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Increased dietary cholesterol does not increase plasma low density lipoprotein when accompanied by an energy-restricted diet and weight loss

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■ **Abstract** *Background* Diets enriched with dietary cholesterol, frequently from eggs, have been shown to produce a small but variable increase in plasma low density lipoprotein (LDL) cholesterol. There is evidence to suggest that energy-restricted diets, that may contain a relatively high proportion of fat and cholesterol, can attenuate the cholesterol-raising effect of dietary cholesterol on plasma LDL. *Aim of the study* To determine the combined effects of increased dietary cholesterol and weight loss produced by energy restriction on plasma LDL cholesterol and lipoproteins. *Methods* A randomized, controlled, parallel study was performed in two groups of free-living volunteers on an energy-restricted diet for 12 weeks, one group was instructed to consume two eggs a day ($n = 24$), the other, to exclude eggs ($n = 21$). Dietary advice on energy restriction was based on the British Heart Foundation guidelines on how to lose weight for men and women. *Results* Energy intake fell by 25 and 29% in the egg-fed and non-egg-fed

groups, resulting in a moderate weight loss of 3.4 kg ($P < 0.05$) and 4.4 kg ($P < 0.05$), respectively. The daily intake of dietary cholesterol increased significantly in the egg-fed group from 278 to 582 mg after 6 weeks. The concentration of plasma LDL cholesterol decreased in the non-egg-fed groups after 6 weeks ($P < 0.01$) and in the egg-fed and non-egg-fed at 12 weeks relative to baseline. There were no other significant changes in plasma lipoproteins or LDL particle size. *Conclusions* An increased intake of dietary cholesterol from two eggs a day, does not increase total plasma or LDL cholesterol when accompanied by moderate weight loss. These findings suggest that cholesterol-rich foods should not be excluded from dietary advice to lose weight on account of an unfavorable influence on plasma LDL cholesterol.

■ **Key words** dietary cholesterol – LDL cholesterol – energy restriction – weight loss

Introduction

Numerous studies have shown that an increased intake of dietary cholesterol can increase plasma low density lipoprotein (LDL) cholesterol [5, 4, 7, 8, 13, 25]. However, the effect of increased dietary cholesterol on LDL has been relatively small in comparison to the well established LDL-raising effects of dietary saturated fat [5]. It is also highly variable between individuals [7, 8, 12], and, on the strength of evidence from prospective cohort studies, does not translate into an increased risk of CHD [11, 15, 16]. Nevertheless, dietary advice to lower blood cholesterol continues to recommend avoidance of cholesterol-rich foods, on the pretence that they will increase plasma cholesterol and are thus inappropriate for cardiovascular health. In the face of the current epidemic of obesity, weight loss achieved through changes in lifestyle remains central to the treatment and prevention of CHD [14]. Low carbohydrate diets that are relatively high in cholesterol but effective in promoting weight loss, at least in the short term, are reported to produce neutral, or in some cases even favorable rather than adverse effects on plasma cholesterol and lipid profile [6, 21, 24]. This observation suggests that the energy deficit that produces weight loss and/or the weight loss itself may counter affect the cholesterol-raising effects of increased dietary cholesterol. To test this hypothesis, the present study compared the combined effects of two energy-restricted diets, with and without added dietary cholesterol (2 eggs/day), on weight loss, plasma lipids and lipoproteins.

Experimental methods

A total of 67 healthy male and female volunteers, aged 18–55 years, were screened in response to an advertisement in local newspapers. Exclusion criteria for subject selection included a BMI > 35, plasma total cholesterol >6.5 mmol/l and plasma triglyceride >3 mmol/l. Subjects also had to be free of any medical condition or medication that would adversely affect lipid metabolism or prevent them from adhering to the study protocol, and be willing to eat eggs. Subjects had to refrain from consuming dietary supplements or cholesterol-lowering ‘functional’ foods, chiefly products containing plant sterols or enriched with soy protein, 2 weeks prior to baseline measurements, and for the duration of the intervention. Additional exclusion criteria included being on an existing diet or having lost >3 kg in weight in the preceding 2 months. Of the 67 subjects screened, 14 failed to meet the entry criteria due to hyperlipidemia or medication likely to influence lipid metabolism. The remaining 53 were randomized to ei-

ther an energy-restricted diet with two eggs per day or the same energy-restricted diet with no eggs, both for 12 weeks. All subjects gave their informed consent in writing, and the study design was approved by the University of Surrey’s Ethical Committee.

■ Study design and diets

The study was a dietary intervention with an ‘open’ design in which subjects were randomly assigned to one of two parallel dietary interventions; an energy-restricted diet which included two eggs per day for 12 weeks, and the same energy-restricted diet without eggs (‘control’) for 12 weeks. The rationale for using two eggs a day was based on this delivering approximately 400 mg cholesterol over and above baseline intakes. This intake of dietary cholesterol represents an upper physiological limit, beyond which cholesterol absorption in the gut may be diminished [18]. Two eggs a day also represented an increased intake of cholesterol that the subjects could easily incorporate into their diet. The energy-restricted diet was based on the British Heart Foundation guidelines for losing weight for men and women [22]. The diet aimed to reduce energy intake by between 2 and 4 MJ (500–1,000 kcal) per day, chiefly by limiting portion size and the consumption of both dietary fat and carbohydrate. The egg-fed group was provided with medium-sized eggs from a single supplier throughout the study. All subjects completed an initial 7-day food diary to establish their habitual dietary intake, after which, each subject received dietetic counselling and an individualized diet plan to follow for 12 weeks. Weight loss was monitored during the study by regular (weekly to fortnightly) meetings with a dietician. A further 7-day food diary was completed after 6 weeks of dietary intervention as a check on dietary compliance.

■ Study visits and blood samples

To standardize both short and longer term variation in dietary intake within and between subjects, each subject was provided with a low fat (fat <5% energy) supermarket ‘ready-meal’ as a pre-visit meal to consume by 20.00 h the night before their baseline, 6- and 12-week visits. Subjects attended the clinical investigation unit at the University of Surrey in the morning between 8 and 11 am, after a 12-h fast. Height, weight and waist circumference were measured. Blood pressure was measured by automated plethysmography. Percentage body fat was measured by bioelectrical impedance (Tanita, UK). Blood was drawn from an antecubital vein and collected into tubes containing either EDTA, fluoride oxalate or clot accelerant for the

immediate separation of plasma and serum, which were stored at -80°C prior to laboratory analysis.

■ Lipid and lipoprotein analyses

Total plasma cholesterol and triglyceride were measured by commercially available enzymatic assays (Randox, UK). LDL and HDL cholesterol were measured directly using 'LDL and HDL Direct' kits (Randox, UK) with the use of a SpACE autoanalyzer (Alfa Wassermann, Woerden, Netherlands). LDL was also calculated using the Friedewald formula. LDL subclass distribution was measured by iodixanol density gradient centrifugation using LDL density as a surrogate of LDL particle size as previously described [2]. Plasma glucose and insulin were measured by standard, commercially available colorimetric and ELISA assays, respectively. With the exception of serum insulin, which had within and between assay variation of 5 and 8%, respectively, within and between assay variation for plasma lipids, lipoproteins and apolipoproteins was $<2\%$.

■ Statistical analysis

To take account of the possibility that the addition of two eggs may influence weight-loss, the study was designed to test two possible outcomes; that the cholesterol-enriched, energy-restricted diet could either be 'equivalent' or 'superior' in its effects on plasma LDL cholesterol to that of the energy-restricted diet alone. To test for an 'equivalent' effect it was necessary to pre-define the largest and clinically acceptable difference in plasma LDL cholesterol between diets. Any increase in the concentration of LDL cholesterol above the normal day-to-day variation ($>2\%$) would have been considered unacceptable. Alternatively, in the event of the cholesterol-enriched diet producing additional weight loss, above that achieved with an energy-restricted diet alone, this would be deemed a 'superior' effect that would require a pre-study calculation of the size of statistical difference between dietary treatments after 12 weeks [17]. A sample size for each group of 25 was calculated to provide 80% power of detecting a significant difference in body weight. This was based on a predicted loss in body weight that would accompany an energy restriction of 4.18 MJ/day ($\sim 1,000$ kcal/day) for 12 weeks. This estimate included an allowance for a subject drop-out of 25%, and for 50% of the subjects to under achieve the target weight loss.

All biochemical data, body weight and percentage body fat were logarithmically transformed for statis-

Table 1 Baseline characteristics of subjects

Characteristics	Egg-fed ($n = 24$)	Non-egg-fed ($n = 21$)
Age (years)	44.9 (8.4)	43.0 (10.5)
Sex (M/F)	8/17	6/15
Weight (kg)	84.2 (15.9)	83.2 (15.7)
Body mass index (kg/m^2)	30.1 (3.7)	29.0 (4.2)
Waist circumference (cm)	97.5 (13.6)	90.1 (12.1)
Blood pressure (mmHg)		
Systolic	120 (13)	126 (20)
Diastolic	79 (7)	78 (10)
Plasma glucose (mmol/l)	5.78 (0.65)	5.90 (0.53)
Plasma cholesterol (mmol/l)	5.21 (0.79)	5.30 (1.16)

Values are arithmetic and geometric (plasma cholesterol) means (SD)

tical analysis and the results expressed as geometric means \pm standard deviation (SD). Differences between the egg-fed and non-egg-fed groups were examined by ANOVA with time as a repeated measure using a General Linear Model (GLM) with 'treatment' (egg-fed vs. non-egg-fed) and 'time' (0, 6, 12 weeks) as factors. Within and between group differences between baseline and 12 weeks were determined by one and two-sample students *t* test, respectively, with a Bonferroni correction for multiple comparisons. All statistical tests were performed using Minitab (Minitab Inc, US, Release 13.1).

Results

A total of 53 volunteers met the study entry criteria and were randomly assigned to either the energy-restricted diet with no eggs (control, $n = 26$) or the energy-restricted diet plus two eggs per day ($n = 27$). Eight subjects withdrew during the course of the study for personal reasons or illness ($n = 5$), pregnancy ($n = 1$) or failure to adhere to the study protocol ($n = 2$). The remaining 45 volunteers successfully completed the study within the egg-fed ($n = 24$) and non-egg-fed groups ($n = 21$). There were no significant differences between dietary groups in age, weight or concentration of plasma lipids and lipoproteins at baseline. The baseline characteristics of all participants are shown in Table 1.

■ Dietary intakes

Both the energy-restricted diets in the egg-fed and non-egg-fed groups produced significant reductions in energy intake of 25 and 29%, respectively ($P < 0.01$) (Table 2). While a mean energy deficit of approximately 2,000 kJ/day (~ 500 kcal/day) was produced by a decrease in the intake of both dietary carbohydrate (egg-fed -24% , non-egg-fed -19%) and

Table 2 Daily dietary intakes of subjects at baseline and after 6 weeks of intervention

	Egg-fed (<i>n</i> = 24)			Non-egg-fed (<i>n</i> = 21)		
	Base line	6 weeks	Change (%)	Baseline	6 weeks	Change (%)
Energy (kJ × 10 ⁻³)	9.43 (2.40)	7.21 (2.4)**	-25	8.58 (2.23)	6.10 (1.50)**	-29
Total fat (g)	82 (35)	55 (28)*	-33	68 (17)	36 (13)**†	-47
Saturated (g)	38 (26)	21 (10)*	-45	28 (9)	14 (7)**†	-50
Polyunsaturated (g)	13 (5)	12 (8)	-8	13 (4)	8 (3)**†	-38
Monounsaturated (g)	31 (11)	22 (12)*	-29	26 (8)	14 (4)**††	-46
Cholesterol (mg)	278 (143)	582 (138)**	109	258 (93)	144 (67)**††	-44
Total carbohydrate (g)	289 (59)	220 (67)*	-24	271 (80)	219 (115)	-19
Total protein (g)	86 (21)	81 (22)	-6	76 (15)	68 (13)**†	-11
Alcohol (g)	26 (26)	16 (24)	-38	24 (28)	14 (18)*	-42

Values are arithmetic means

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ within group differences by Student's paired *t* test. † $P < 0.05$, †† $P < 0.01$ between group differences by Student's unpaired *t* test

fat, a reduction in dietary fat made the greatest contribution to this effect, most noticeably in the non-egg-fed group alone (egg-fed -33%, non-egg-fed -47%). Both groups consumed less saturated ('S') fat, whereas the non-egg-fed group consumed proportionally less poly ('P') and monounsaturated fat. This resulted in an increase in the P:S ratio in both groups [0.45–0.59 egg-fed ($P < 0.05$), 0.51–0.61 non-egg-fed (n.s.)]. There was no change in the intake of dietary protein in the egg-fed group but a decrease in protein intake in the non-egg fed group, and similar reductions in the intake of alcohol in both groups (egg-fed -38%, non-egg-fed -42%, $P < 0.05$). The intake of dietary cholesterol was increased by more than twofold in the egg-fed group to 582 mg/day (+109%, $P < 0.01$), and decreased in the non-egg-fed group, who were advised to exclude eggs from their diet (-44%, $P < 0.01$).

■ Weight loss

Both the egg-fed and non-egg-fed groups lost a significant amount of body weight on an energy-restricted diet for 12 weeks (Table 3). Significant weight loss was achieved within each group after 6 weeks, and was maintained over the next 6 weeks. There was no significant difference in total body weight or percentage body fat between groups after 6 or 12 weeks. Both the egg-fed and non-egg-fed groups had lost similar amounts of body fat by the end of the study (-2 kg egg-fed, -3 kg non-egg-fed).

■ Plasma lipids and lipoproteins

The concentration of plasma lipids, lipoproteins and apolipoproteins all tended to decrease over time on

Table 3 Body weight, plasma lipids, lipoproteins and apoproteins at baseline and after 6 and 12 weeks of dietary intervention

	Egg-fed (<i>n</i> = 24)			Non-egg-fed (<i>n</i> = 21)		
	Baseline	6 weeks	12 weeks	Baseline	6 weeks	12 weeks
Weight (kg)	81.0 (16.6)	78.6 (16.0)†	77.6 (16.0)†	80.7 (15.3)	77.3 (14.8)†	76.4 (14.7)†
Body fat (%)	34.9 (7.9)	34.3 (7.2)	33.3 (7.9)	33.6 (7.4)	32.5 (8.4)	31.2 (8.9)
Lipids (mmol/l)						
Total cholesterol	5.34 (1.14)	5.29 (1.15)	4.99 (1.24)	5.20 (0.99)	4.63 (0.94)	4.98 (0.91)
LDL cholesterol	3.02 (0.76)	2.97 (0.78)	2.85 (0.78)	2.95 (0.66)	2.72 (0.49)*	2.85 (0.51)
HDL cholesterol	1.12 (0.28)	1.08 (0.31)	0.97 (0.30)	1.08 (0.36)	0.92 (0.35)	1.10 (0.32)
Triglyceride	1.20 (0.56)	1.23 (0.62)	1.11 (0.44)	1.28 (0.59)	1.17 (0.48)	1.21 (0.72)
LDL peak density (g/l)	1.0243	1.0243	1.0237	1.0252	1.0250	1.0251
%sdLDL (median)	14.8 (10.1)	15.7 (12.4)	13.5 (10.7)	18.4 (16.2)	18.5 (17.2)	20.1 (13.5)
Apoproteins (g/l)						
Apoprotein A-I	1.28 (0.27)	1.21 (0.27)	1.12 (0.28)	1.19 (0.22)	1.09 (0.20)	1.18 (0.20)
Apoprotein B	1.03 (0.25)	1.17 (0.25)	1.18 (0.20)	1.04 (0.19)	1.25 (0.18)	1.14 (0.23)

Values are geometric means of log transformed data. LDL cholesterol was calculated by Friedewald formula

* Treatment effect ($P < 0.01$) by General Linear Model, with a post hoc Tukey pairwise comparison; and † within group differences ($P < 0.05$) after 6 and 12 weeks versus baseline, by Student's paired *t* test with Bonferroni correction for multiple comparisons

the energy-restricted diet in both the egg-fed and non-egg-fed groups, but there were no significant dietary effects between the groups (Table 3). The concentration of total plasma cholesterol fell over 12 weeks in both the egg-fed and non-egg-fed groups as a result of a decrease in plasma LDL cholesterol, as measured directly and by calculation. While LDL cholesterol decreased from baseline within both groups after 6 weeks, only the decrease in the non-egg-fed group was significant (change from baseline at 6 and 12 weeks for egg-fed: -1.7 and -5.6% and non-egg-fed: -7.8% ($P < 0.01$) and -3.9%). There was no change in LDL particle size, as measured by the peak LDL density, or the relative proportion of small, dense LDL, either between groups or within groups over time. Although the concentration of plasma triglyceride, HDL cholesterol, and apolipoproteins A-I and B decreased in parallel with the fall in LDL cholesterol, there was no significant change in these measures, either between or within groups over time (Table 3). There were also no significant differences in the concentration of either plasma glucose or insulin either between the egg-fed and non-egg fed groups or over time (data not shown).

The increase in the ratio of dietary polyunsaturated to saturated fat ($P:S$ ratio) was inversely associated with the decrease in LDL cholesterol at the end of the study ($r = -0.56$, $P < 0.05$). However, there was no evidence of any other significant relationships between dietary intake (difference in total fat or fatty acids within or between groups), weight loss and plasma lipids or lipoproteins, or between the changes in these variables.

Discussion

An increase in dietary cholesterol from two eggs a day, in combination with energy restriction, was accompanied by a decrease in plasma LDL cholesterol that, was approximately similar to that produced by an energy-restricted diet alone. This observation provides evidence to suggest that either the energy restriction and/or the resultant weight loss, may counter affect any potential plasma cholesterol-raising effects of increased dietary cholesterol.

Previous intervention studies with dietary cholesterol that controlled for the confounding effects of total and saturated fat, established that intakes of cholesterol between 100 and 700 mg/day produce a small but highly variable increase in plasma cholesterol between individuals (0.04–0.07 mmol/l plasma cholesterol per 100 mg of additional dietary cholesterol) [4, 5, 8, 7, 13, 25]. Nevertheless, this effect does not appear to translate into a link between dietary

cholesterol and increased risk of developing CHD [11, 15, 16]. In contrast, the growing prevalence of obesity presents the greatest risk to cardiovascular health in the UK, as the fattest country in Europe, with over half the adult population being overweight and a quarter clinically obese [20]. The dietary advice provided in this study aimed to produce weight loss through reductions in both dietary fat and carbohydrate. Even though the energy restriction in both groups was achieved, for the most part, through a reduced intake of fat, the effects of this diet are not comparable with that of more extreme diets that have excluded energy as predominantly carbohydrate or fat. On the other hand, the higher cholesterol content of the egg-fed group is comparable to the high cholesterol content of some commercially renowned, low carbohydrate diets. The long-term safety and efficacy of low, and very low carbohydrate diets on cardiovascular health are contentious, not only because they tend to be high in dietary saturated fat and cholesterol and thus have the capacity to increase plasma LDL cholesterol, but also because the long-term effects have not been studied. However, weight loss on these diets has not generally been accompanied by significant increases in plasma LDL cholesterol [6, 21, 24]. This suggests that energy restriction may mitigate against any cholesterol-raising potential of these high fat diets. No studies to date have examined how energy restriction and/or weight loss influence the effect of dietary cholesterol on plasma LDL. In the present study, only the non-egg-fed group showed a significant reduction in plasma LDL cholesterol after 6 weeks on an energy-restricted diet. Since the change in LDL cholesterol, and other measures, paralleled the decrease in body weight over time, it is reasonable to assume that these changes were produced in response to the energy deficit and/or the weight loss. However, there was no evidence of any relationships between energy intake, body weight or weight loss and changes in plasma lipids or lipoproteins to support this assumption. Moreover, with the exception of a decrease in the $P:S$ ratio, there was also no evidence to link changes in the intake of total and saturated fat that with the decreases in plasma LDL cholesterol. This may be explained, in part, by the small size of the study groups in relation to the between subject variability associated with changes in plasma lipids and body weight. Moreover, in the absence of additional control groups (weight-maintained, egg-fed and non-egg-fed groups), the study was unable to fully discriminate between the effects of weight loss and dietary cholesterol on plasma cholesterol.

Dietary advice and counselling based on the British Heart Foundation's guidelines to lose weight [22] was successful in achieving an energy deficit ($\sim 2,000$ kJ or ~ 500 kcal/day) and in promoting moderate weight

loss in both groups. The amount of weight loss at 6 weeks corresponded to the energy deficit in both groups, and was also close to that predicted to be a feasible amount of weight to lose over 12 weeks.

However, the reduced amount of weight lost between 6 and 12 weeks strongly suggests that the energy deficit was not maintained over this period. This is a frequent finding in weight loss studies that can result from decreased motivation and dietary compliance, and highlights the lack of experimental control in studies with an 'open' design, and the need to consider the results with care.

In view of the high satiety index of eggs [10] and recent evidence to suggest that eggs may increase satiety [23], it was of interest to see whether the inclusion of eggs in an energy-restricted diet could promote additional weight loss when compared with an energy-restricted diet alone. However, since the non-egg consumers lost marginally more weight than the egg-fed group, there was no evidence to suggest that eggs *per se* can promote weight loss. The effects of food in general to increase satiety may depend on the time of day and the form in which the food, including eggs, is consumed [23]. The present study, was neither designed, nor sufficiently well controlled or statistically powered to test the effect of a food on satiety.

The concentration of plasma LDL cholesterol is regulated, to a large extent, by the rate at which it is

removed from the plasma compartment. This in turn is regulated by levels of intracellular cholesterol, which are tightly controlled by the activity of membrane LDL receptors that bind and remove circulating LDL particles from the plasma [1]. Weight loss produced by an energy-restricted, low carbohydrate diet has been shown to lower LDL cholesterol by increasing the removal of LDL particles from the plasma by increasing the activity of LDL receptors [19]. Conversely, the LDL-raising properties of dietary cholesterol and saturated fat have, in part, been attributed to their capacity to suppress the activity of LDL receptors [3]. It is conceivable that the decreases in plasma LDL in the present study resulted from the energy restriction and/or weight loss over riding a suppressive effect of the increased dietary cholesterol on this receptor pathway.

In conclusion, this study demonstrates that increasing dietary cholesterol by consuming two eggs a day, produces no increase in plasma LDL when accompanied by energy restriction and moderate weight loss. The latter may also counteract any cholesterol-raising potential of increased dietary cholesterol, at least in the short term. These findings support the view that cholesterol-rich foods should not be excluded from an energy-restricted diet on account of producing an unfavorable effect on blood cholesterol [9].

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