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Egg consumption as part of an energy-restricted high-protein diet improves blood lipid and blood glucose profiles in individuals with type 2 diabetes

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Abstract

The role of dietary cholesterol in people with diabetes has been little studied. We investigated the effect of a hypoenergetic high-protein high-cholesterol (HPHchol) diet compared to a similar amount of animal protein (high-protein low-cholesterol, HPLchol) on plasma lipids, glycaemic control and cardiovascular risk markers in individuals with type 2 diabetes. A total of sixty-five participants with type 2 diabetes or impaired glucose tolerance (age 54·4 (SD 8·2) years; BMI 34·1 (SD 4·8) kg/m^2; LDL-cholesterol (LDL-C) 2·67 (SD 0·10) mmol/l) were randomised to either HPHchol or HPLchol. Both hypoenergetic dietary interventions (6–7 MJ; 1·4–1·7 Mcal) and total carbohydrate:protein:fat ratio of 40:30:30 % were similar but differed in cholesterol content (HPHchol, 590 mg cholesterol; HPLchol, 213 mg cholesterol). HPHchol participants consumed two eggs per d, whereas HPHchol participants replaced the eggs with 100 g of lean animal protein. After 12 weeks, weight loss was 6·0 (SD 0·4) kg ($P<0·001$). LDL-C and homocysteine remained unchanged. All the subjects reduced total cholesterol (−0·3 (SD 0·1) mmol/l, $P<0·001$), TAG (−0·4 (SD 0·1) mmol/l, $P<0·001$), non-HDL-cholesterol (HDL-C, −0·4 (SD 0·1) mmol/l, $P<0·001$), apo-B (−0·04 (SD 0·02) mmol/l, $P<0·01$), HbA1c (−0·6 (SD 0·1) %, $P<0·001$), fasting blood glucose (−0·5 (SD 0·2) mmol/l, $P<0·01$), fasting insulin (−1·7 (SD 0·7) mIU/l, $P<0·01$), systolic blood pressure (−7·6 (SD 1·7) mmHg, $P<0·001$) and diastolic blood pressure (−4·6 (SD 1·0) mmHg; $P<0·001$). Significance was not altered by diet, sex, medication or amount of weight loss. HDL-C increased on HPHchol (+0·02 (SD 0·02) mmol/l) and decreased on HPLchol (−0·07 (SD 0·03) mmol/l, $P<0·05$). Plasma folate and lutein increased more on HPHchol ($P<0·05$). These results suggest that a high-protein energy-restricted diet high in cholesterol from eggs improved glycaemic and lipid profiles, blood pressure and apo-B in individuals with type 2 diabetes.

Key words: **Type 2 diabetes: Lipid profiles: Blood glucose: Weight-reducing diets: Eggs**

The increased prevalence of type 2 diabetes has resulted in it becoming a leading public health problem[1]. Although there is a strong genetic predisposition to the development of type 2 diabetes, obesity is a significant contributor and it is a strong independent risk factor for CVD[2]. The risk of mortality from CVD is two to four times higher in this population[3]. Approximately, 80 % of individuals presenting with type 2 diabetes are overweight or obese, which makes long-term weight management an essential component in the reduction of diabetes-associated morbidity.

Increasingly the literature supports the efficacy of high-protein weight loss dietary patterns in improving cardiovascular risk factors particularly in individuals with insulin resistance (IR) including type 2 diabetes[4–8]. Inexpensive protein sources such as eggs could potentially contribute to higher protein dietary patterns, but concerns about excessive dietary cholesterol intake in people at cardiovascular risk raise questions about limitations of egg consumption.

There are only two epidemiological studies published that have reported the effects of eggs or dietary cholesterol on the risk of CHD in people with diabetes. Both the studies showed increased CHD risk with increasing dietary cholesterol intakes; however, there was no distinction between individuals with type 1 or type 2 diabetes[9,10].

The most recent meta-analysis of seventeen studies by Weggemans *et al.*[11] conducted in individuals with normoglycaemia demonstrated that the addition of 100 mg dietary cholesterol/d increased LDL-cholesterol (LDL-C) by 0·05 mmol/l. Two small studies from this meta-analysis[11]

Abbreviations: CRP, C-reactive protein; HbA1c, glycosylated Hb; HDL-C, HDL-cholesterol; HOMA2, homoeostatic model assessment; HPHchol, high-protein high-cholesterol; HPLchol, high-protein low-cholesterol; IR, insulin resistance; LDL-C, LDL-cholesterol; TC, total cholesterol.

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in individuals with impaired glucose metabolism examining the effect of 800 mg/d of dietary cholesterol from eggs bear comment. Dietary cholesterol had little effect either on LDL-C or on total cholesterol (TC) in thirty-one overweight, insulin-resistant individuals over 4 weeks[12] but increased TC and LDL-C in ten individuals with insulin-dependent diabetes over 3 weeks[13]. A further 4-week study in insulin-sensitive individuals consuming four eggs per d showed a significant increase in non-HDL-cholesterol (HDL-C) and inflammatory markers in insulin-sensitive individuals, which was not observed in lean or obese insulin-resistant individuals[14].

Clinical research on dietary cholesterol and diabetes management is very limited, and there are no clinical intervention trials that have investigated the role of egg consumption in people with type 2 diabetes. We conducted a clinical trial to examine the role of eggs in the context of a high-protein weight-reducing dietary pattern in individuals with type 2 diabetes. We hypothesised that the daily consumption of two eggs per d (containing 400 mg of dietary cholesterol) compared to an equivalent animal protein source (meat, chicken or fish) low in both saturated fat and cholesterol would similarly improve the lipid profile, cardiovascular risk markers and glycaemic control under conditions of weight loss (6–7 MJ/d) in individuals with type 2 diabetes.

Experimental methods

Subjects

A total of eighty-two participants (aged 20–75 years; BMI 25–40 kg/m^2; glycosylated Hb (HbA1c) < 9 %) were recruited through public advertisement to participate in a 12-week outpatient clinical trial. Volunteers were assumed to have diabetes if fasting fingerprick glucose was > 6·1 mmol/l. Diabetes status was confirmed through the analysis of fasting blood glucose and the 2 h oral glucose tolerance test[15]. Of those who completed, fourteen had impaired glucose tolerance or impaired fasting glucose and six had fasting sugar levels between 5 and 6 mmol/l. Participants were excluded if they had a malignancy, a history of liver, kidney or gastrointestinal disease, or if they consumed food that could interfere with the study (e.g. plant sterol margarines and psyllium). The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all the subjects provided a written informed consent, and all experimental procedures were approved by the human ethics committees of the Commonwealth Scientific and Industrial Research Organisation and the University of Adelaide. Several participants were taking medication, hypoglycaemic medication, (*n* 38), lipid-lowering medication (*n* 34) and blood pressure medication (*n* 34). No participants were taking exenatide. As type 2 diabetes is a chronic disease that requires long-term medical attention, both to limit the development of its devastating complications and to manage them when they do occur, participants taking medication were not excluded from the study provided they maintained a stable dose of medication throughout the study.

Experimental design

In a parallel design, the subjects were allocated to two groups so that the groups were matched for baseline BMI, sex, age, HbA1c, LDL-C and use of metformin, and then the groups were randomly assigned to consume either an energy-restricted high-protein high-cholesterol (HPHchol) or an isoenergetic high-protein low-cholesterol (HPLchol) 12-week dietary pattern using the Clinstat MS DOS program (http://www-users.york.ac.uk/~mb55/soft/soft.htm). The study was conducted on an outpatient basis and was conducted according to the guidelines laid down in the Declaration of Helsinki. The trial registry number (ACTRN) was ACTRN012606000475549.

At baseline (week 0) and after the intervention (week 12), subjects attended the CSIRO outpatient clinic after an overnight fast, during which body mass, height and resting blood pressure were measured before a venous blood sample being drawn for determination of blood lipids, glucose, insulin, HbA1c, C-reactive protein (CRP) and apo-B, homocysteine, vitamin B_{12}, lutein, folate and carotenoids. Throughout the intervention, subjects also attended the clinic fortnightly for a body weight measurement and dietary consult only. Apart from the prescribed dietary intervention, participants were asked to maintain their usual lifestyle throughout the study.

Dietary intervention

The participants consumed one of the two isoenergetic dietary treatments (30 % energy restriction; approximately 6000 kJ (1400 kcal)). Both the hypoenergetic dietary interventions contained a total carbohydrate:protein:fat ratio of 40:30:30 %, but differed primarily in the source of animal protein and cholesterol content (HPHchol, 590 mg cholesterol; HPLchol, 213 mg cholesterol). In the HPHchol dietary pattern, the participants were instructed to consume two eggs per d, whereas participants in the HPLchol diet were instructed to avoid eggs and replace them with 100 g of lean protein (meat, chicken or fish).

Participants attended the Clinical Research Unit every 2 weeks for individual consultations with a dietitian where they were provided with meal plans, instruction on dietary requirements, method for recording food intake and need for compliance. (Kitchen scales were provided; DZC 5000A; Procon Technology, Brisbane, QLD, Australia). Dietary analysis of food records was calculated for 3 d (two midweek and one weekend day) in each 2-week period.

A total of 18 d of records were averaged and analysed using 'Foodworks version 4' software (Xyris Software

1998–2005, Highgate Hill, QLD, Australia) to estimate dietary intake over the 12 weeks. The software is based on Australian Food Composition tables and food manufacturers' data.

Body weight and composition

Body height was measured fortnightly to the nearest 0·1 cm using a stadiometer (SECA, Hamburg, Germany) with subjects barefoot, in the free-standing position. Body weight was measured in the fasting state with subjects without shoes, wearing light clothing using calibrated electronic digital scales (Mercury, AMZ 14, Tokyo, Japan) to the nearest 0·05 kg. BMI was calculated as weight (kg) divided by height (m) squared.

Body composition was assessed using bioelectrical impedance measurements. Measurements were performed using an ImpediMed SFB7, version 6 instrument with individuals lying supine (with palms down and arms positioned by their sides slightly ajar from the body and legs slightly apart) to determine fat mass and fat-free mass. Duplicate measurements were averaged and recorded as the measured value.

Blood pressure

Resting blood pressure was measured by automated oscillometry (Dinamap™ 845XT/XT-IEC, Tampa, FL, USA), with subjects in an upright seated position in the fasted state.

Biochemical analysis

The fasting blood samples were collected at weeks 0 and 12 in tubes with NaF/EDTA for glucose, insulin and homocysteine, tubes with K_3EDTA for plasma carotenoids and lutein, and in tubes without additives for folate, vitamin B_{12}, lipids, creatinine, apo-B and CRP measurements. On a separate occasion, an oral glucose tolerance test at weeks 0 and 12 was conducted. Collection of a fasting venous blood sample for glucose and insulin was followed by the ingestion of 75 g of glucose or 82·5 g of glucose monohydrate in a 300 ml drink of water (Glucaid, Histo-Labs, Riverstone, NSW, Australia) over the course of 5 min[16]. At 2 h post ingestion, a second venous blood sample was collected.

Serum and plasma were isolated by centrifugation at 2000 ***g*** for 10 min at 5°C (Beckman GS-6R centrifuge, Menlo Park, CA, USA) and frozen at −20°C. Biochemical assays were performed in a single assay at the completion of the study.

Serum TC, HDL-C, TAG, total apo-B (B100 and B48), CRP and plasma glucose were measured on a Roche Hitachi 902 auto-analyzer (Roche Diagnostics, Indianapolis, IN, USA) using standard Roche enzymatic kits (Roche Diagnostics, Basel, Switzerland) and control sera. A modified Friedewald[17] equation was used to calculate LDL-C.

Insulin was determined in duplicate using a RIA kit (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden). The homoeostatic model assessment (HOMA2) was used as a surrogate measure of insulin sensitivity (HOMA2 %S) and β-cell function (HOMA2 %B) based on fasting glucose and insulin concentrations[18]. HOMA2-IR is the reciprocal of HOMA2 %S (100/HOMA2 %S). The University of Oxford HOMA calculator was used to calculate the results (http://www.dtu.ox.ac.uk/homacalculator/index.php). The results were expressed as a percentage of the normal reference population. These measures correspond well, but are not necessarily equivalent, to the non-steady-state estimates of β-cell function and insulin sensitivity derived from stimulatory models such as the hyperinsulinaemic clamp, the hyperglycaemic clamp, the intravenous glucose tolerance test and the oral glucose tolerance test.

HbA1c, folate and total plasma homocysteine were analysed using a certified reference laboratory (Institute of Medical and Veterinary Science (Adelaide)).

Plasma extractions and HPLC chromatography for plasma carotenoids and vitamins A and E were performed according to the method of Yang & Lee[19]. Minor modifications to this method were derived from Khachik *et al.*[20]. Details of the isocratic separations and quality control are described elsewhere[21].

Physical activity

Physical activity levels were measured before and after the intervention using a Baecke questionnaire[22].

Statistical analysis

Before hypothesis testing, data were examined for normality. Differences in baseline values were compared using a one-way ANOVA for continuous variables and Pearson's χ^2 test for categorical variables. The effect of the dietary intervention was assessed using repeated-measures ANOVA with time as a within-subject factor and diet and sex as between-subject factors. ANCOVA was used to adjust for weight loss, age and medication (lipid lowering, blood pressure and hypoglycaemic medication as categorical variables). Final systolic blood pressure and diastolic blood pressure were compared using a one-way ANOVA adjusted for baseline values. Subjects with TAG concentrations > 4 mmol/l were excluded from the LDL-C analysis. Participants with CRP > 10 mg/l at either baseline or week 12 were excluded from the analysis, as this was indicative of possible inflammation from intercurrent infection (five from each group). Based on the work of Parker *et al.*[23], we calculated that we needed seventeen subjects in each group to see a 10 % difference in the change in LDL-C between groups with 80 % power ($\alpha = 0{\cdot}05$). Statistical analysis was performed using SPSS for Windows 14.0 software (SPSS, Inc., Chicago, IL, USA) with statistical significance set at an α level of $P < 0{\cdot}05$. All the data are

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presented as means with their standard errors unless otherwise stated. The missing data were not replaced.

Results

Participants

Of the eighty-two participants enrolled in the study, seven individuals withdrew before the commencement period. A further ten subjects withdrew throughout the study; five subjects from the HPHchol diet, citing work commitments (*n* 2), unable to comply with the diet (*n* 1) and health reasons not related to the study (*n* 2), while five subjects withdrew from the HPLchol diet, citing personal reasons (*n* 2), inability to comply with the diet (*n* 1), lost contact (*n* 1) and time commitments (*n* 1). A total of sixty-five participants completed all the aspects of the 12-week intervention; thirty-one participants (76 %) in the HPHchol and thirty-four participants (83 %) in the HPLchol group. Details are outlined in Table 1.

Dietary analysis

There were no significant differences in usual dietary composition between the two diet groups at baseline. This included egg intake before the study (*n* 65, $P=0{\cdot}6$).

The self-reported macronutrient composition of the diets derived from subjects' daily weighed food records over the intervention showed that there were no significant differences between total energy, %carbohydrate, alcohol and fibre between the dietary groups. As anticipated, %total fat, %saturated fat and dietary cholesterol were significantly higher in the HPHchol group (589 (SD 4) mg HPHchol *v.* 227 (SD 4) mg HPLchol, $P<0{\cdot}001$), and dietary protein was slightly but significantly lower (28·1 (SD 0·4) % HPHchol *v.* 31·4 (SD 0·4) % HPLchol, $P<0{\cdot}001$). Egg consumption and compliance for the HPHchol group were 13·8 (SD 0·2) eggs/week (96·6 %) and those for the HPLchol group (no eggs at all) were 98·5 (SD 1·3) % (Table 2). Protein compliance was checked via the dietary checklists completed everyday.

Weight and fat loss

Overall, there was a significant net weight loss over the intervention of 6·0 (SD 0·4) kg ($P<0{\cdot}001$). There were no significant differences by diet allocation with a net weight loss of 5·3 (SD 3·6) kg (5·6 (SD 3·2) %) in the HPHchol group and 6·5 (SD 3·2) kg (6·7 (SD 3·0) %) in the HPLchol group ($P=0{\cdot}2$). There were no sex differences in either total or percentage of weight lost: 6·0 (SD 0·6) % in men and 6·4 (SD 0·5) % in women ($P=0{\cdot}4$). The use of diabetes medications did not alter these relationships: 5·9 (SD 4·3) % in the HPHchol group and 6·9 (SD 4·2) % in the HPLchol group ($P=0{\cdot}3$) (Table 3).

There was a significant overall decrease in fat-free mass and fat mass over the intervention (3·4 (SD 0·6) kg; $P<0{\cdot}000$ and 2·6 (SD 0·7) %, $P<0{\cdot}001$). Both these relationships were not affected by diet allocation, sex or medications.

Biochemical analysis

Blood lipids and cardiovascular markers. Overall, LDL-C and homocysteine were not significantly affected by the intervention or diet allocation regardless of whether the volunteers had proven type 2 diabetes or had impaired fasting glucose/impaired glucose tolerance. There was a significant diet × time interaction with the plasma HDL-C response with an increase observed on HPHchol and a decrease observed on HPLchol, which was retained after adjustment for weight loss ($P=0{\cdot}03$). When the LDL-C

Table 1. Baseline characteristics*
(Mean values and standard deviations)

	HPHchol		HPLchol	
	Mean	SD	Mean	SD
Age (years)	59·8	8·4	59·1	8·0
BMI (kg/m^2)	33·4	4·6	34·8	5·0
Baseline wt (kg)	94·4	15·5	96·8	14·8
SBP (mmHg)	135·4	13·3	129·8	13·5
DBP (mmHg)	74·6	9·2	72·8	8·4
HbA1c (%)	7·0	1·0	7·0	1·0
Smokers (yes/ no)	0/31		1/33	
Male/female (total)	13/18 (31)		16/18 (34)	
Lipid medication (yes/no)	14/17		13/21	
Diabetes medication (yes/no)	16/15		13/21	

HPHchol, high-protein high-cholesterol; HPLchol, high-protein low-cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated Hb.
* Statistics was performed using a one-way ANOVA. There were no significant differences between treatments.

Table 2. Average dietary daily intake
(Mean values and standard deviations)

	Average dietary daily intake*			
	HPHchol (*n* 31)		HPLchol (*n* 34)	
	Mean	SD	Mean	SD
Energy (kJ)†	6136·9	965·9	6012·4	751·5
Protein (% energy)‡	28·1	2·2	31·4	2·0
Fat (% energy)§	31·3	4·2	27·8	3·6
Carbohydrate (% energy)¶	39·7	4·6	39·8	3·4
Saturated fat (% energy)¶	9·4	2·2	8·2	1·6
Cholesterol (mg)‡	589·5	24·1	227·5	21·9
Folate (μg)§	363·9	73·0	337·6	68·2

HPHchol, high-protein high-cholesterol; HPLchol, high-protein low-cholesterol.
* Dietary analysis of food records was calculated for 3 d in each 2-week period. A total of 18 d of records were averaged and analysed using 'Foodworks version 4' software (Xyris Software 1998–2005, Highgate Hill, QLD, Australia) to estimate dietary intake over the 12 weeks. Student's independent *t* tests were used to compare dietary data.
† 1 kJ = 0·239 kcal.
‡ $P<0{\cdot}001$.
§ $P<0{\cdot}005$.
¶ $P>0{\cdot}01$.

Table 3. Lipid, cardiovascular, glycaemic and nutritional plasma markers*
(Mean values with their standard errors)

	Baseline		Week 12		Change		*P*	
	Mean	SEM	Mean	SEM	Mean	SEM	Diet × time	Time†
TC (mmol/l)								
HPHchol	4·66	0·18	4·60	0·18	−0·07	0·09	0·09	<0·001
HPLchol	4·81	0·15	4·51	0·15	−0·28	0·50		
TAG (mmol/l)								
HPHchol	1·77	0·16	1·36	0·16	−0·44	0·13	0·8	<0·001
HPLchol	1·80	0·16	1·39	0·16	−0·42	0·13		
LDL-cholesterol (mmol/l)								
HPHchol	2·66	0·13	2·80	0·13	0·14	0·23	0·2	0·9
HPLchol	2·76	0·13	2·71	0·13	−0·04	0·07		
HDL-cholesterol (mmol/l)								
HPHchol	1·21	0·05	1·24	0·05	0·02	0·02	0·01	0·2
HPLchol	1·24	0·05	1·17	0·05	−0·07	−0·03		
TC:HDL-C‡								
HPHchol	4·02	0·23	3·88	0·23	−0·23	0·18	0·5	0·08
HPLchol	4·04	0·18	3·97	0·18	−0·06	0·09		
Non-HDL-C (mmol/l)§								
HPHchol	3·45	0·17	3·36	0·17	−0·09	0·08	0·3	0·007
HPLchol	3·57	0·14	3·34	0·14	−0·23	0·08		
apo-B (g/l)								
HPHchol	0·87	0·03	0·84	0·03	-0·03	0·18	0·4	0·003
HPLchol	0·87	0·03	0·83	0·03	−0·05	0·02		
CRP (mg/l)¶								
HPHchol	2·64	0·35	2·97	0·35	0·32	0·34	0·8	0·08
HPLchol	3·19	0·38	3·41	0·38	−0·21	0·42		
FBG (mmol/l)								
HPHchol	7·29	0·26	6·96	0·26	−0·32	0·15	0·3	0·005
HPLchol	7·87	0·43	7·14	0·43	−0·73	0·32		
2 h Glucose (mmol/l)								
HPHchol	13·03	0·60	10·30	0·60	−2·39	0·58	0·1	<0·001
HPLchol	12·86	0·86	11·19	0·86	−1·95	−0·46		
Insulin (mIU/l)								
HPHchol	12·41	1·23	11·17	1·23	−1·23	0·93	0·4	0·003
HPLchol	12·01	1·10	9·85	1·10	−2·17	0·58		
HOMA2 %S								
HPHchol	54·9	5·4	63·0	5·3	8·9	3·6	0·1	0·003
HPLchol	56·9	5·4	66·0	5·4	9·0	3·6		
HOMA2 %B								
HPHchol	83·3	6·4	82·9	6·4	−0·3	3·0	0·8	0·6
HPLchol	83·1	6·5	82·6	6·5	−0·4	2·9		
HOMA2-IR								
HPHchol	2·4	0·1	2·1	0·1	-0·3	2·5	0·4	0·004
HPLchol	2·4	0·1	2·2	0·1	−0·2	2·2		
HbA1c%								
HPHchol	6·95	0·17	6·49	0·17	−0·45	0·10	0·06	<0·001
HPLchol	7·10	0·19	6·29	0·19	−0·81	0·14		
SBP (mmHg)‖								
HPHchol	136·3	2·3	125·7	2·3	−10·6	2·5	0·2	<0·001
HPLchol	128·8	2·2	124·4	2·2	−4·5	−1·6		
DBP (mmHg)**								
HPHchol	75·3	1·6	68·9	1·6	−6·4	1·8	0·7	<0·001
HPLchol	72·4	1·4	69·4	1·4	−3·0	1·1		
Lutein (μg/ml)								
HPHchol	0·16	0·01	0·23	0·01	0·08	0·01	0·02	<0·001
HPLchol	0·17	0·01	0·21	0·01	0·04	0·01		
Folate (nmol/l)								
HPHchol	23·0	1·2	28·4	1·2	5·0	0·9	0·048	0·001
HPLchol	24·4	1·6	26·8	1·6	2·0	1·2		
Homocysteine (μmol/l)								
HPHchol	8·1	0·4	8·3	0·4	0·3	0·2	0·9	0·4
HPLchol	7·6	0·3	7·8	0·3	0·2	0·2		
Vitamin B_{12} (pmol/l)								
HPHchol	282	14	315	14	35	12	1	<0·001
HPLchol	334	22	367	22	34	22		
α-Carotene (μmol/l)								
HPHchol	0·06	0·01	0·12	0·01	0·05	0·01	0·1	<0·001
HPLchol	0·07	0·07	0·15	0·01	0·07	0·02		

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Table 3. *Continued*

	Baseline		Week 12		Change		*P*	
	Mean	SEM	Mean	SEM	Mean	SEM	Diet × time	Time†
β-Carotene (μmol/l)								
HPHchol	0·19	0·04	0·36	0·04	0·18	0·04	0·2	<0·001
HPLchol	0·19	0·02	0·29	0·02	0·13	0·02		
Lycopene (μmol/l)								
HPHchol	0·27	0·02	0·25	0·02	0·01	0·01	0·8	0·2
HPLchol	0·26	0·02	0·25	0·02	0·01	0·01		

HPHchol, high-protein high-cholesterol (*n* 31); HPLchol, high-protein low-cholesterol (*n* 34); TC, total cholesterol; CRP, C-reactive protein; FBG, fasting blood glucose; HOMA2%S, homoeostatic model assessment insulin sensitivity; HOMA2%B, homoeostatic model assessment β-cell function; HOMA-IR, homoeostatic model assessment insulin resistance; HbA1c, glycosylated Hb; SBP, systolic blood pressure; DBP, diastolic blood pressure.

* There were no significant differences in variables between the two diets at baseline.

† Main effect of time by repeated-measures AVOVA with all time points as within-subject variables over both treatments.

‡ Significant time × weight loss interaction ($P=0·023$) with repeated-measures ANOVA with all time points as a within-subject factor and weight loss as a covariate.

§ Significant time × weight loss interaction ($P<0·001$) with repeated-measures ANOVA with all time points as a within-subject factor and weight loss as a covariate.

¶ Participants with CRP > 10 mg/l at either baseline or week 12 were excluded from the analysis, as this was indicative of possible inflammation from intercurrent infection (five from each group).

‖ Final SBP was assessed using a one-way ANOVA with covariate adjustment for baseline values.

** Final DBP was assessed using a one-way ANOVA with covariate adjustment for baseline values.

responses to the two dietary patterns were plotted, the profile of changes was similar with no clear indication that the participants in the HPHchol diet could be classed as 'hyper-responders' to dietary cholesterol (data not shown). Removing individuals who had been taking statins from the analysis for blood lipids did not change the significance of these results.

Non-HDL-C decreased over time ($P<0·01$). These relationships were strengthened with adjustment for weight loss ($P<0·001$ and $P=0·02$, respectively). Removing individuals who had been taking statins from the analysis for blood lipids did not change the significance of these results.

Overall, there was a significant effect of time on the intervention and TC ($P<0·001$) with no effect of diet. Removing individuals who had been taking statins from the analysis for blood lipids did not change the significance of these results. Plasma TAG and apo-B were decreased equally effectively with weight loss on both dietary interventions ($P<0·001$ and 0·005, respectively). Sex or medications did not alter these relationships.

Overall, there was no change in CRP for individuals free from infection (CRP < 10 mg/l). Close examination of the data above and below 3 mg/l showed that starting CRP did not influence the change in CRP. Weight loss, sex or medications did not alter these relationships.

Homocysteine remained constant over the intervention and did not vary by diet.

Glycaemic control. Overall, fasting blood glucose was significantly reduced by approximately 4% with weight loss on both dietary patterns ($P=0·005$) independent of diet. Two-hour glucose in the oral glucose tolerance test was significantly reduced by approximately 18% with weight loss ($P<0·001$). Further analyses were performed to assess the impact of baseline HbA1c. Individuals with both good and poor glycaemic control (HbA1c < 7% (*n* 28) or > 7% (*n* 34), respectively) experienced significant reductions in HbA1c ($P<0·001$ for both), which were independent of diet. Fasting insulin decreased 6% in the HPHchol group and 18% in the HPLchol group with weight loss ($P<0·005$ for time; $P=0·4$ for diet × time). HOMA2%S increased significantly over the intervention by 9% ($P<0·005$). HOMA2%B remained constant over the intervention at 83 (SD 1·1)%. HOMA2-IR decreased significantly over time. There was no effect of diet or sex on HOMA2%S, HOMA2%B or HOMA2-IR. Removal of four individuals who changed their medications did not change the significance of these results (three HPHchol individuals and one HPLchol individual).

Blood pressure. There was a significant effect of weight loss on systolic blood pressure and diastolic blood pressure reduction ($P<0·001$ for both). There were no differences between the interventions in either systolic blood pressure or diastolic blood pressure after adjustment for baseline blood pressures, blood pressure medications, sex or amount of weight lost.

Nutritional analysis

Plasma folate and lutein increased on both dietary interventions ($P<0·001$ and $P=0·001$, respectively) with more pronounced increases in the HPHchol group than in HPLchol group ($P<0·05$ and 0·05 diet × time interaction, respectively). Plasma vitamin B_{12}, α-carotene and β-carotene all increased similarly over time on both the dietary patterns ($P<0·001$ for all) with no differential effect of diet. There were no changes in homocysteine and lycopene concentrations over time. Weight loss, sex or medications did not alter these relationships.

Physical activity

Physical activity levels remained relatively constant before and after the intervention (2·4 (SD 0·1) and 2·5 (SD 0·1), $P > 0{\cdot}05$, respectively). Weight loss, sex or medications did not alter these relationships.

Discussion

The key findings of the present study, in the context of a weight-reducing high-protein diet, were that a diet high in dietary cholesterol from eggs did not adversely affect blood lipid profiles or cardiovascular risk factors in individuals with type 2 diabetes and improved HDL-C more effectively than a diet containing isoenergetic alternative animal sources of protein that was low in dietary cholesterol.

One of the findings of the study was a pronounced improvement in HDL-C in the HPHchol group compared with the HPLchol group. A 1% increase in HDL-C has been associated with a 3% decrease in CVD risk[24], although there is no evidence that a rise in HDL-C through dietary means reduces CVD risk. The increase in HDL-C in response to dietary cholesterol was also observed in the meta-analysis by Weggemans *et al.*[11] and the recent study by Mutungi *et al.*[25]. It is possible that in the present study, dietary cholesterol also mediated the increase in HDL-C response. It is also possible that the increase in HDL-C may have been due to the increased fat content (3·5%) in the HPHchol dietary pattern.

Traditionally, raised LDL-C has been seen as a key risk factor for CVD[26], with some elevation of LDL-C required for initiation and progression of atherosclerosis[27]. Lowering of LDL-C through aggressive drug treatment has produced significant reductions in CHD[28]. Furthermore, some, but not all, interventions with elevated intakes of saturated fats and to a lesser extent dietary cholesterol have been shown to increase LDL-C levels[29,30] by suppressing hepatic LDL receptor activity[29]. The present study showed that there was a minimal change in LDL-C over time, which was not influenced by dietary treatment (with or without eggs). As insulin-resistant subjects are less responsive to dietary cholesterol than normal subjects[25,30], it is not surprising that we observed a similar effect in overweight individuals with type 2 diabetes.

Hyper-responders to dietary cholesterol have previously been defined as individuals producing a response in TC higher than 0·06 mmol/l for each additional 100 mg of dietary cholesterol, with elevation of both large LDL-C and HDL-C and maintenance of the LDL-C:HDL-C ratio[31,32]. A 4-week study of fifty-one pre-menopausal women with initial plasma cholesterol concentrations ranging from 3·62 to 5·17 mmol/l showed that 40% of the participants were hyper-responders to 640 mg additional dietary cholesterol per d[33]. The present study showed no clear indication of 'hyper-responders' to dietary cholesterol when the LDL-C profiles were plotted.

Increased levels of TAG (reviewed in Austin[34]) and TC[35] have independently been associated with increased CHD mortality. The present study found that plasma TAG and TC levels decreased over time on both dietary interventions.

The present research suggests that non-HDL-C (a measure of the concentration of cholesterol in the apo-B-containing lipoproteins)[28,36,37], apo-B (a measure of the number of atherogenic particles)[36,38], the ratio of TC to HDL-C[39,40] and HDL-C lipoproteins[36] are emerging as secondary targets for CVD[26,41,42], although there is much debate on which is the best marker. Both the dietary patterns significantly reduced non-HDL-C cholesterol over time. The lack of change in the TC:HDL-C ratio following weight loss is supported by the majority of studies conducted in the general population[11].

Other markers of atherosclerotic risk are apo-B, CRP and homocysteine. Apo-B levels improved similarly over the intervention on both the diets, irrespective of the levels of dietary cholesterol. CRP did not change probably because the weight loss was not great enough, while homocysteine was not altered.

Both the 12-week dietary interventions produced a reduction in weight of 6·2% over the intervention, which was of similar magnitude to that observed by Wing *et al.*[43]. Improvements in insulin sensitivity (HOMA2-IR, HOMA2%S and fasting insulin) and glycaemic control (HbA1c, fasting blood glucose and the postprandial response to glucose (as measured by oral glucose tolerance test)) were also observed irrespective of the type of diet or the use of hyperglycaemic medication.

Hyperglycaemia and, specifically, impaired insulin action have been shown to result in abnormalities in lipoprotein production, and circulation of more atherogenic remnant particles in individuals with diabetes[44] predominantly due to peripheral IR and increased flux of fatty acids to the liver; these conditions lead to increased VLDL production and impaired peripheral lipolysis. While LDL-C levels did not change over the course of the intervention, TC, TAG, non-HDL-C, apo-B levels and systolic and diastolic blood pressure, all risk factors for CHD, decreased on both the HPHchol and HPLchol dietary patterns. As there are no studies that have examined the effect of energy-balanced dietary patterns high in dietary cholesterol in type 2 diabetes, we are unable to speculate whether the improvements in HPHchol would be observed under conditions of weight maintenance or whether the lipid profiles might rebound after a new stable weight was attained.

Plasma folate and lutein increased on both the dietary interventions with more pronounced increases in HPHChol reflecting their dietary composition and supporting dietary compliance.

Systolic blood pressure and diastolic blood pressure, both markers of CVD risk[45], were also reduced with weight loss. The present findings are also supported by those of Mutungi *et al.*[25] and Yancy *et al.*[46].

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Limitations to the study include possible differential changes in LDL-C particle size. Many of the participants were also taking several medications. We attempted to control for this in the subanalyses. Additional studies in individuals with type 2 diabetes, conducted over a longer time period with a range of participants with varying nationalities, would need to be performed before nutritional recommendations could be made. It also remains unknown if the outcomes of the present study will be the same with a weight-maintenance diet.

The strengths of this outpatient-based study include the use of a dietary pattern, which have been shown to increase weight loss and fat loss, and to improve insulin sensitivity and blood lipid profiles[4–8]. Exceptional compliance across both the dietary patterns we believe was, in part, to be due to personalised individual fortnightly dietary consultations.

In conclusion, the present results show that in the context of a high-protein, low-saturated fat energy-restricted diet, two eggs per d did not adversely affect blood lipid profiles in individuals with type 2 diabetes and improved HDL-C, folate and lutein more effectively than a diet containing isoenergetic alternative animal sources. These results suggest that a high-protein energy-restricted diet high in cholesterol from eggs may have nutritional benefits and assist in metabolic control in individuals with type 2 diabetes.

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