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Lipoprotein metabolism in patients with anorexia nervosa: a case–control study investigating the mechanisms leading to hypercholesterolaemia

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Hypercholesterolaemia is a common finding in patients with anorexia nervosa (AN). To investigate the type, frequency and pathophysiological mechanisms of changes in lipoprotein metabolism in AN we performed a cross-sectional study in fifty-eight female patients (mean age 24·2 years, BMI 15·3 (SD 1·5) kg/m^2) and fifty-eight healthy age-matched controls (CO; BMI 22·2 (SD 1·7) kg/m^2). Total cholesterol and LDL-cholesterol were higher in AN (5·5 (SD 1·3) *v.* 5·0 (SD 0·8) mmol/l, $P=0·023$; 3·6 (SD 1·1) *v.* 3·2 (SD 0·7) mmol/l, $P=0·025$ respectively). LDL particles were significantly more enriched in cholesterol and triacylglycerol in AN. In multiple regression analysis with LDL-cholesterol as the dependent and BMI, total body fat (%), lathosterol:cholesterol ratio (endogenous cholesterol synthesis), 7α-hydroxy-4-cholesten-3-one (bile acid synthesis), non-esterified glycerol, free triiodothyronine and free thyroxine as independent variables, BMI was the only significant predictor in CO (R^2 0·36, overall $P=0·001$). In AN the variability of LDL-cholesterol was significantly predicted by total body fat, free thyroxine, BMI, free triiodothyronine and non-esterified glycerol (R^2 0·55, overall $P<0·001$). Subgroup analysis between restricting (AN-R) and binge-eating–purging patients (AN-B) indicated that in AN-R changes in lipoproteins, BMI and total body fat were more pronounced. AN-R patients had lower bile acid synthesis than AN-B ($P=0·02$). We conclude that elevated cholesterol concentrations in AN are generally due to an increase in LDL-cholesterol, which is mostly determined by the severe loss of body fat and the resulting changes in thyroid hormones, increased lipolysis and decreased endogenous cholesterol synthesis with resulting decrease in LDL removal. The clinical subtype of AN plays a major role in the mechanisms leading to hypercholesterolaemia.

Anorexia nervosa: Lipoprotein metabolism: Hypercholesterolaemia

Anorexia nervosa (AN) is a psychiatric disease with a high incidence in young women. Its main characteristic, self-induced weight loss, causes abnormalities in a number of organ systems and in multiple metabolic pathways and results in significant changes in body composition with severe loss of body fat. As a surprising metabolic consequence, elevated serum cholesterol (Chol) levels are often found, as first described by Klinefelter (1965) and later confirmed by several other authors (Blendis & Crisp, 1968; Crisp *et al.* 1968; Mordasini *et al.* 1978). The food intake of these patients is usually hypoenergetic; the food consumed has a higher proportion of energy from protein and lower proportions from carbohydrate and fat compared with that consumed by control subjects. Thus, a low concentration of total Chol and LDL-Chol in serum would be expected. The precise mechanisms underlying the increases in blood lipids are unknown, but a number of assumptions have been made. For instance, it was suggested that a diet rich in Chol (Crisp *et al.* 1968) or that carbohydrate binging in bulimic patients (Blendis & Crisp, 1968; Crisp *et al.* 1968; Mordasini *et al.* 1978) might be responsible. Nestel (1974) proposed that hypercholesterolaemia may reflect reduced Chol and bile acid turnover, and Mordasini *et al.* (1978) showed increased amounts of LDL as the major component of elevated Chol and suggested that they result from substantial mobilization of body fat for energy supply.

According to a recent long-term follow-up study, the mortality from AN (which is mainly related to undernutrition) is significant (Zipfel *et al.* 2000). The contribution of

Abbreviations: AN, anorexia nervosa; AN-B, binge eating–purging type of anorexia nervosa; AN-R, restricting type of anorexia nervosa; Chol, cholesterol; CO, healthy control subjects; TBF, total body fat; T3, triiodothyronine; T4, thyroxine; TG, triacylglycerol; 7α-HCO, 7α-hydroxy-4-cholesten-3-one.

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cardiovascular complications to the causes of mortality are unclear, but since increased total Chol and LDL-Chol levels are associated with an elevated risk for arteriosclerotic diseases, it is of clinical interest to investigate the type and frequency of lipoprotein disorders in AN in more detail. Moreover, it would be desirable to have better insights into the basic mechanisms that lead to changes in lipoprotein metabolism.

Due to specific characteristics of the disease, long-term studies documenting dietary intakes, applying controlled dietary regimens or performing invasive sampling are very difficult to carry out. In order to achieve reliable data and a reasonable sample size, we decided that a cross-sectional study design with differential metabolite assessment of lipid metabolism would be most appropriate. We therefore designed a case–control study in fifty-eight anorexic women who were age- and height-matched with fifty-eight healthy women serving as the control group (CO). The aim of the present study was to determine the lipoprotein profile in AN compared with CO, with LDL-Chol as the primary study variable, as well as to study mechanisms leading to changes in LDL-Chol levels. Therefore, we investigated multiple variables of lipoprotein metabolism and composition, Chol metabolism, eating behaviours, body composition and endocrine functions.

Experimental design

Study design

The present multi-centre study was performed in white women and had a cross-sectional case–control study design. Each participant was examined once. First, the patient or volunteer had to complete a questionnaire about her disease history: menstruation, self perception of the body, influence on life of eating behaviour, eating habits, regulation of body weight, binges and purging behaviour, intake of medications and hormones. This was followed by measurements of pulse rate, blood pressure, body temperature, height and weight, and by bioelectrical impedance analysis. Thereafter, a fasting blood sample (about 70 ml) was taken in a sitting position after about 15 min of sitting.

Experimental subjects

Fifty-eight patients with AN, age range from 14 to 55 (mean 24·2) years, were studied. The women were diagnosed according to the International Classification of Diseases Code 10 and Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Association IV criteria, and had BMI $\leq 17{\cdot}5\,kg/m^2$. Known somatic causes for malnutrition and other diseases that could interfere with the inclusion criteria of the study were excluded by history. All patients were examined before initiating psychotherapy and all were hospitalized at the time point of the examination. They were recruited through collaboration with eating-disorder management programmes in three centres in Germany. All participants were postmenarchal. Thirty-four patients (59 %) had had secondary amenorrhoea for at least 3 months. Twenty-three patients (40 %) were taking oral contraceptives to regulate their menstrual cycle. The information on hormone intake was missing in one case. The patients were divided in two groups according to Diagnostic and Statistical Manual of Mental Disorders IV criteria: (1) the 'restricting type', which has not regularly engaged in binge-eating or purging behaviour (i.e. self-induced vomiting or the misuse of laxatives, diuretics or enemas) during the current episode of AN (AN-R, *n* 30); (2) the 'binge-eating–purging' type that has regularly engaged in binge-eating or purging behaviour (i.e. self-induced vomiting or the misuse of laxatives, diuretics or enemas) during the current episode of AN (AN-B, *n* 28).

CO consisted of fifty-eight healthy women (age range 17–53 (mean 25·5) years), who were pair-matched according to age (± 3 years) and height (± 0·04 m). Recruitment of CO was done by local publicity. The examination was arranged after an initial telephone interview asking for weight, height, age and information on the ovarian cycle. Inclusion criterion was a BMI $20–25\,kg/m^2$. None of the CO had a history of AN, other eating disorders, recent changes in body weight, obesity or any other metabolic disturbances that could interfere with the study objectives. They were examined between the 5th and the 12th day of their ovarian cycle (follicular phase) to control for the effects of cycle stage on biochemical, hormonal and body composition variables. None had secondary amenorrhoea. Thirty-one were taking oral contraceptive agents (53 %).

Ethical approval for the study protocol was granted by the Ethics Committee, Faculty of Medicine, University of Bonn. Written informed consent was obtained from each subject after the purpose and procedures of the study had been explained. The study was conducted in accordance with the guidelines proposed in The Declaration of Helsinki.

Body variables and anthropometrical methods

The clinical measurements included the determination of body axillar temperature, blood pressure and pulse rate, and height and weight using standard research methods. To determine body composition, as fat-free mass (kg, %), total body fat (TBF; kg, %) and total body water (kg, %), we used bioelectrical impedance analysis (Multi Frequency Analyzer B. I. A. 2000-M; Data Input, Hofheim, Germany) in combination with the manufacturer's software Nutri4.

Laboratory methods

Lipoproteins. Blood was drawn from a large antecubital vein after a short tourniquet time, centrifuged immediately; lipoproteins were assayed on the same day. VLDL, LDL and HDL were separated using sequential density ultracentrifugation of serum (500 μl). Chol was measured as total Chol in serum and in VLDL and LDL lipoproteins (VLDL-Chol and LDL-Chol) using the CHOD-PAP enzymatic colorimetric kit (Boehringer Mannheim, Mannheim, Germany). HDL-Chol was determined with the same kit after precipitation of ApoB-containing lipoproteins with phosphor tungstic acid (Boehringer Mannheim). Triacylglycerol (TG) was measured in serum and in VLDL, LDL and

Table 1. Baseline characteristics and anthropometric data for the subjects
(Mean values and standard deviations for fifty-eight subjects per group)

Variable	CO		AN		Difference *v.* CO			Statistical significance of effect: *P**
	Mean	SD	Mean	SD	Difference %	Mean	95 % CI	
Weight (kg)	62·4	6·3	42·8	5·6	−32	−19·7	−21·8, −17·5	< 0·001
Systolic blood pressure (mmHg)	111	14	92	9·4	−17	−19	−24, −14	< 0·001
Diastolic blood pressure (mmHg)	70	7	61	7	−14	−9·7	−12, −7·0	< 0·001
BMR (kJ)	5541	267	4876	351	−12	−665	−778, −548	< 0·001
BMI (kg/m^2)	22·2	1·7	15·3	1·5	−31	−6·9	−7·5, −6·3	< 0·001
Total body fat kg	18·1	3·8	5·3	3·1	−71	−12·8	−14·1, −11·5	< 0·001
%	28·9	3·8	12·0	6·2	−59	−17·0	−18·8, −15·1	< 0·001
Lean body mass kg	44·3	3·4	37·4	4·0	−16	−6·9	−8·3, −5·5	< 0·001
%	71·2	3·7	88·0	6·2	+24	+17	+15, +19	< 0·001
Total body water litres	32·5	4·5	27·4	2·9	−16	−5·0	−6·0, −4·0	< 0·001
%	52·2	2·7	64·4	4·6	+24	+12	+14, +11	< 0·001

CO, healthy control subjects; AN, patients with anorexia nervosa.
*Student's unpaired *t* test (two sided).

HDL fractions (VLDL-TG, LDL-TG, HDL-TG) using the GPO-PAP enzymatic kit (Boehringer Mannheim).

Non-cholesterol sterols. The plant sterols campesterol (24-methyl-cholesterol) and sitosterol (24-ethyl-cholesterol), and the endogenous Chol precursor lathosterol, were determined by GLC as previously described (Björkhem *et al.* 1987). Data are expressed as μg/mg Chol. 7α-Hydroxy-4-cholesten-3-one (7α-HCO) was determined in serum using HPLC as previously described by Axelson *et al.* (1988).

Other lipid variables. Determination of the ApoB concentrations was done in total serum and in VLDL and LDL lipoprotein fractions using kinetic nephelometry with the Beckmann-ARRAY®-360-System (Beckmann Instruments, Munich, Germany). The analysis of lipoprotein(a) was performed with an ELISA test kit (Immuno, Heidelberg, Germany). Serum concentrations of NEFA were measured with a half-micro test and serum concentration of non-esterified glycerol with a UV-test (Boehringer Mannheim).

Fasting leptin concentration was measured using the DSL-10-23100 ACTIVE™ Human Leptin ELISA kit (Diagnostic System Laboratories, Webster, TX, USA). The sensitivity of this assay was 0·5 mg/ml and the inter-assay CV was 4·6 %.

The haematological, endocrine, clinical chemistry variables, coagulation tests and serum protein electrophoresis analysis were performed using routine laboratory methods in the Bonn University Clinical Chemistry core laboratory.

Statistical analyses

Statistical processing was done by computer using the SPSS/PC 8.0.1 program (SAS Institute, Cary, NC, USA). Normality of variable distribution was assessed by the Kolmogorov–Smirnov test and by analysis of skewness and kurtosis. Values of the AN and CO were compared by Student's *t* test for parametric independent samples or Mann–Whitney U-test for non-parametric samples. A P value $< 0{\cdot}05$ was regarded as significant. All tests were performed two-sided. Relationships between variables were determined by Pearson's correlation coefficient for parametric samples and Spearman rank correlation coefficient for non-parametric samples. Partial correlations were performed to describe the linear relationship between two variables while controlling for the effects of one additional variable. Multiple regression analyses were performed to determine the relationship of several variables to LDL-Chol concentrations. All results are given as means and standard deviations unless otherwise indicated. For comparisons between groups, mean differences and 95 % CI are shown.

Results

Clinical and body composition data

The clinical characteristics and anthropometric data of the fifty-eight women with AN and fifty-eight CO women are shown in Table 1. The duration of the illness from its first manifestation ranged from 0·6 to 42·0 (median 4·3) months. The weight loss since manifestation of the disease was 12·3

Table 2. Lipoprotein profiles, lipoprotein composition and lipid metabolism variables in patients with anorexia nervosa (AN) and healthy control subjects (CO)*
(Mean values and standard deviations for fifty-eight subjects per group)

	CO		AN		Difference *v.* CO			
Variable	Mean	SD	Mean	SD	%	Mean	95% CI	Statistical significance of effect: *P*†
Lipoprotein profile								
Total Chol (mmol/l)	5·03	0·77	5·48	1·28	8·9	+0·45	+0·06, + 0·84	0·023
LDL-Chol (mmol/l)	3·20	0·71	3·59	1·07	11·9	+0·38	+0·005, + 0·72	0·025
HDL-Chol (mmol/l)	1·43	0·29	1·53	0·46	7·0	+0·1	−0·04, +0·25	0·15
VLDL-Chol (mmol/l)	0·30	0·20	0·34	0·22	13·3	+0·04	−0·04, + z0·12	0·31
Total serum TG (mmol/l)	1·0	0·42	0·998	0·44	0·5	+0·005	+0·15, + 0·16	0·96
VLDL-TG (mmol/l)	0·59	0·35	0·58	0·35	−1·7	−0·01	+0·14, + 0·12	0·88
LDL-TG (mmol/l)	0·28	0·08	0·31	0·10	10·7	+0·03	0·00, + 0·07	0·045
HDL-TG (mmol/l)	0·15	0·05	0·15	0·04	2·7	+0·004	−0·01, +0·02	0·59
Total serum ApoB (mg/l)	820	210	890	280	+8·1	+67	−25, + 160	0·15
LDL-ApoB (mg/l)	530	190	460	220	−12	−63	−140, +13	0·10
VLDL-ApoB (mg/l)	50	31	45	31	−10	−5	−16, + 6	0·38
Lp(a) (mg/l)	190	250	250	320	+28	+53	−51, + 16	0·31
Lipoprotein composition								
Total chol : total apoB	2·48	0·57	2·48	0·56	+0·1	+0·002	−0·21, +0·21	0·98
LDL-Chol : LDL-ApoB	2·67	1·45	3·87	2·9	+45	+1·2	+0·36, + 2·1	0·001
VLDL-Chol : VLDL-ApoB	2·85	2·95	3·68	2·56	+29	+0·8	−0·2, + 1·8	0·003
TG : total ApoB	1·11	0·52	1·06	0·58	−4·0	−0·04	−0·25, + 0·16	0·66
LDL-TG : LDL ApoB	0·54	0·35	0·79	0·72	+46	+0·25	+0·04, + 0·46	0·003
VLDL-TG : VLDL-ApoB	13·4	11·5	15·9	12·1	+19	+2·5	−1·8, + 6·9	0·11
Lipid Variables‡								
Non-esterified glycerol (mg/l)	10·8	5·3	8·1	3·8	−25	−2·7	−4·4, −1·0	0·002
NEFA (mg/l)	38·6	22·6	36·6	26·8	−11	−4	−13, + 4·0	0·66
r-Lathosterol	1·08	0·35	0·83	0·29	−23	−0·25	−0·37, −0·13	<0·001
r-Cholestanol	2·25	0·44	2·69	0·65	+19	+0·44	+0·23, + 0·64	<0·001
r-Campesterol	2·38	1·05	2·99	1·18	+26	+0·62	+0·2, + 1·03	0·004
r-Sitosterol	2·12	1·86	2·21	1·03	+4·2	+0·09	−0·47, + 0·64	0·15
7α-HCO (ng/ml)	18·5	11·4	20·1	15·5	+9·0	+1·7	−3·4, + 6·7	0·7
Leptin (ng/ml)	10·33	7·56	1·28	1·76	−88	−9	−11·1, −7·0	<0·001

Chol, cholesterol; TG, triacylglycerol; Lp(a), lipoprotein(a); 7α-HCO, 7α-hydroxy-4-cholesten-3-one.
* For details of subjects and procedures, see Table 1 and pp. 960–961.
† Student's unpaired *t* test (two-sided).
‡ Values for non-Chol sterols are given as ratios (μg/mg) with Chol.

Table 3. Comparison of lipoproteins and associated variables between patients with restricting-type (R-AN) or binge eating–purging-type (AN-B) anorexia nervosa*
(Mean values and standard deviations)

	AN-B (*n* 28)		AN-R (*n* 30)		Difference *v.* CO			
Variable	Mean	SD	Mean	SD	%	Mean	95 % CI	Statistical significance of effect: *P*†
Lipoprotein profile								
Total Chol (mmol/l)	5·52	0·94	5·46	1·55	−1·1	−0·06	−0·74, +0·62	0·85
LDL-Chol (mmol/l)	3·63	0·78	3·55	1·3	−2·2	−0·08	−0·65, +0·49	0·77
HDL-Chol (mmol/l)	1·47	0·31	1·6	0·57	+8·8	+0·13	−0·11, +0·37	0·27
VLDL-Chol (mmol/l)	0·40	0·26	0·28	0·15	−20	−0·08	+0·65, +0·49	0·043
Total serum TG (mmol/l)	1·09	0·53	0·92	0·27	−14·7	−0·16	−0·38, +0·06	0·16
VLDL-TG (mmol/l)	0·69	0·45	0·49	0·19	−28·6	−0·197	−0·38, −0·01	0·040
LDL-TG (mmol/l)	0·296	0·096	0·32	0·10	+10·1	+0·03	−0·02, +0·08	0·296
HDL-TG (mmol/l)	0·15	0·04	0·16	0·04	+4·7	+0·007	−0·01, +0·03	0·53
Total serum ApoB (mg/l)	877	244	901	307	+2·7	+24	−126·2, + 174·2	0·75
LDL-ApoB (mg/l)	513	218	419	209	−18·4	−94·2	−206·4, + 18·1	0·098
VLDL-ApoB (mg/l)	53·5	32·6	37·4	28·0	−30·1	−16·1	−32·0, −0·1	0·048
Lp(a) (mg/l)	220	260	269	372	+22·3	+49·1	−120·9, + 219·1	0·44
Lipoprotein composition								
Total Chol : total ApoB	2·55	0·71	2·42	0·36	−5·1	−0·13	−0·44, + 0·17	0·38
LDL-Chol : LDL-ApoB	3·55	3·19	4·17	2·63	+17·5	+0·62	−0·92, + 2·15	0·198
VLDL-Chol : VLDL-ApoB	3·08	1·12	4·23	3·33	+37·7	1·16	−0·14, + 2·48	0·22
TG: total ApoB	1·17	0·75	0·96	0·28	−17·9	−0·21	−0·52, + 0·10	0·18
LDL-TG : LDL-ApoB	0·69	0·83	0·88	0·58	+28·3	+0·195	−0·18, + 0·57	0·027
VLDL-TG : VLDL-ApoB	13·05	7·04	18·57	15·07	+42·9	+5·52	−0·65, + 11·69	0·19
Lipid Variables‡								
Non-esterified glycerol (mg/l)	8·98	4·5	7·2	2·7	−20·0	−1·8	−3·8, + 0·2	0·083
NEFA (mg/l)	39·98	28·0	33·4	25·6	−16·5	−6·6	−20·7, + 7·5	0·35
r-Lathosterol	0·85	0·29	0·82	0·29	−3·5	−0·03	−0·18, + 0·12	0·69
r-Cholestanol	2·64	0·48	2·73	0·78	+3·4	+0·09	−0·25, + 0·44	0·59
r-Campesterol	3·16	1·44	2·84	0·87	−10·1	−0·32	−0·96, + 0·32	0·32
r-Sitosterol	2·21	1·14	2·21	0·94	−0·1	−0·002	−0·55, + 0·55	0·74
7α-HCO (ng/ml)	23·89	16·77	16·45	13·54	−31·1	−7·44	−15·5, + 0·63	0·049
Leptin (ng/ml)	1·65	2·11	0·94	1·31	−43·0	−0·71	−1·64, + 0·23	0·21

CO, healthy control subjects; Chol, cholesterol; TG, triacylglycerol; Lp(a), lipoprotein(a); 7α-HCO, 7α-hydroxy-4-cholesten-3-one.
* For details of subjects and procedures, see Table 1 and pp. 960–961.
† Student's unpaired *t* test (two-sided).
‡ The values for non-Chol sterols are given as ratios (μg/mg) with Chol.

Table 4. Endocrine variables in patients with anorexia nervosa (AN) and healthy control subjects (CO)*
(Mean values and standard deviations for fifty-eight subjects per group)

Variable	Laboratory reference value	CO		AN		Difference *v.* CO			Statistical significance of effect: *P*†
		Mean	SD	Mean	SD	%	Mean	95 % CI	
Cortisol (μg/l)	80–250	270	140	320	150	+20	+54	−1·6, +11	0·057
Insulin (mU/l)	3·0–17·0	9·4	4·0	6·2	2·5	−34	−3·1	−4·4, −1·8	<0·001
C-peptide (ng/ml)	0·33–3·81	2·39	0·7	2·10	0·76	−12	−0·28	−0·56, −0·001	0·049
Thyroid-stimulating hormone (μU/ml)	0·27–4·20	2·04	1·13	1·87	1·02	−8·5	−0·17	−0·58, +0·23	0·39
Free triiodothyronine (pg/l)	1·8–4·6	3·32	0·55	2·30	0·57	−31	−1·02	−1·23, −0·81	<0·001
Free thyroxine (ng/l)	9–19	13·0	1·7	11·3	1·5	−13	−1·7	−2·3, −1·1	<0·001

*For details of subjects and procedures, see Table 1 and pp. 960–961.
†Student's unpaired *t* test (two-sided).

(SD 6·3) kg (29·2 (SD 16·0) %). There was no difference in body temperature and pulse rate, while AN patients had significantly lower systolic and diastolic blood pressure readings, and a lower BMR. Age and height were not different due to the matched pairs design. As expected, body weight, BMI (range: CO 20·0–25·1, AN 10·6–17·5 kg/m^2) and the related body composition variables were significantly different between the groups. There was no relationship between body weight or BMI and duration of the illness.

Comparing the subgroups of AN, AN-R patients had BMI values 4·5 % lower than AN-B patients (14·9 (SD 1·3) *v.* 15·6 (SD 1·6) kg/m^2, $P=0{\cdot}084$). AN-R patients had significantly less TBF than AN-B (4·4 (SD 2·8) *v.* 6·3 (SD 3·1) kg, $P=0{\cdot}015$), corresponding to 28 % less TBF (10·1 (SD 5·8) *v.* 14·0 (SD 6·1) %, $P=0{\cdot}016$). In addition, changes in the % fat-free mass and % total body water were significantly more pronounced in AN-R than in AN-B patients (fat-free mass 86·0 (SD 6·1) *v.* 89·9 (SD 5·8) %, $P=0{\cdot}016$; total body water 63·0 (SD 4·4 *v.* 65·8 (SD 4·3) %, $P=0{\cdot}016$).

Lipoprotein profiles

The results of the lipoprotein analyses are given in Table 2. Total Chol and LDL-Chol were significantly higher in the AN group as a whole compared with CO (+9 and + 12 % respectively). In AN, 36 and 40 % of the total Chol and LDL-Chol values respectively were above the 75th percentile of the CO, while only 17 % total Chol and 12 % LDL-Chol levels were below the 25th percentile. AN had significantly higher LDL-TG concentrations (+11 %) than CO. The other variables were not significantly different between the groups. Comparing AN subgroups (Table 3), AN-R had 20 % lower VLDL-Chol concentrations and 29 % lower VLDL-TG concentrations compared with AN-B. AN-R had 30 % lower VLDL-ApoB concentrations than AN-B.

Lipoprotein particle composition

Calculations of Chol:ApoB or TG:ApoB ratios is widely accepted in the estimation of particle composition, since a lipoprotein particle always contains only one molecule ApoB and a large but variable number of other lipid molecules. In doing so, we found that the ratios of serum total Chol:ApoB and TG:ApoB were virtually identical (Table 2). In LDL, however, we found significantly increased Chol:ApoB and TG:ApoB in AN (+45 and + 46 % respectively), indicating more Chol and TG load per LDL particle. Similar changes, although not so pronounced, could be observed in VLDL composition (Table 2). With regard to comparison of the AN subgroups (Table 3), the TG:ApoB ratio in LDL was 28 % higher than in AN-R.

Lipid metabolism variables

Table 2 shows the comparison of variables of lipid metabolism for AN and CO. The concentrations of non-Chol

sterols in serum that can be used to evaluate Chol metabolism are expressed as ratios to Chol (μg/mg) to correct for relative changes in Chol concentrations. The ratio of lathosterol as indicator of Chol synthesis (Kempen *et al.* 1988) was significantly decreased (−23%) in AN compared with CO. The ratio of campesterol, an indicator of Chol absorption (Miettinen *et al.* 1990), was significantly increased (+26%) in AN patients. 7α-HCO, an indicator of bile acid synthesis (Axelson *et al.* 1988), was not different between the two groups. Non-esterified glycerol concentrations were significantly decreased (−25%) in AN patients and leptin, as potential afferent signal of body fat stores that is produced mainly in adipose tissue (Auwerx & Staels, 1998), was significantly reduced in AN (−88%). The comparison of the lipid metabolism variables between the AN-R and AN-B subgroups (Table 3) revealed significantly lower 7α-HCO levels in AN-R (−31%).

Endocrine variables

The results of the endocrine tests are shown in Table 4. Cortisol was significantly increased in AN patients compared with CO (+20%). Insulin and C-peptide were significantly lower (−34 and −12% respectively). There was no difference in thyroid-stimulating hormone concentrations, but free triiodothyronine (T3) and free thyroxine (T4) were significantly decreased (−31 and −13%

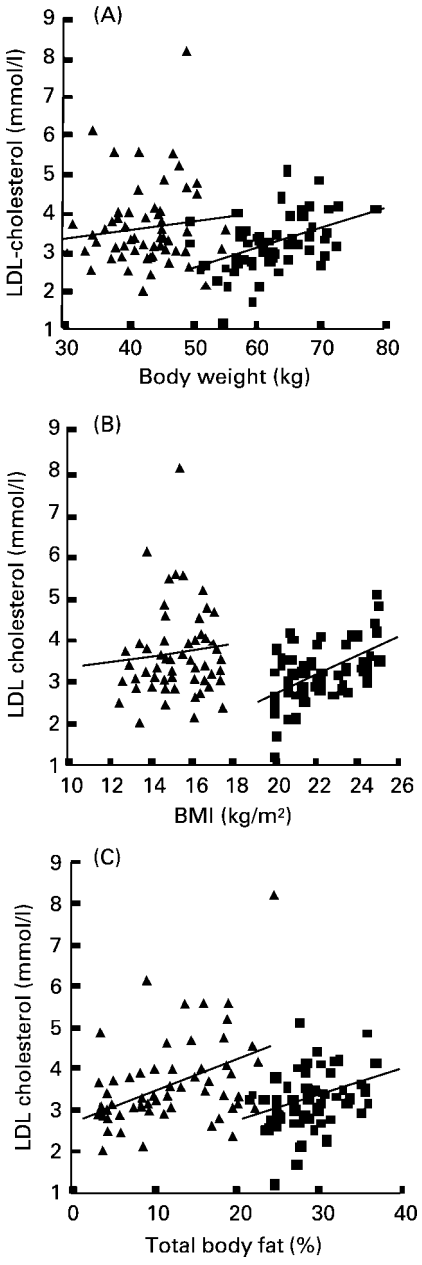

Fig. 1. Correlation between LDL-cholesterol and body weight ((A); healthy control subjects (CO) r 0·47, $P<0·001$; patients with anorexia nervosa (AN) r 0·1, $P=0·47$), BMI ((B); CO r 0·54, $P<0·001$; AN r 0·10, $P=0·46$) or total body fat ((C); CO r 0·36, $P=0·006$; AN r 0·40, $P=0·002$). ■, CO; ▲, AN. For details of subjects and procedures, see Table 1 and pp. 960–961.

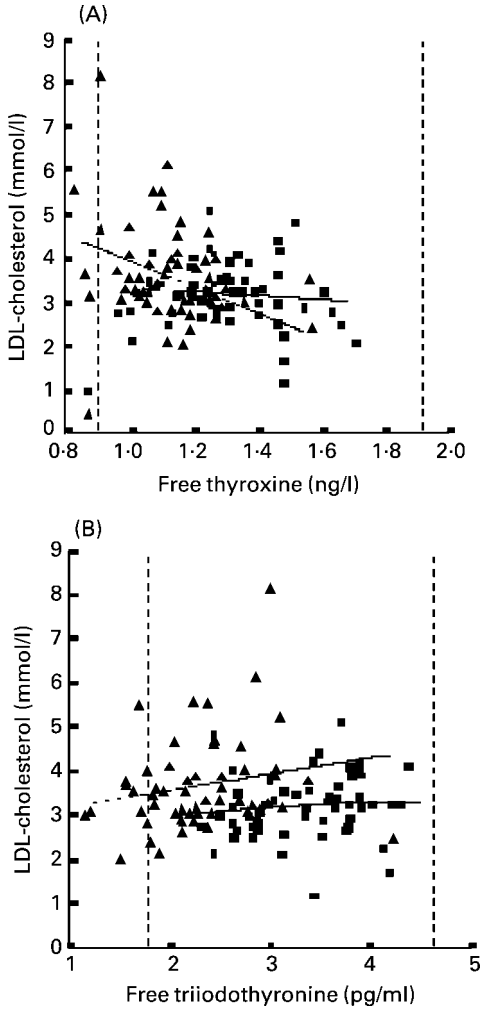

Fig. 2. Correlation between LDL-cholesterol and free thyroxine ((A); healthy control subjects (CO) r 0·13, $P<0·35$; patients with anorexia nervosa (AN) r −0·36, $P=0·006$) or free triiodothyronine ((B); CO r 0·14, $P=0·29$; AN r 0·23, $P=0·099$). ■, CO; ▲, AN; ---, upper and lower limits of normal range in laboratory. For details of subjects and procedures, see Table 1 and pp. 960–961.

respectively). Nine of the AN patients had free T3 values slightly below the lower range of normal (low T3 syndrome) and three had decreased free T4 values, but none had grossly decreased thyroid hormone levels or clinical evidence of hypothyroidism. All healthy controls were euthyroid. There were no significant differences between the AN subgroups.

Univariate regression analyses and partial correlations

LDL-Chol in CO had a significant correlation with weight (r 0·47, $P<0·001$), BMI (r 0·54, $P<0·001$) and TBF (%) (r 0·36, $P=0·006$) (Fig. 1). Partial correlation of LDL-Chol with BMI under control of TBF (%) resulted in a significant relationship (r 0·44, $P=0·001$), but the correlation of LDL-Chol with TBF (%) under control of BMI did not become significant (r 0·034, $P=0·452$). In AN, LDL-Chol correlated significantly with TBF (%) (r 0·40, $P=0·002$), but not with BMI (r 0·10, $P=0·46$) or with weight (r 0·10, $P=0·47$). Partial correlation of LDL-Chol to TBF (%) was not significantly influenced by controlling for BMI but became stronger (r 0·50, $P<0·001$), and the relationship of LDL-Chol with BMI reached a significant inverse correlation coefficient under control of TBF (%) (r −0·33, $P=0·011$).

LDL-Chol correlated significantly inversely in AN with free T4 (r −0·36, $P=0·006$) and weakly positively with free T3 (r 0·225, $P=0·099$), but there were no significant relationships of both variables to LDL-Chol in CO (Fig. 2).

Multiple regression analysis

We performed a multiple regression analysis with LDL-Chol as dependent variable and BMI, TBF (%), r-lathosterol, 7α-HCO, non-esterified glycerol, free T3, and free T4 as independent variables. This model reached in the CO group a significance level of $P=0·0023$, with a coefficient of determination R^2 0·362, F 3·993. BMI had the largest influence with a significance level $P=0·0011$, while none of the other variables reached predictive significance, not even as a trend. In the AN group, the model reached a significance level $P<0·001$, with R^2 0·545 and F 7·88. The most important predictor variables were TBF (%) ($P<0·001$) and free T4 ($P<0·001$), but also BMI ($P=0·004$), free T3 ($P=0·004$) and non-esterified glycerol ($P=0·005$).

Discussion

In the present case–control study in fifty-eight female patients with AN and fifty-eight healthy CO subjects we found elevated total Chol concentrations in AN compared with CO, as first reported by Klinefelter (1965). The higher concentrations were based on higher LDL-Chol levels, as also described in previous studies (Mordasini *et al.* 1978), but not confirmed by others (Arden *et al.* 1990; Mehler *et al.* 1998). The mean difference between CO and AN in our present study was rather small, in contrast to markedly elevated Chol levels found in reasonably large cohorts in early reports in the literature (Klinefelter, 1965; Blendis & Crisp, 1968; Crisp *et al.* 1968; Mehler *et al.* 1998; Mordasini *et al.* 1978). A high degree of selection of patients of certain subgroups in these studies could be the reason. The range of total Chol levels (3·65–10·12 mmol/l) and of the LDL-Chol concentrations (1·97–8·11 mmol/l) was larger than in CO (total Chol 1·17–5·10 mmol/l, LDL-Chol 2·82–6·81 mmol/l), a phenomenon that has also been previously reported (Crisp *et al.* 1968; Halmi & Falk, 1981). The metabolic causes for elevated LDL-Chol were unknown until now.

It is safe to say that increased endogenous Chol synthesis is rather unlikely to be responsible for the higher values in AN, because the lathosterol:Chol ratio, which is accepted as an indicator of whole-body Chol synthesis (Kempen *et al.* 1988), was significantly lower in the AN compared with CO. This has recently also been reported by others (Feillet *et al.* 2000), although in a smaller number of patients.

Mordasini *et al.* (1978) suggested that the mobilization of body fat during phases of weight loss could be responsible for the higher LDL-Chol concentrations. Our present results support this hypothesis, because the results of our multiple regression analyses indicate that BMI and TBF (%) are, besides the thyroid hormones and non-esterified glycerol, important predictor variables for the LDL-Chol levels, accounting for about 55% of their variability. Furthermore, TBF seemed to be the most important independent variable in multiple regression analysis and under its influence the relationship between LDL-Chol and BMI in partial correlation analyses became significantly inverse. Thus, with lower BMI the LDL-Chol concentrations increased in AN. It is therefore conceivable that pronounced weight loss causes an increased flux of peripheral Chol to the liver, so that the hepatocytes are enriched with Chol and Chol synthesis is downregulated, as was shown in the present study. Earlier studies (Miettinen, 1968; Montoye *et al.* 1966) and more recent studies (Phinney *et al.* 1991) showed that phases of weight loss, which were always related to reduced energy intake, caused mobilization of Chol. Savendahl & Underwood, (1999) demonstrated that increased concentrations of Chol, LDL-Chol and ApoB in fasting non-obese adults were associated with weight loss. Other studies showed no change (Vaisman *et al.* 1990) or decreased (Gower *et al.* 2002) Chol concentrations. The contradictory reports can be explained by differences in time points of the examination of the subjects in the course of their disease (Phinney *et al.* 1991) and in gender, age, obesity, diet and state of health (Savendahl & Underwood, 1999).

It can be assumed that an increased Chol flux to the liver reduced the activity of LDL receptors and the catabolism of LDL lipoproteins could be prolonged. Teng *et al.* (1983) showed that a reduced LDL catabolism influenced the content of Chol in LDL lipoproteins. Interestingly, the LDL-Chol:LDL-ApoB and LDL-TG:LDL-ApoB ratios were significantly higher in AN than in CO. This was more pronounced in AN-R patients, who also had significantly lower TBF compared with AN-B. In addition, the VLDL-Chol:VLDL-ApoB ratio was significantly higher in AN. In a case report of an AN patient with severe hyperlipoproteinaemia, a subnormal LDL receptor activity was found, together with high plasma LDL, which skewed to

less dense fraction and Chol-rich VLDL (Homma *et al.* 2002). The higher LDL-TG:LDL-ApoB ratio of our present AN patients could be explained by decreased TG hydrolysis because of decreased activities of hepatic TG lipase and/or lipoprotein lipase, probably because of loss of metabolically active peripheral tissues. In accordance with this hypothesis, Mordasini *et al.* (1978) found in a selected group of four patients with AN significantly decreased activities of both enzymes. Furthermore, we showed that AN patients had a significantly lower BMR than CO. Lipoprotein composition influences their susceptibility to oxidation. LDL-TG have been shown to correlate with their susceptibility to oxidation and with the overall severity and rate of progression of coronary atherosclerosis (Regnstrom *et al.* 1992). Further investigation is necessary to prove if AN patients have altered lipoprotein composition and higher levels of oxidized LDL, which has been shown to play a pivotal role in the development of atherosclerosis (Regnstrom *et al.* 1992).

Our findings of decreased free T4 and free T3 in AN patients in comparison with CO are in agreement with the findings of other investigators in AN (Tamai *et al.* 1986). In the AN group, free T4 correlated significantly inversely with total ApoB and LDL-Chol concentrations. It is known that decreased free T4 and/or free T3 concentrations are associated with elevated LDL-Chol levels (Diekman *et al.* 2000), which is mainly attributable to decreased Chol catabolism (de Bruin *et al.* 1993) due to changes in LDL receptor activity (van der Wal *et al.* 1998). It should be pointed out, however, that none of the subjects in our present study was clinically or subclinically hypothyroid, but low thyroid hormone concentrations are among the most powerful predictors for elevated LDL-Chol in AN, as indicated by the multiple regression analysis models.

The endogenous hormone insulin also influences LDL-Chol by modulation of the expression of LDL receptors (Duvillard *et al.* 2003). In the present study, the correlation of insulin with LDL-Chol reached statistical significance only in CO, but not in AN (results not shown). Because of these results and the fact that the mean value of insulin in AN was in the normal range and only four AN patients had insulin levels slightly below the lower range, we do not suppose a significant effect of insulin on LDL-Chol concentrations in the present AN group.

The intake of hormones also affects lipoprotein metabolism (Wiegratz *et al.* 1998). Comparison of AN patients taking or not taking oral contraceptives did not show differences of lipoprotein concentrations. In CO, the concentrations of TG, LDL-TG and HDL-TG were significantly higher in women who took hormones (results not shown). This demonstrates the influence of the oestrogens of the hormone preparations that have been shown to increase TG concentrations (Wiegratz *et al.* 1998). For AN patients we suppose that an effect of hormone intake could not be demonstrated because of the stronger effect of metabolism changes due to undernutrition.

One of the most important aspects of our present study for understanding Chol metabolism in AN is the necessity to distinguish between subtypes of AN: AN-R and AN-B. As AN-B eat foods rich in carbohydrate and fat (and thus rich in Chol) during the binge episodes, this could provide a further contribution to the higher Chol concentration seen in these subjects (Zoppi *et al.* 1993; Sullivan *et al.* 1998). Other studies have also suggested a relationship between this bulimic behaviour and hypercholesterolaemia (Crisp *et al.* 1967; Blendis & Crisp, 1968; Affenito *et al.* 1997; Case *et al.* 1999). It could be argued that purging does not lead to absorption of the food consumed, but it has been shown previously that a substantial amount of energy is absorbed despite vomiting (Kaye *et al.* 1993). The higher concentrations of VLDL-TG and VLDL-Chol in AN-B could be a result of the influence of binge-eating during the present study. Other studies have also found higher TG concentrations (Sanchez-Muniz *et al.* 1991; Case *et al.* 1999) and Chol levels (Blendis & Crisp, 1968) in the so-called 'bulimic' type of AN patients. Increases in total Chol and LDL-Chol are seen when high-fat diets are given to dieters or to subjects on low-fat diets (Stone, 1994). This could be due to a markedly decreased tolerance to high Chol intake (Lacombe *et al.* 1986). We suggest that the periods of binging in our AN-B patients contributed, in addition to the mobilisation of Chol from peripheral tissues, to the higher LDL-Chol levels. In this context, the significantly higher plant sterol campesterol:Chol ratio, an indicator of Chol absorption (Miettinen *et al.* 1990), does not reflect a higher absorption of Chol in the whole AN group compared with CO. Nevertheless in AN-B, the inverse correlation of campesterol with lathosterol (results not shown) indicates that Chol absorption reduced Chol synthesis. In AN-R patients, campesterol was correlated significantly positively with lathosterol (results not shown), which indicates that the serum concentration of the plant sterol reflects its own absorption (Miettinen *et al.* 1990).

Another aspect of Chol metabolism to be considered is its degradation to bile acids. Nestel (1974) proposed that reduced bile acid formation could be responsible for hypercholesterolaemia observed in his AN patients. We therefore analysed 7α-HCO, which is closely correlated with total bile acid synthesis in human subjects (Axelson *et al.* 1988). Our AN patients showed, as a whole group, no significantly different concentrations compared with the CO group, but the division into the AN-R and AN-B subgroups revealed significantly higher concentrations in the AN-B compared with the AN-R patients. One possible explanation for this observation could be that the binge-eating episodes lead to a higher activity of cholesterol 7α-hydroxylase, but the literature showed only a partial stimulating effect of Chol intake on the activity of this enzyme in human subjects (Lin & Connor, 1980). We propose that purging leads to an interruption of the enterohepatic circulation, which has been shown to increase bile acid production (Angelin, 1991). Thus, in the AN-B patients the increased degradation of Chol to bile acids did not lead to a depletion of the Chol pool in the hepatocytes followed by increase of Chol synthesis and/or number of LDL receptors as under normal circumstances (Brown & Goldstein, 1986; Angelin, 1991), because low lathosterol:Chol ratios and elevated LDL-Chol levels were similar in AN-B and AN-R. The lower concentration of the bile acid precursor in the AN-R group could be due

to continuously reduced energy consumption, which can also be seen under acute energy restriction (Mazzella *et al.* 1995). Under these circumstances, the need for new bile and bile acids could be maximally reduced and bile flux and bile acid circulation would be downregulated. Furthermore, more efficient reabsorption of bile acids due to prolonged intestinal passage time (Bertolotti *et al.* 1995) could lead to the inhibition of cholesterol 7α-hydroxylase (Björkhem *et al.* 1987).

In conclusion, the results of the present study show that AN patients have higher LDL-Chol compared with healthy volunteers, although the differences were smaller than expected on the basis of the existing literature. A final answer to the reasons for increased Chol concentrations in AN can still not be given, although some possibilities can be proposed. It is clear that differences in the lipid metabolism of AN and CO would have been disguised if the clinical appearance of the eating disorder had not been taken into account. In both AN subgroups the weight loss and, as a consequence, the loss of TBF contributed to the higher Chol levels. Furthermore, the decrease in thyroid hormones levels as adaptive responses to chronic starvation in order to conserve metabolic fuels are clearly important factors influencing Chol metabolism. Particularly in the AN-R group, the decreased bile acid synthesis and therefore the decreased Chol catabolism could contribute to the higher LDL-Chol levels. In the AN-B group, the frequent number of binges probably leads to higher ingestion of Chol, which contributes to the inhibition of the Chol synthesis. Enhanced bile acid synthesis as a consequence of the purging behaviour does not seem to result in lower Chol levels. The clinical relevance of dyslipidaemia for CVD risk in AN remains to be established. AN seems to be a helpful model to study mechanisms of lipid disorders in human subjects *in vivo*.

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References

Affenito SG, Lammi-Keefe CJ, Vogel S, Backstrand JR, Welch GW & Adams CH (1997) Women with insulin-dependent diabetes mellitus (IDDM) complicated by eating disorders are at risk for exacerbated alterations in lipid metabolism. *Eur J Clin Nutr* **51**, 462–466.

Angelin B (1991) Regulation of hepatic cholesterol metabolism in man. *Ann Med* **23**, 177–180.

Arden MR, Weiselberg EC, Nussbaum MP, Shenker IR & Jacobson MS (1990) Effect of weight restoration on the dyslipoproteinemia of anorexia nervosa. *J Adolesc Health Care* **11**, 199–202.

Auwerx J & Staels B (1998) Leptin. *Lancet* **351**, 737–742.

Axelson M, Aly A & Sjovall J (1988) Levels of 7 alpha-hydroxy-4-cholesten-3-one in plasma reflect rates of bile acid synthesis in man. *FEBS Lett* **239**, 324–328.

Bertolotti M, Spady DK & Dietschy JM (1995) Regulation of hepatic cholesterol metabolism in the rat in vivo: effect of a synthetic fat-free diet on sterol synthesis and low-density lipoprotein transport. *Biochim Biophys Acta* **1255**, 293–300.

Björkhem I, Miettinen T, Reihner E, Ewerth S, Angelin B & Einarsson K (1987) Correlation between serum levels of some cholesterol precursors and activity of HMG-CoA reductase in human liver. *J Lipid Res* **28**, 1137–1143.

Blendis LM & Crisp AH (1968) Serum cholesterol levels in anorexia nervosa. *Postgrad Med J* **44**, 327–330.

Brown MS & Goldstein JL (1986) A receptor-mediated pathway for cholesterol homeostasis. *Science* **232**, 34–47.

Case T, Lemieux S, Kennedy SH & Lewis GF (1999) Elevated plasma lipids in patients with binge eating disorders are found only in those who are anorexic. *Int J Eat Disord* **25**, 187–193.

Crisp AH, Blendis LM & Pawan GL (1968) Aspects of fat metabolism in anorexia nervosa. *Metabolism* **17**, 1109–1118.

Crisp AH, Lace A, Blendis LM & Pawan GL (1967) Some aspects of fat metabolism in anorexia nervosa. *Proc Nutr Soc* **26**, 32–35.

de Bruin TW, van Barlingen H, Linde-Sibenius TM, van Vuurst de Vries AR, Akveld MJ & Erkelens DW (1993) Lipoprotein(a) and apolipoprotein B plasma concentrations in hypothyroid, euthyroid, and hyperthyroid subjects. *J Clin Endocrinol Metab* **76**, 121–126.

Diekman MJ, Anghelescu N, Endert E, Bakker O & Wiersinga WM (2000) Changes in plasma low-density lipoprotein (LDL)- and high-density lipoprotein cholesterol in hypo- and hyperthyroid patients are related to changes in free thyroxine, not to polymorphisms in LDL receptor or cholesterol ester transfer protein genes. *J Clin Endocrinol Metab* **85**, 1857–1862.

Duvillard L, Florentin E, Lizard G, Petit JM, Galland F, Monier S, Gambert P & Verges B (2003) Cell surface expression of LDL receptor is decreased in type 2 diabetic patients and is normalized by insulin therapy. *Diabetes Care* **26**, 1540–1544.

Feillet F, Feillet-Choloudray C, Bard JM, Parra HJ, Favre E, Kabuth B, Fruchart JC & Vidailhet M (2000) Plasma cholesterol and endogenous cholesterol synthesis during refeeding in anorexia nervosa. *Clin Chim Acta* **294**, 45–56.

Gower BA, Weinsier RL, Jordan JM, Hunter GR & Desmond R (2002) Effects of weight loss on changes in insulin sensitivity and lipid concentrations in premenopausal African American and white women. *Am J Clin Nutr* **76**, 923–927.

Halmi KA & Falk JR (1981) Common physiological changes in anorexia nervosa. *Int J Eat Disord* **1**, 27.

Homma Y, Homma K, Iizuka S & Iigaya K (2002) A case of anorexia nervosa with severe hyperlipoproteinemia. *Int J Eat Disord* **32**, 121–124.

Kaye WH, Weltzin TE, Hsu LK, McConaha CW & Bolton B (1993) Amount of calories retained after binge eating and vomiting. *Am J Psychiatry* **150**, 969–971.

Kempen HJ, Glatz JF, Gevers Leuven JA, van der Voort HA & Katan MB (1988) Serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. *J Lipid Res* **29**, 1149–1155.

Klinefelter HF (1965) Hypercholesterolemia in anorexia nervosa. *J Clin Endocrinol Metab* **25**, 1520–1521.

Lacombe CR, Corraze GR, Nibbelink MM, Boulze D, Douste-Blazy P & Camare R (1986) Effects of a low-energy diet associated with egg supplementation on plasma cholesterol and lipoprotein levels in normal subjects: results of a cross-over study. *Br J Nutr* **56**, 561–575.

Lin DS & Connor WE (1980) The long term effects of dietary cholesterol upon the plasma lipids, lipoproteins, cholesterol absorption, and the sterol balance in man: the demonstration of feedback inhibition of cholesterol biosynthesis and increased bile acid excretion. *J Lipid Res* **21**, 1042–1052.

Mazzella G, Cipolla A, Villanova N, Polimeni C, Sipahi A, Parini P, Fusaroli P, Festi D & Roda E (1995) Changes in biliary lipid secretion and cholic acid kinetics induced by diet, diet plus simvastatin and diet plus ursodeoxycholic acid in obese subjects. *Ital J Gastroenterol* **27**, 441–445.

Mehler PS, Lezotte D & Eckel R (1998) Lipid levels in anorexia nervosa. *Int J Eat Disord* **24**, 217–221.

Miettinen TA (1968) Fecal steroid excretion during weight reduction in obese patients with hyperlipidemia. *Clin Chim Acta* **19**, 341–344.

Miettinen TA, Tilvis RS & Kesaniemi YA (1990) Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am J Epidemiol* **131**, 20–31.

Montoye HJ, Epstein FH & Kjelsberg MO (1966) Relationship between serum cholesterol and body fatness. An epidemiologic study. *Am J Clin Nutr* **18**, 397–406.

Mordasini R, Klose G & Greten H (1978) Secondary type II hyperlipoproteinemia in patients with anorexia nervosa. *Metabolism* **27**, 71–79.

Nestel PJ (1974) Cholesterol metabolism in anorexia nervosa and hypercholesterolemia. *J Clin Endocrinol Metab* **38**, 325–328.

Phinney SD, Tang AB, Waggoner CR, Tezanos-Pinto RG & Davis PA (1991) The transient hypercholesterolemia of major weight loss. *Am J Clin Nutr* **53**, 1404–1410.

Regnstrom J, Nilsson J, Tornvall P, Landou C & Hamsten A (1992) Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. *Lancet* **339**, 1183–1186.

Sanchez-Muniz FJ, Marcos A & Varela P (1991) Serum lipids and apolipoprotein B values, blood pressure and pulse rate in anorexia nervosa. *Eur J Clin Nutr* **45**, 33–36.

Savendahl L & Underwood LE (1999) Fasting increases serum total cholesterol, LDL cholesterol and apolipoprotein B in healthy, nonobese humans. *J Nutr* **129**, 2005–2008.

Stone NJ (1994) Secondary causes of hyperlipidemia. *Med Clin North Am* **78**, 117–141.

Sullivan PF, Gendall KA, Bulik CM, Carter FA & Joyce PR (1998) Elevated total cholesterol in bulimia nervosa. *Int J Eat Disord* **23**, 425–432.

Tamai H, Mori K, Matsubayashi S, Kiyohara K, Nakagawa T, Okimura MC, Walter RM Jr, Kumagai LF & Nagataki S (1986) Hypothalamic–pituitary–thyroidal dysfunctions in anorexia nervosa. *Psychother Psychosom* **46**, 127–131.

Teng B, Thompson GR, Sniderman AD, Forte TM, Krauss RM & Kwiterovich PO Jr (1983) Composition and distribution of low density lipoprotein fractions in hyperapobetalipoproteinemia, normolipidemia, and familial hypercholesterolemia. *Proc Natl Acad Sci USA* **80**, 6662–6666.

Vaisman N, Sklan D & Dayan Y (1990) Effect of moderate semi-starvation on plasma lipids. *Int J Obes* **14**, 989–996.

van der Wal AM, Bakker O & Wiersinga WM (1998) The decrease of liver LDL receptor mRNA during fasting is related to the decrease in serum T3. *Int J Biochem Cell Biol* **30**, 209–215.

Wiegratz I, Jung-Hoffmann C, Gross W & Kuhl H (1998) Effect of two oral contraceptives containing ethinyl estradiol and gestodene or norgestimate on different lipid and lipoprotein parameters. *Contraception* **58**, 83–91.

Zipfel S, Lowe B, Reas DL, Deter HC & Herzog W (2000) Long-term prognosis in anorexia nervosa: lessons from a 21-year follow-up study. *Lancet* **355**, 721–722.

Zoppi G, Cinquetti M & Luciano A (1993) Hypercholesterolemia in anorexia nervosa: probable cause. *Acta Med Auxol* **25**, 201.