human study, we have examined the impact of a selective increment in dietary fat saturation levels on lipoprotein turnover (6). Our results indicate that the hypercholesterolemic effect of this manipulation derives primarily from a decrease in the plasma clearance rate of LDL, although the rise in LDL apoprotein synthesis that occurred in some subjects indicates that saturated fat feeding may have multiple effects on the metabolism of this lipoprotein. Another study of this kind has reached similar conclusions (11).

Recent advances in our understanding of LDL metabolism have revealed the existence of at least two distinct mechanisms for catabolism of the lipoprotein *in vivo*. One is the high affinity receptor pathway originally described by Goldstein and Brown (12). Its activity is tightly regulated at a level dictated by the cell's cholesterol requirement. When demand for the sterol rises, for example in the liver during accelerated bile acid synthesis (13-15), receptor-mediated LDL catabolism is stimulated. The other pathway functions independent of the high affinity receptor but at present we do not know where it is located or how it is controlled, although some reports have suggested that the reticuloendothelial system is involved (16, 17). In this study we have extended our observations on the influence of diet on LDL metabolism by examining the effect of cholesterol supplementation on the synthesis and receptor-dependent and -independent catabolism of LDL in a group of healthy normolipemic subjects.

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TABLE I
Dietary Consumption During Control and Test Periods

	Protein	Calories as carbohydrate	Fat	P/S ratio*	Cholesterol	Cal/kg body wt/d
		%			mg/d	
Control phase	12.4±2.2	50.3±6.8	37.3±5.4	0.17±0.02	180±110	26.0±7.8
Cholesterol feeding	15.1±3.1	47.1±6.5	37.8±5.4	0.17±0.05	1470±80	25.6±6.4

* Ratio of polyunsaturated/saturated fat.

METHODS

Subjects and diets. Seven healthy normolipemic volunteers (three males, four females) gave informed consent to the study. Their ages ranged from 21 to 28 yr. None showed clinical or laboratory evidence of renal, hepatic, endocrine, hematologic, or cardiovascular dysfunction. They received no drugs (including the contraceptive pill) with the exception of KI (360 mg/d in divided doses), which was given for 3 d before and throughout each turnover to inhibit thyroidal uptake of radioiodide.

All subjects were examined on an outpatient basis which, as we have shown earlier (18), provides appropriate steady-state conditions for LDL kinetic analysis. The regular dietary habit of each subject was determined by weighing and recording his food consumption over a period of 7 d. The resulting data were used to calculate (19) individual intakes of calories, protein, carbohydrate, fat (including polyunsaturated/saturated fat ratios) and cholesterol (Table I).

On average, each person consumed a diet containing 26.0 Cal/kg body wt, which included 180±110 mg (mean±1 SD) of cholesterol per day. LDL turnover studies were performed while the subjects were consuming this diet and their adherence to the regimen was confirmed by regular dietetic interview. Thereafter their daily cholesterol intake was raised to 1,470±80 mg by adding six eggs to their diet and adjusting their protein and fat consumption in order to maintain constant their intake of these components (Table I). Particular attention was paid to the total intake of calories and the quality and quantity of dietary fat. In each phase of the study the subjects' body weight and plasma lipid and lipoprotein values remained constant (Table II) indicating that steady-state conditions were maintained. This experimental design has already been shown (20) to produce a satisfactory hypercholesterolemic response.

The study conformed to the requirements of the Ethical Committee of Glasgow Royal Infirmary.

Turnover protocol. The investigation was performed in two parts. In the first, low cholesterol phase, the plasma clearance rates of ¹²⁵I-native and ¹³¹I-cyclohexanediol-treated LDL were measured as described previously (13, 21). Essentially, autologous LDL ($d = 1.030$ – 1.050 kg/liter) was prepared by rate zonal ultracentrifugation and divided into two aliquots which were labeled separately with ¹²⁵I and ¹³¹I using iodine monochloride (22). The latter was then treated with 1.2 cyclohexanediol (23) to block the arginyl residues on its protein moiety and provide a tracer for receptor-independent LDL catabolism. In an earlier study (24) we have compared the properties of this tracer with those of other modified lipoproteins. The resulting data support its use in man although we still do not know how each human tissue handles it in comparison to native LDL. Approximately 0.5 mg (25 μ Ci) of each preparation was sterilised by membrane filtration and injected in rapid sequence into the bloodstream of the donor. A 10-min blood sample was collected and there-

after fasting plasma specimens were obtained at daily intervals over the next 14 d. Plasma radioactivity clearance curves were constructed for each isotope and analyzed by the procedure of Matthews (25). This gave fractional clearance rates for native and cyclohexanediol-treated LDL as measured respectively by the disappearances of iodine-125 and iodine-131 radioactivities from the plasma compartment. On the assumption that the chemically modified lipoprotein is degraded only by receptor-independent pathways, receptor-mediated clearance could then be estimated as the difference between these two values (21). The plasma LDL apoprotein (apoLDL)¹ concentration (26) was determined from serial LDL cholesterol measurements (27) and the particle total cholesterol/protein ratio. This was used to calculate the absolute clearance rate of the protein (in milligrams per kilogram body weight per day). Under the steady-state conditions that pertained during the study, this value was taken to be the synthetic rate of apoLDL. During each turnover, LDL was isolated by rate zonal ultracentrifugation and its content of protein, triglyceride, cholesterol (free and esterified), and phospholipid measured as described previously (6).

The above procedure was repeated following 4 wk of high cholesterol feeding (6 eggs/d) when new steady-state LDL cholesterol levels would have been attained (28, 29). The results obtained on the two occasions were compared by paired Student's *t* test.

RESULTS

The plasma cholesterol concentration was on average 29% higher during the period of high cholesterol intake (Table II). This increment was due to 18% ($P < 0.01$) and 41% ($P < 0.001$) rises in the cholesterol content of the HDL and LDL fractions. Individual responses to the egg diet varied from a small change in LDL cholesterol of 0.3 mmol/liter to a maximum of almost 2.0 mmol/liter. VLDL cholesterol levels did not change although total plasma triglyceride fell significantly (18%, $P < 0.01$) and hence the mean VLDL cholesterol/plasma triglyceride ratio rose. Cholesterol feeding did not alter the composition of LDL (Table III) and so the rise in the cholesterol content of this fraction was attributable to a greater number of LDL particles in the circulation.

The high cholesterol diet raised (by 39%, $P < 0.001$) the apoLDL concentration to a new steady-state level

¹ Abbreviation used in this paper: apoLDL, LDL apoprotein.

TABLE II
Effects of Cholesterol Feeding (6 eggs/d) on Plasma Lipids and Lipoproteins in Seven Healthy Subjects

Subject No	Body weight		Plasma triglyceride*		Plasma cholesterol		VLDL cholesterol		LDL cholesterol		HDL cholesterol	
	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed
	kg		mmol/liter		mmol/liter		mmol/liter		mmol/liter		mmol/liter	
M ₁	77.1±0.4	79.0±0.2	1.36±0.29	1.11±0.14	5.46±0.27	6.62±0.34	0.58±0.11	0.43±0.17	3.49±0.31	4.64±0.34	1.40±0.07	1.54±0.16
M ₂	66.7±0.6	68.1±0.8	1.09±0.24	0.93±0.04	5.92±0.46	7.35±0.50	0.48±0.24	0.52±0.10	3.90±0.43	4.98±0.34	1.52±0.12	1.88±0.16
M ₃	70.4±0.2	71.2±0.3	0.97±0.43	0.80±0.21	4.00±0.14	4.58±0.31	0.27±0.12	0.29±0.14	2.32±0.22	2.65±0.17	1.42±0.19	1.68±0.11
F ₁	62.1±0.7	64.2±0.2	1.23±0.41	0.91±0.17	5.03±0.22	7.42±0.17	0.41±0.19	0.50±0.19	2.95±0.08	4.91±0.33	1.67±0.22	2.01±0.19
F ₂	56.7±0.2	58.2±0.2	1.14±0.16	0.93±0.16	4.44±0.22	6.12±0.25	0.51±0.13	0.41±0.27	2.56±0.22	4.06±0.19	1.36±0.07	1.64±0.10
F ₃	56.4±0.8	57.0±0.6	0.76±0.23	0.55±0.12	5.94±0.45	8.20±0.28	0.32±0.28	0.37±0.09	3.61±0.53	5.29±0.31	2.01±0.23	2.54±0.20
F ₄	55.3±0.8	55.5±0.7	0.78±0.12	0.79±0.11	4.81±0.23	5.59±0.25	0.35±0.28	0.12±0.03	2.57±0.21	3.61±0.23	1.78±0.13	1.88±0.07
Mean±1 SD			1.05±0.22	0.86±0.17	5.09±0.73	6.56±1.23	0.42±0.11	0.38±0.14	3.06±0.61	4.31±0.93	1.59±0.24	1.88±0.33
Paired <i>t</i> test			<0.01		<0.01		NS		<0.001		<0.01	

* To convert to milligrams per deciliter, multiply by 88.5.

† To convert to milligrams per deciliter, multiply by 38.7.

TABLE III
Effects of Cholesterol Feeding (6 eggs/d) on LDL Composition

Subject No.	Grams per 100 grams									
	Triglyceride		Free cholesterol		Cholesteryl esters		Phospholipid		Protein	
	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed
M ₁	2.5	2.8	9.2	8.4	38.4	42.7	24.8	23.0	25.0	23.1
M ₂	3.8	1.3	10.8	10.6	41.0	46.3	23.0	18.6	21.3	23.1
M ₃	3.1	2.8	9.6	10.8	37.3	35.2	21.8	24.1	28.2	27.0
F ₁	3.8	2.3	10.7	9.1	37.5	44.0	22.3	19.9	25.7	24.8
F ₂	2.4	3.1	9.0	8.7	40.4	39.5	23.7	24.1	24.5	24.6
F ₃	2.5	1.5	10.8	9.3	43.8	37.6	20.9	27.9	22.0	23.6
F ₄	2.1	3.2	10.8	10.0	42.4	35.8	20.7	28.4	24.1	22.5
Mean±1 SD	2.9±0.7	2.4±0.8	10.1±0.8	9.6±0.9	40.1±2.5	40.2±4.3	22.5±1.5	23.7±3.7	24.4±2.3	24.1±1.5

(Table IV). This derived from a combination of two effects. First, synthesis of the protein (which equals the total absolute catabolic rate) rose consistently and significantly (by 23%, $P < 0.01$). At the same time, overall fractional catabolism fell (10%, $P < 0.05$). However no change was observed in the fractional clearance of the cyclohexanedione-modified lipoprotein that serves as a marker of receptor-independent pathways. Hence, one would predict a fall in the fractional clearance of apoLDL by the receptor route, and this on the whole was observed, although statistical significance was not achieved. When the clearance rates by both routes were expressed in absolute terms it was apparent (Table IV) that during the cholesterol feeding phase a significantly increased amount of LDL was degraded by receptor-independent mechanisms but not by the receptor pathway itself.

DISCUSSION

It is widely accepted that dietary cholesterol supplementation raises plasma cholesterol but until now the mechanism behind this effect has remained obscure. Following absorption, cholesterol is packaged in chylomicra and is ultimately assimilated by the liver (30), which recognises the sterol-rich chylomicron remnant. Thus, the first tissues to expand their cholesterol pools in response to the diet are probably the intestine and liver. Conceptually, this should have two effects. First, endogenous synthesis of the sterol in these organs should fall and secondly, according to Goldstein and Brown (12), their assimilation of plasma cholesterol in the form of LDL should diminish due to suppression of the high affinity receptor pathway. The first effect is well documented. The amount of cholesterol absorbed has been reported to increase linearly with that

presented in the diet (31–33) and this results in suppression of endogenous sterol synthesis, particularly in the liver (33, 34). In addition, excess sterol is reexcreted in the bile so that many individuals in this way are able to compensate to some extent for the increased cholesterol load (33). Undoubtedly this contributes to the recognised variability in the response of lipoprotein cholesterol to dietary sterol supplementation (20, 28, 29, 33). Evidence for suppressed intestinal and hepatic LDL receptor activity in response to cholesterol feeding is much more difficult to obtain. Nevertheless, recent studies have suggested that where the liver expresses significant receptor activity, cholesterol feeding will suppress it. This is seen in beagle puppies or in cholestyramine-treated adult dogs fed a cholesterol-rich diet (35) or infused with cholesterol-containing lymph chylomicrons (36). However, its relevance to man, whose hepatic receptors may be normally suppressed (35), is open to question and indeed our subjects showed no significant fall in the amount of LDL cleared by the receptor route.

So, elevation of the hepatic cholesterol pool, which presumably occurred during egg feeding, seemed to have a greater impact on LDL synthesis than on its catabolism. Synthesis rose 23% and accounted for most of the increment in circulating LDL during dietary cholesterol supplementation. This should have reduced the receptor-mediated fractional clearance of LDL so that a constant absolute amount of cholesterol was delivered to extrahepatic tissues by the physiologically controllable receptor pathway (37). This in fact occurred in a study of lymphocyte LDL receptors (20) and was the tendency in our group of seven subjects (Table IV) although the response was variable. Therefore, where the LDL pool is elevated in response to cholesterol feeding the receptor-independent catabolic

TABLE IV
Effects of Cholesterol Feeding (6 eggs/d) on apoLDL Metabolism

Plasma apoLDL			ApoLDL fractional catabolism*						ApoLDL absolute catabolic rate†					
Subject No.			(a) Total		(b) Receptor mediated		(c) Receptor independent		(a) Total		(b) Receptor mediated		(c) Receptor independent	
	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed	Control	Cholesterol fed
	mg/dl		pools/d						mg/kg/d					
M ₁	104	137	0.295	0.298	0.106	0.079	0.189	0.219	12.5	15.9	4.5	4.1	8.0	11.8
M ₂	112	141	0.330	0.282	0.105	0.124	0.225	0.158	15.0	15.7	4.8	6.9	10.2	8.8
M ₃	76	87	0.385	0.363	0.250	0.151	0.135	0.212	11.7	12.5	7.6	5.2	4.1	7.3
F ₁	105	162	0.298	0.279	0.121	0.121	0.177	0.158	12.8	17.8	5.2	7.7	7.6	10.1
F ₂	83	132	0.436	0.355	0.215	0.182	0.221	0.173	14.8	18.4	7.3	9.4	7.5	9.0
F ₃	108	160	0.306	0.241	0.136	0.088	0.170	0.153	13.3	15.3	5.9	5.6	7.4	9.7
F ₄	81	118	0.405	0.401	0.203	0.194	0.202	0.207	13.1	19.0	6.6	9.2	6.5	9.8
Mean±1 SD	95±15	133±26	0.351±0.060	0.317±0.060	0.162±0.060	0.134±0.040	0.188±0.030	0.183±0.030	13.3±1.2	16.4±2.2	6.0±1.2	6.9±2.0	7.7±1.7	9.5±1.4
Paired t test	P < 0.001		P < 0.05		NS		NS		P < 0.01		NS		P < 0.02	

* The fraction of the apoLDL pool catabolized per day.

† The product of the fractional catabolic rate and the plasma apoLDL pool size expressed per kilogram of body weight.

pathway handles more of the sterol. It is arguable that such an effect is detrimental since this "scavenger" pathway has been implicated in the pathogenesis of atherosclerosis (37).

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