Sequential changes in plasma lipoproteins and body fat composition during polyunsaturated fat feeding in man

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1. The early effects of a moderate polyunsaturated fat diet on the composition of circulating lipoproteins and adipose tissue fatty acids were measured in five healthy adults.

2. The fatty acid content and gross composition of the three major plasma lipoprotein fractions altered within 7 d of treatment. The response of depot fat was slower but did show a significant and progressive change after 14 d on the diet.

3. The efficiency of the moderate diet in changing the composition of the lipoproteins suggests that it should be equally effective in altering their metabolic handling.

Substitution of polyunsaturated for saturated fat in the human diet causes profound changes in the chemical composition (Kayden *et al.* 1963; Spritz & Mishkel, 1969) and physical properties (Morrisett *et al.* 1977) of the plasma lipoproteins which become apparent within 6–8 h of diet modification (Kayden *et al.* 1963) and are complete by 14 d (Spritz & Mishkel, 1969). These changes have important effects on lipoprotein metabolism. In an earlier series of studies we have shown that ingestion of a highly polyunsaturated fat diet promotes the catabolism of plasma low-density lipoproteins (LDL) and reduces the rate of synthesis of the major high-density-lipoprotein (HDL) apoprotein, apolipoprotein A-I (Shepherd *et al.* 1978; Shepherd *et al.* 1980). The present report extends our observations on this therapeutic regimen by examining the sequential changes which a more moderate diet produces over a 35 d period in the composition of the plasma lipoproteins and the fatty acid content of human adipose tissue.

METHODS

Subjects

Five healthy adult females (aged 20–22 years) were studied. None had clinical or biochemical evidence of cardiac, hepatic, renal or endocrine disease. No medications were given for 4 weeks before and throughout the study.

Diets

All subjects were instructed to describe, weigh and record their daily food intake over a 7 d period, and were supplied with dietary scales and fluid measures to achieve more accuracy wherever possible. This information was used to calculate (Table 1) the average daily energy consumption of each subject as protein, carbohydrate, polyunsaturated fats and other fats (Paul & Southgate, 1978). An energetic diet of higher polyunsaturated fat content was then prepared for each subject by substitution of as much safflower seed oil for saturated fat as was consistent with palatability. The percentage of dietary energy as carbohydrate, fat and protein was maintained as in the preliminary study (Table 1). This involved eating

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Table 1. *Compositions of control and polyunsaturated fat diets eaten by five normal adult female subjects*

Subject no.	Mean daily energy intake (MJ)	Energy intake (%) as:				Polyunsaturated fat: saturated fat	
		Protein	Carbohydrate	Ethanol	Fat	Control diet	Test diet
1	9·68	13	39	5	43	0·14	1·96
2	6·46	19	46	1	34	0·47	1·11
3	8·73	10	42	3	45	0·16	2·00
4	6·20	21	48	3	28	0·16	1·88
5	8·99	12	40	0	48	0·12	1·64

the same foods each day excepting fruit, lean meat and vegetables which were provided on exchange lists to give variety. The polyunsaturated diet maintained the mean body-weight of each subject within 2·0 % of the starting value throughout the 35 d of the study.

Sample handling

Before beginning the diet, and on days 7, 14, 21 and 35 of diet therapy, a 50 ml plasma sample and 50 mg (approximately) adipose tissue biopsy was collected from each subject following a 14 h fast and analysed as described later.

Plasma analysis

Very-low-density lipoproteins (VLDL), LDL and HDL (densities (g/l); 0·95–1·006, 1·006–1·063 and 1·063–1·21 respectively) were isolated by sequential ultracentrifugation as described by Havel *et al.* (1955) and purified by a further wash as their upper limit density. The chemical composition of each fraction was then determined by estimation of free and esterified cholesterol (Boehringer Cholesterol Kit 15732, Boehringer Mannheim Biochemicals, Mannheim, West Germany), triglyceride (Boehringer Triglyceride Kit 126012), phospholipid (Bartlett, 1959) and protein (Lowry *et al.* 1951). Finally, a portion of each fraction was extracted with organic solvents and subjected to fatty acid analysis (Morrisett *et al.* 1977) by gas–liquid chromatography.

Adipose tissue analysis

Subcutaneous adipose tissue biopsies were removed at weekly intervals from the abdominal wall of each subject through 5 mm incisions made alternately on the right and left side midway between the anterior superior iliac spine and the umbilicus. The specimens were hydrolysed by treatment with 1·0 M-methanolic sodium hydroxide for 30 min at 100° and the liberated fatty acids esterified using 140 g boron trifluoride/l methanol and analysed by gas–liquid chromatography (Morrisett *et al.* 1977).

Statistical analysis

Comparisons of lipoprotein and adipose tissue compositions before and after institution of the polyunsaturated fat diet were made by paired Student's *t* test.

Ethical considerations

Each volunteer gave informed consent to the project which was in accord with the principles of the Ethical Committee of the Royal Infirmary, Glasgow.

RESULTS

Lipoprotein compositions

Ingestion of the polyunsaturated-fat diet produced rapid and significant changes in the compositions of the circulating lipoproteins of all subjects (Tables 2, 3 and 4).

Table 2. *Sequential changes in very-low-density lipoprotein composition (g/kg) during polyunsaturated fat feeding in healthy adult female subjects*

(Mean values and standard deviations for five subjects)

Gross lipoprotein composition

Sampling time (d on diet)	Free cholesterol		Cholesteryl esters		Triglyceride		Phospholipid		Protein	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	40	11	79	48	633	66	139	33	110	26
7	33	17	108	64	524	39	220**	24	146*	17
14	40	10	87	31	592	66	203**	19	97	16
21	37	13	89	26	563	80	213*	59	98	23
35	36	6	39	21	613	106	221**	28	91	7

Fatty acid composition

Fatty acid / Sampling time (d on diet)	12:0		14:0		16:0		16:1		18:0		18:1		18:2		18:3		20:4		Others	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	26	19	41	10	175	42	86	24	75	20	262	22	146	15	52	14	82	34	54	13
7	30	14	20**	3	179	13	83	24	49	3	245	27	246*	56	45	21	61	26	41	28
14	17	17	20*	4	186	21	84	42	48	8	237	19	284**	43	42	16	57	24	24	19
35	45	42	31	13	164	39	74	20	56	11	196*	32	293***	43	37	28	65	17	39	20

Values were significantly different from those for day 0 (paired *t* test): $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table 3. *Sequential changes in low-density lipoprotein composition (g/kg) during polyunsaturated fat feeding in healthy adult female subjects*

(Mean values and standard deviations for five subjects)

Gross lipoprotein composition

Sampling time (d on diet)	Free cholesterol		Cholesteryl esters		Triglyceride		Phospholipid		Protein	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	104	16	431	51	53	11	208	14	224	14
7	91	15	312**	43	63	33	239	32	294**	41
14	74**	9	339**	34	73	37	248	37	268**	15
21	90	8	360*	33	64	27	218	37	268***	11
35	101	15	360*	12	60	4	210	27	268***	17

Fatty acid composition

Fatty acid / Sampling time (d on diet)	12:0		14:0		16:0		16:1		18:0		18:1		18:2		18:3		20:4		Others	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	10	9	18	18	161	22	96	8	62	5	205	28	306	44	52	24	70	12	20	16
7	4	2	5*	1	149	22	69**	12	47**	6	170	12	398**	35	60	11	67	15	28	9
14	24	14	14	7	142	11	32***	5	44	17	148*	20	451**	49	49	27	70	24	28	14
35	8	2	6*	1	151	30	52**	11	50*	12	157*	36	398**	33	54	29	85	29	41	22

Values were significantly different from those for day 0 (paired *t* test): $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Table 4. *Sequential changes in high-density lipoprotein composition (g/kg) during polyunsaturated fat feeding in healthy adult female subjects*

(Mean values and standard deviations for five subjects)

Gross lipoprotein composition

Sampling time (d on diet)	Free cholesterol		Cholesteryl esters		Triglyceride		Phospholipid		Protein	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	48	17	204	29	33	6	267	37	448	21
7	40	15	170	52	27	12	268	47	493*	36
14	30	12	161	41	31	5	284	26	494*	35
21	44	15	159*	25	23	11	265	67	509*	41
35	35	8	185	50	42	28	272	34	466	66

Fatty acid composition

Fatty acid / Sampling time (d on diet)	12:0		14:0		16:0		16:1		18:0		18:1		18:2		18:3		20:4		Others	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	36	19	25	17	146	21	74	5	82	26	151	14	259	31	61	15	110	13	56	22
7	23	16	14	6	174	8	76	7	87	12	181	19	236	14	60	29	100	7	49	9
14	16	5	9	4	156	11	61	8	74	11	140	15	404***	32	27	6	79	16	34	8
35	39	28	29	15	138	12	53	17	53	12	127*	8	418*	79	45	9	62	15	36	3

Values are significantly different than those for day 0 (paired *t* test): $*P < 0.05$, $***P < 0.001$.

Table 5. *Sequential changes in adipose tissue fatty acid composition (g/kg) during polyunsaturated fat feeding in healthy adult female subjects*

(Mean values and standard deviations for five subjects)

Fatty acid / Sampling time (d on diet)	12:0		14:0		16:0		16:1		18:0		18:1		18:2		18:3		20:4		Others	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	22	11	54	13	209	21	75	16	61	11	385	27	114	27	46	16	34	19	trace	
7	29	21	55	22	203	23	79	16	60	6	365	46	116	20	48	5	34	17	11	3
14	26	17	56	14	208	24	81	17	58	14	361	39	128*	28	48	11	34	10	trace	
21	28	16	50	8	201	30	80	17	60	15	355*	25	126*	24	55	9	46	10	trace	
35	19	9	52	6	206	26	72	12	58	14	344*	27	138**	29	55	16	57**	32	trace	

Values were significantly different from those for day 0 (paired *t* test): $*P < 0.05$, $**P < 0.02$.

In VLDL (Table 2) the percentage of fatty acid 18:2 rose within 7 d from a control value of $14{\cdot}6 \pm 1{\cdot}5$ (mean $\pm$ 1 SD) to $24{\cdot}6 \pm 5{\cdot}6$ ($P < 0{\cdot}05$) and reached a maximum of $29{\cdot}3 \pm 4{\cdot}3$ ($P < 0{\cdot}001$) by day 35 (Table 2). This increment was associated with reciprocal changes in more saturated fatty acids. Specifically, the level of the most abundant fatty acid in this lipoprotein, 18:1, fell from $26{\cdot}2 \pm 2{\cdot}2$ to $19{\cdot}6 \pm 3{\cdot}2\%$ ($P < 0{\cdot}05$) over the 35 d of diet therapy. Similar smaller reductions were also seen in 14:0, 16:1 and 18:0 although in the instance of the last two, statistical significance was not reached. Alterations in the over-all composition of VLDL accompanied these changes in fatty acid content and primarily affected the hydrophilic, phospholipid-rich surface coat of the particles. The percentage phospholipid rose from $13{\cdot}9 \pm 3{\cdot}3$ to a maximum of $22{\cdot}1 \pm 2{\cdot}8$ by day 35 ($P < 0{\cdot}01$). There was no concomitant reduction in the percentage of core components (cholesterol, cholesteryl esters and triglyceride) in the fraction.

LDL (Table 3) showed the greatest compositional change in response to polyunsaturated fat ingestion. Again, the largest increment in over-all fatty acid composition was seen in the 18:2 fraction which rose from a control value of $30{\cdot}6 \pm 4{\cdot}4$ of the total fatty acid content to a maximum of $45{\cdot}1 \pm 4{\cdot}9\%$ ($P < 0{\cdot}01$) by day 14 (Table 3). A fall in the percentage of 14:0, 16:0, 18:0 and 18:1 accompanied this rise.

Major structural alterations also affected the LDL fraction as a result of the treatment (Table 3). In particular, the percentage of cholesteryl esters fell from $43{\cdot}1 \pm 5{\cdot}1$ to $31{\cdot}2 \pm 4{\cdot}3$ ($P < 0{\cdot}01$) by day 7 and stabilized at $36{\cdot}0 \pm 1{\cdot}2$ at day 35. In parallel with this reduction in core components there was an increase ($P < 0{\cdot}001$) in the percentage protein in the fraction. Consequently, the polyunsaturated diet produced a significant decrease in cholesterol:protein in LDL, in keeping with the proposal that the mean particle diameter had fallen during treatment. This factor, calculated by the method of Shen *et al.* (1977), was 192 Å on day 0 and fell to 167 Å by day 35.

The fatty acid spectrum in HDL, (Table 4) like those of the other major plasma lipoprotein fractions, was significantly influenced by diet therapy. The percentage of fatty acid 18:2 again rose during treatment (from $25{\cdot}9 \pm 3{\cdot}1$ to $41{\cdot}8 \pm 7{\cdot}9$; $P < 0{\cdot}05$) at the expense of the more saturated fatty acids (Table 4). Although there was a consistent reduction in 16:0, 16:1, 18:0 and 18:1, this only reached significance in the instance of the latter. These changes again reflect the fatty acid composition of the diet. Over all, the effects of the diet on HDL composition were minimal (Table 4) and were largely confined to the protein component of the particle which showed an increase from 44·8 to a mean maximal value of 50.9 on day 21.

Adipose tissue fatty acid compositions

The response of adipose tissue to the 35 d of diet modification was much less than that of the lipoproteins (Table 5). Again, however, the changes reflected the dietary fat composition. After 2 weeks' treatment there was a small but significant increase in the percentage of fatty acid 18:2 which rose to a maximum by day 35. Fatty acid 20:4 also rose significantly after 5 weeks treatment. These increments seemed to occur at the expense of the more saturated fatty acids although only that fall apparent in 18:1 was statistically significant.

DISCUSSION

In two earlier studies we examined the influence of polyunsaturated-fat diets on the chemical composition and metabolism of HDL (Shepherd *et al.* 1978) and LDL (Shepherd *et al.* 1980). We intentionally used a diet which was an extreme example of that normally employed in clinical practice in order to maximize its perturbative effect on the lipoproteins. This diet did not take palatability into account and is unlikely to be acceptable to most patients as

long-term therapy. The present study was designed to determine whether our previous findings still apply when a more acceptable, less extreme diet is used.

Our results indicate that whereas the relative abundance and saturation level of fat in the diet of normal individuals shows substantial variation (cf. subjects nos. 2 and 5 ,Table 1), it is possible by limited manipulation of this dietary component alone to cause significant changes in the chemical composition of the circulating lipoproteins. Further, these changes agree qualitatively with the results produced by extreme diet modification, inasmuch as both the fatty acid saturation level and the gross chemical composition of the lipoproteins are affected. Since these two factors contribute to the microscopic fluidity of the lipoproteins, it is not surprising that dietary manipulations of the type described here should be accompanied by an increase in the fluidity of the particles (Morrisett *et al.* 1977; Shepherd *et al.* 1978), providing a rational explanation for the changes in lipoprotein metabolism which accompany polyunsaturated fat feeding (Shepherd *et al.* 1978; Shepherd *et al.* 1980).

Safflower seed oil, the source of dietary polyunsaturated fatty acids used in this study, contained (g/kg) 670 18:2, 160 18:1, 80 16:0 and trace amounts of other fatty acids on gas–liquid chromatographic analysis. The effects which it produced on the fatty acid content of the circulating lipoproteins reflects this composition. In all lipoprotein fractions 18:2 was most affected by treatment; and the increment which it exhibited was consistently associated with a reduction in fatty acid 18:1.

The changes which occurred in lipoprotein composition were qualitatively similar to those reported for LDL and HDL during administration of the extreme diet. The LDL fraction became depleted in cholesterol and relatively enriched in protein, so that the cholesterol:protein fell as a result of treatment. The phospholipid content of the particle rose, but did not reach the level of significance observed previously (Shepherd *et al.* 1980). The diet-induced alteration in HDL composition was confined to an enrichment of its protein content. This contrasts with the fall in protein and increase in phospholipid reported during administration of the extreme diet (Shepherd *et al.* 1978). The measured increase in VLDL phospholipid during polyunsaturated fat administration suggests that the diet may have caused a fundamental change in the structure of these particles. Since phospholipid constitutes a major component of the surface coat of the lipoprotein, its enrichment is in accord with the proposal that the mean particle diameter is reduced during treatment. In fact its calculated value (from Shen *et al.* 1977) fell from 382 Å on day 0 to 330 Å by day 35.

It is now well established that the composition of human adipose tissue is influenced by diet (Miettinen *et al.* 1972). It is also appreciated that the response of adipose tissue to diet modification is substantially delayed, in contrast to changes in blood lipids, and varies with sampling site (Pittet *et al.* 1979). By sampling from a fixed location on the anterior abdominal wall we have shown (Table 5) that although the diet-induced change in fatty acid composition of the adipose tissue biopsy is slow, measurable effects become increasingly apparent after 14 d of treatment. These first involve an enrichment of 18:2 in the fat stores with displacement of 18:1. Consequently, in adipose tissue and in circulating lipoproteins, 18:1 behaves more like a saturated than an unsaturated fatty acid.

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