Modification in the composition and metabolic properties of human low density and high density lipoproteins by different dietary fats

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Abstract The chemical composition and metabolism of lipoproteins in a population of Benedictine nuns were studied after 5-month periods during which the predominant dietary fats were sunflower oil, peanut oil, palm oil, or milk fats (butter and cream). The population was divided into three groups. The control group (C) included twelve subjects selected at random by taking two subjects per age pool among those with plasma cholesterol < 230 mg/dl. Groups H_1 and H_2 were selected at random in the same way, among those with plasma cholesterol > 230 mg/dl. Each group comprised six subjects and differed from each other in the amount of plasma cholesteryl esters, i.e., below and above the mean value of group C, for H_1 and H_2, respectively. Changes in the degree of saturation of the predominant fat of the diet were associated with changes in both the chemical composition of lipoproteins and their cellular metabolism studied in fibroblast cultures. No significant difference between the normocholesterolemic subjects of group C and the "high risk" subjects of groups H_1 and H_2 was found.—**Baudet, M. F., C. Dachet, M. Lasserre, O. Esteva, and B. Jacotot.** Modification in the composition and metabolic properties of human low density and high density lipoproteins by different dietary fats. *J. Lipid Res.* 1984. **25:** 456–468.

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Many studies have been carried out on the relationship between the degree of saturation of dietary fatty acids and the concentration of plasma cholesterol. Only a few of them concerned the relationship between the nature of the fat and plasma lipoproteins (1–5). Some of the reports are in disagreement (6, 7). Although the mechanisms of their metabolic effects are not completely understood, polyunsaturated fats have a well-established hypocholesterolemic action. Moreover, they induce a fall in the plasma triglycerides (8), an increased excretion of bile acids (9), a modification in the acyl groups of triglycerides (10), and an increase in polyunsaturated fatty acids associated with lipoproteins (11–13). It can be assumed from these results that polyunsaturated fats produce modifications in the physical properties of lipoproteins (13). Engelberg (8) reported that the fall in plasma triglycerides was correlated with the enhancement of lipoprotein catabolism. Other studies (14, 15) suggested a relationship between LDL apoprotein catabolism and the fatty acid composition of the lipoprotein.

The aim of this work was to study the effect of the main dietary lipids, ingested during a 5-month period, on the composition and the structure of plasma lipoproteins, and on the interactions between LDL and HDL and cultured cells (fibroblasts). The lipoproteins were obtained from Benedictine nuns (16). The regularity of the diet and the perfect adherence of this population to the dietary prescriptions must be emphasized. Under these conditions the dietary fats were perfectly controlled, and the other nutrients remained practically invariable.

The following dietary fats were used: sunflower oil, peanut oil, palm oil, and milk fats (butter and cream).

MATERIALS AND METHODS

Materials

Sodium [^{125}I]iodide solubilized in NaOH (15.5 mCi/μg), [1-^{14}C]acetate (43 mCi/mmol), [7(n)-^{3}H]-cholesterol (in toluene) (58 Ci/mmol), [1α,2α-(n)-^{3}H]cholesterol (in toluene), [1-^{14}C]oleic acid (in toluene) were purchased from Amersham (Cardiff, England). The RPMI 16/40 medium, fetal calf serum, and L-glutamine were purchased from Eurobio (Paris, France). The penicillin-streptomycin solution (50,000 U/ml) and the trypsin were obtained from Bio-Mérieux (Charbonnière-les Bains, France), and the fungizone was from Squibb Laboratories (Princeton, NJ).

Abbreviations: BSA, bovine serum albumin; LDL, low density lipoproteins; HDL, high density lipoproteins.

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Population

The population was composed of 69 Benedictine nuns who rigorously adhered to the dietary instructions. Informed consent was obtained from all the subjects. The mean age at the beginning of the study was 46 ± 11 years (range 26–70 years). Subjects with metabolic disturbances (diabetes, plasma cholesterol > 300 mg/dl, plasma triglycerides > 200 mg/dl) were not included in this study. The remaining subjects (**Table 1**) were divided into six age pools (in steps of 8 years); in each age pool two subjects were selected at random from among those having a plasma cholesterol > 230 mg/dl and two others from among those with a plasma cholesterol < 230 mg/dl. Thus two groups each of twelve subjects were constituted, a hypercholesterolemic group H and a control group C.

A previous study of the serum lipids of each subject of groups H and C (**Table 2**) showed that group C was homogeneous, whereas group H showed a bi-model distribution and divided into two sub-groups (each of six subjects); H_1 having low levels of plasma cholesteryl esters (below the mean value of group C) and H_2 having high levels of plasma cholesteryl esters (above the mean value of group C).

Diets

Four test diets were given successively during 5-month periods. The order of the different diets was randomized. In each diet 30% of the calories was from fat. Fat other than the main dietary fat was reduced in so far as possible and represented only one-third of the ingested lipid. The remaining two-thirds was composed of one of the tested fats, sunflower oil, palm oil, peanut oil, and milk fats. The diet comprised 16% protein and 54% carbohydrate, the whole representing 2,000 calories per day on average.

The P/S ratios of the diets were as follows: sunflower oil, 1.75; palm oil, 0.31; peanut oil, 0.68; and milk fat, 0.09. The fatty acid composition of these fats is shown in **Table 3.** The cholesterol intake during the sunflower oil, palm oil, and peanut oil diets was 300 mg per day. The cholesterol intake during the milk fat diet was 400 mg per day.

TABLE 1. Initial clinical data

Subject Number	Weight	Age	Plasma Cholesterol	Plasma Triglycerides	HDL Cholesterol
	kg	*yr*		*mg/dl*	
Group C					
1	56	26	155	50	45
2	44	33	195	40	72
3	57	35	155	25	66
4	69	35	190	45	38
5	54	42	195	85	90
6	65	42	155	45	63
7	48	44	180	65	61
8	47	48	175	50	73
9	61	54	200	60	62
10	53	56	225	65	72
11	46.5	67	225	65	74
12	60	69	210	50	49
Mean ± SD	55.04 ± 7.82	45.91 ± 13.43	188.33 ± 25.16	53.75 ± 15.39	63.75 ± 14.34
Group H					
13	54	26	255	35	65
14	61	28	260	50	55
15	58	32	240	70	55
16	55	33	240	35	65
17	64	36	285	90	101
18	68	44	240	165	47
19	59	52	295	70	71
20	59	52	265	80	69
21	60	58	280	105	55
22	71	60	260	185	47
23	65	66	295	85	66
24	63	68	280	80	59
Mean ± SD	61.41 ± 5.03	46.25 ± 15.03	266.25 ± 20.57	87.50 ± 46.19	62.91 ± 14.39

TABLE 2. Fatty acids of serum cholesteryl esters

Fatty Acids	Group C	Group H	Group H_1	Group H_2
	μg/ml of serum ± SD			
14:0	4.73 ± 2.65	5.06 ± 1.35	4.02 ± 1.02	6.09 ± 0.64
16:0	42.65 ± 8.8	44.52 ± 12.3	34 ± 3.7[a]	55.05 ± 7.3[b]
16:1	11.86 ± 5.15	13.61 ± 5.77	9.46 ± 4.79[c]	17.77 ± 2.9[d]
18:0	5.89 ± 3.51	4.95 ± 1.94	4.95 ± 2.61	4.96 ± 1.21
18:1	58.21 ± 13.22	56.92 ± 16.33	42.46 ± 7.5	71.38 ± 5.3
18:2	184.7 ± 35	219.23 ± 66.57	160.86 ± 19.5[a]	277.6 ± 34[b]
20:4	21.05 ± 6	25.75 ± 9.67	17.64 ± 5.6[a]	33.86 ± 3.5[b]

Fatty acids of serum cholesteryl esters at the beginning of the study. Mean levels of fatty acids (μg/ml) of serum cholesteryl esters in the twelve subjects of each of the experimental groups (control group, C; high risk group, H) when the community was maintained on the usual diet (before the beginning of the test diets). Group H shows two different populations, each of six subjects; H_1, high mean level of serum cholesteryl esters; H_2, low mean level of serum cholesteryl esters, as compared with the mean level in group C. The significant differences are as follows: a vs b, $P < 0.001$; c vs d, $P < 0.01$ (Student's *t* test).

Preparation of lipoproteins and lipids

Blood samples were obtained by venous puncture after a 12-hr fasting period. Pools C, H_1, and H_2 were constituted from aliquots of sera of subjects of each group. The lipid levels of these pooled sera are given in **Table 4.**

The lipoproteins were isolated by ultracentrifugation according to Havel, Eder, and Bragdon (17). After removal of chylomicrons by preliminary centrifugation, VLDL was isolated at 1.006 g/ml, LDL at 1.019–1.063 g/ml, and HDL at 1.085–1.21 g/ml. Each lipoprotein fraction was washed at its equilibrium density, then dialyzed against 0.9% NaCl, pH 7.4, containing 0.01% EDTA at 4°C. The purified lipoproteins were sterilized by filtration on Millipore membranes (0.45 μm) and stored at 4°C.

TABLE 3. Fatty acid composition of the tested dietary fats

Fatty Acids	Sunflower Oil	Palm Oil	Peanut Oil	Milk Fats
	weight %			
4:0				3.4
6:0				1.9
8:0				1.26
10:0				2.89
12:0		0.17		3.62
14:0		0.85		11.8
14:1				0.36
16:0	6.19	21.79	11.44	32.61
16:1	0.09	0.32	0.10	2.5
18:0	4.74	2.10	3.54	12.47
18:1	16.96	57.71	41.32	24.9
18:2	70.61	15.64	35.63	0.64
18:3	0.20	0.32	0.23	1.51
20:0	0.30	0.19	1.48	
20:1	0.17	0.20	1.04	
22:0		0.09	3.25	
22:1		0.04	0.17	
22:2				
24			1.42	
Polyunsaturated/ Saturated	6.3	0.6	1.7	0.1

The lipoproteins (**Table 5**) of a pool of control sera obtained from ten normolipidemic subjects on an ad libitum diet (45% lipid calories, 15% protein calories, and 40% carbohydrate calories) were isolated in the same way. The LPDS (lipoprotein-deficient serum) was prepared from this pool of sera by ultracentrifugation at 1.25 g/ml, then dialyzed for 48 hr against 0.9% NaCl, pH 7.4, containing 0.01% EDTA, filtered on a Millipore filter (0.45 μm), and stored at −20°C.

Analytical procedures

Proteins were determined according to Lowry et al. (18). The free and esterified cholesterols were assayed by the catalase method (Boeringher Mannheim kit). The triglycerides were measured by the glycerol 3-phosphate-oxidase method (triglycerides G-test kit from Biolyon). The phospholipids were assayed according to the method of Van Gent and Roseleur (19).

Each lipoprotein fraction was analyzed by SDS polyacrylamide gel electrophoresis (20).

Cell cultures

The normal fibroblasts used in this study came from skin biopsies of healthy human donors, 30–35 years old. The cells were maintained at 37°C in the presence of air–CO_2 (95:5%) in Falcon flasks (75 cm^2) containing 30 ml of RPMI medium 16/40 supplemented with penicillin (100 U/ml), streptomycin (100 U/ml), fungizone (50 μg/ml), containing 1% (v/v) of 200 mM L-glutamine, and 10% fetal calf serum.

TABLE 4. Mean lipid levels of pooled sera from subjects in different test groups

Diet	Cholesterol	Triglyceride	Phospholipid
		mg/dl	
Ad libitum diet (n = 10)	188 ± 19	58 ± 23	196 ± 15
Sunflower oil diet			
C (n = 12)	163 ± 17	44 ± 15	228 ± 17
H_1 (n = 6)	175 ± 20	79 ± 40	175 ± 21
H_2 (n = 6)	200 ± 16	269 ± 39	204 ± 20
Palm oil diet			
C (n = 12)	175 ± 30	66 ± 18	146 ± 17
H_1 (n = 6)	221 ± 22	70 ± 39	175 ± 36
H_2 (n = 6)	219 ± 19	114 ± 88	201 ± 28
Peanut oil diet			
C (n = 12)	182 ± 18	41 ± 11	156 ± 16
H_1 (n = 6)	221 ± 26	77 ± 54	220 ± 29
H_2 (n = 6)	226 ± 17	78 ± 58	184 ± 22
Milk fat diet			
C (n = 12)	217 ± 33	49 ± 14	247 ± 18
H_1 (n = 6)	272 ± 20	95 ± 46	52 ± 20
H_2 (n = 6)	260 ± 28	80 ± 53	56 ± 19

Diets: C, control; H_1, high mean level of serum cholesteryl esters; H_2, low mean level of serum cholesteryl esters.

For the experiments cells from the stock flasks were dissociated with 0.05% trypsin–0.05% EDTA solution and were seeded at a concentration of approximately 2.5 $\times 10^5$ cells per flask, into Falcon flasks (25 cm^2) containing 8 ml of the above growth medium. These flasks were used when the cells were confluent (average confluence between 75 and 100%). Fibroblasts were used between the 6th and the 12th passages.

Determination of the binding, internalization, and degradation of lipoproteins by the fibroblasts

These studies were carried out with human LDL from a pool of control sera. The LDL preparation was labeled with ^{125}I according to the technique of MacFarlane (21) modified by Bilheimer, Eisenberg, and Levy (22). Forty-eight hours before the beginning of the experiment, the fibroblasts were washed twice with the Dulbecco solution, then the RPMI-fetal calf medium was replaced by RPMI medium containing 5% LPDS.

The lipoproteins of subjects in groups C, H_1, and H_2 were isolated from pools made up from aliquots of sera of subjects of each group. In order to test the interaction of the LDL and HDL isolated from the pools of plasma of groups C, H_1, and H_2 with the fibroblasts, the LPDS medium was replaced in each flask by 2 ml of fresh LPDS medium containing 10 μg/2 ml of ^{125}I-LDL and 100, 200, or 300 μg of nonlabeled lipoproteins, coming either from the pool of sera of the ad libitum diet group or from the pool of sera of the C, H_1, and H_2 groups. After 4 hr of incubation at 37°C, the binding and internalization were measured as previously described (23). The proteolytic degradation of lipoproteins was calculated by determination of the ^{125}I-monoiodotyrosin liberated in the culture medium after treatment with trichloracetic acid and hydrogen peroxide. The actual degradation corresponded to the difference between the values obtained from the medium with or without fibroblasts, the incubation conditions being identical (24). The corresponding incubations were carried out in triplicate.

The results for binding, internalization, and degra-

TABLE 5. Composition of control lipoproteins (ad libitum diet)

	Overall Composition			Lipid Composition				
				Cholesterol				
	Total Protein	Total Lipid	Protein / Lipid	Total	Free	Ester	Triglycerides	Phospholipids
	weight %			*mg/100 mg of lipoprotein protein*				
VLDL	16	84	0.19	91	58.0	34	320	115
LDL	25	75	0.33	160	46.0	114	15	113
HDL	50	50	1.0	39	6.5	33	5	54

dation were expressed as percentage of the corresponding values obtained with ^{125}I-LDL free of any traces of other lipoproteins.

Determination of cholesterol synthesis

This was done by measuring the incorporation of [1-^{14}C]acetate into free cholesterol as previously described (25). The results were expressed as percentage of the amount of intracellular free cholesterol synthesized in the absence of lipoproteins. Values obtained with LDL from pooled control sera ranged between 38 and 54%.

Determination of the intracellular esterification of LDL-cholesterol

The esterification was measured using a dilute sodium oleate solution according to the method described by Van Harken, Dixon, and Heimberg (26). [1-^{14}C]Oleic acid was diluted in a nonradioactive sodium oleate solution containing 12% BSA in 0.9% NaCl, pH 7.4.

Twenty-four hours before the experiment the cells were placed in LPDS medium. This medium was then replaced with 2 ml of fresh LPDS medium containing 20 μg/ml of LDL derived either from a pool of ad libitum sera or from one of the pools of groups C, H_1, or H_2. After 16 hr of incubation, 40 μl of the [^{14}C]oleate-BSA solution was added to the medium. After 2 hr of incubation, the cells were washed three times with 0.9% NaCl, pH 7.4, EDTA 0.01%, and solubilized in 2 ml of 0.1 N NaOH. Cellular proteins were determined according to Lowry et al. (18). Lipids were extracted according to Folch et al. (27) on an aliquot of NaOH after addition of [1α,2α-(n)-^{3}H]free cholesterol used as an internal standard. The chloroform phase was evaporated to dryness, solubilized with 50 μl of a mixture containing nonradioactive free and esterified cholesterol in solution in chloroform–methanol 2:1, and plated on a thin-layer plate. After development the free cholesterol and cholesteryl ester spots were scraped off and counted in a liquid scintillation counter.

The results are expressed as percentage of the amount of endogenous cholesterol esterified in the presence of 20 μg of LDL from pooled control sera.

Determination of the removal of cellular free cholesterol by HDL

This measurement was made with fetal calf serum labeled with [7(n)-^{3}H]cholesterol (7 μCi/15 ml of fetal calf serum).

Forty-eight hours before the beginning of the experiment, fibroblasts were incubated with RPMI medium containing tritiated fetal calf serum. The medium was discarded, and fibroblasts were washed three times with Dulbecco solution.

The washed fibroblasts of each Falcon flask were incubated with RPMI medium without fetal calf serum, and 250 μg/ml of HDL from the pooled control sera, or from the pooled sera of C, H_1, and H_2 groups. After 24 hr incubation at 37°C (28), the extra- and intracellular radioactivity was measured after extraction of the tritiated cholesterol according to Dole (29).

The results were expressed as percentage of the radioactivity of the medium, as compared to the radioactivity of the fibroblasts considered as 100%. The radioactivity in the medium, in the absence of added proteins, was subtracted from each experiment value.

For HDL from pooled control sera, the removal of cellular cholesterol ranged between 24 and 28%.

RESULTS

Whereas the concentrations of VLDL, LDL, and HDL in serum (**Table 6**) did not differ between sunflower oil, peanut oil, and palm oil diets, they were all greatly increased with the milk fat diet. For a given diet, groups C, H_1, and H_2 behaved similarly. The concentration of LDL and especially of VLDL was lower in group C. HDL concentration did not differ from one group to another.

The ratio HDL/VLDL + LDL was always higher in group C, and lower in group H_2 than in group H_1.

Lipoprotein composition (Tables 7, 8, and 9)

In order to obtain a more direct comparison of the composition of the different lipoproteins among the four diet groups, the lipid class content has been expressed as mg/100 mg of protein.

The lipoprotein composition was somewhat different with the milk fat diet, but only minor differences appeared among groups C, H_1, and H_2.

VLDL (Table 7). The proteins–lipid (P/L) ratio was the lowest with the most unsaturated diet, i.e., sunflower oil diet. This ratio increased very slightly with the palm oil and peanut oil diets because of a slight decrease in triglycerides. With the milk fat diet this ratio increased even more; there was a large decrease in free and esterified cholesterol and in phospholipids, and a moderate decrease in triglycerides.

These results are in disagreement with those of Vega et al. (30) and Kuksis et al. (31) who found a decrease in the concentration of each component of VLDL with a polyunsaturated diet as well as no change in their P/L ratio.

LDL (Table 8). Changes observed with the different diets in the three groups of subjects C, H_1, and H_2 were similar to those observed with VLDL. In contrast to the results of Vega et al. (30) and Kuksis et al. (31), a large decrease in the P/L ratio was observed with the most saturated diet; a twofold decrease of this ratio was ob-

TABLE 6. Serum concentration of lipoproteins

	VLDL	LDL	HDL	HDL / VLDL + LDL
	mg of protein / dl of serum			
Sunflower oil diet				
C	3.9	38.2	79.3	1.87
H_1	6.5	42.3	77.2	1.57
H_2	4.9	54.3	82.2	1.38
Palm oil diet				
C	2.0	26.4	62.6	2.2
H_1	5.3	44.6	74.2	1.48
H_2	8.5	44.8	63.5	1.18
Peanut oil diet				
C	2.2	41.0	74.0	1.7
H_1	10.0	54.4	63.7	0.99
H_2	8.1	53.3	57.2	0.93
Milk fat diet				
C	8.1	66.8	107.8	1.43
H_1	14.3	76.2	102.2	1.13
H_2	11.9	74.1	96.1	1.11

served with the milk fat diet. An increase in total cholesterol, mainly esterified cholesterol, accounted for the increase in lipids. Changes in the amounts of triglycerides and phospholipids were not significant.

HDL (Table 9). A decrease in the P/L ratio was observed with the milk fat diet. The decrease in protein was accompanied by an increase in total cholesterol and triglycerides, and a decrease in phospholipids.

With the peanut oil diet, there was a slight increase in proteins accompanied by a decrease in esterified cholesterol as compared with sunflower and palm oil diets. Kuksis et al. (31) and Shepherd et al. (4) found a large decrease of the P/L ratio in HDL with polyunsaturated diets.

As for the apoproteins, the only important change that was observed by SDS-polyacrylamide gel electrophoresis was a large increase in apoA-I of HDL with the most

TABLE 7. Composition of very low density lipoproteins (VLDL)

	Overall Composition			Lipid Composition				
			Protein	Cholesterol				
	Total Protein	Total Lipid	Lipid	Total	Free	Ester	Triglycerides	Phosholipids
	weight %			*mg / 100 mg of lipoprotein protein*				
Before diet								
C	12	88	0.13	208	72	136	350	175
H	9	91	0.09	188	80	108	433	385
Sunflower oil diet								
C	15	85	0.17	120	62	58	346	100
H_1	14	86	0.16	142	71	71	407	64
H_2	14	86	0.16	121	72	49	421	71
Palm oil diet								
C	10.5	89.5	0.11	166	74	92	628	57
H_1	17	83	0.20	100	52	48	350	38
H_2	19	81	0.23	81	41	40	302	42
Peanut oil diet								
C	18	82	0.21	138	102	36	244	69
H_1	16.5	83.5	0.19	145	106	39	296	63
H_2	16	84	0.19	143	86	57	325	56
Milk fat diet								
C	22.6	77.4	0.29	64	49	15	273	5
H_1	25	75	0.33	98	47	51	180	22
H_2	22.7	77.3	0.29	82	65	17	231	25

TABLE 8. Composition of low density lipoproteins (LDL)

	Overall Composition			Lipid Composition				
			Protein	Cholesterol				
	Total Protein	Total Lipid	Lipid	Total	Free	Ester	Triglycerides	Phospholipids
	weight %			*mg/100 mg of lipoprotein protein*				
Before diet								
C	30	70	0.42	150	46	104	23	60
H	27	73	0.36	188	104	44	26	55
Sunflower oil diet								
C	29	71	0.40	137	40	97	27	79
H_1	39	61	0.63	89	47	42	25	41
H_2	38	62	0.61	89	36	53	25	48
Palm oil diet								
C	39.5	60.5	0.65	102	45	57	22	27
H_1	43	57	0.75	69	36	33	23	39
H_2	45	55	0.81	75	30	45	18	27
Peanut oil diet								
C	36	64	0.56	112	65	47	15	50
H_1	30	70	0.42	148	54	94	30	55
H_2	35.5	64.5	0.55	109	55	54	22	49
Milk fat diet								
C	22.8	77.2	0.29	250	65	185	27	60
H_1	20	80	0.25	264	89	175	124	22
H_2	22.6	77.4	0.29	289	70	219	26	25

saturated diets, i.e., palm oil and milk fats (**Fig. 1**). Shepherd et al. (4) and Kuksis et al. (31) also observed a decrease in apoprotein A-I with polyunsaturated diets.

Interactions with the fibroblasts

*Binding, internalization, and protein degradation (**Figs. 2 and 3**).* With the sunflower diet, the pattern of LDL-fibroblast interactions was the same in groups C, H_1, H_2 and the ad libitum group. Binding, internalization, and proteolytic degradation of LDL by the fibroblasts were normal. The HDL of the subjects fed the sunflower oil diet and of the ad libitum group did not react with the fibroblast receptors.

TABLE 9. Composition of high density lipoproteins (HDL)

	Overall Composition			Lipid Composition				
			Protein	Cholesterol				
	Total Protein	Total Lipid	Lipid	Total	Free	Ester	Triglycerides	Phospholipids
	weight %			*mg/100 mg of lipoprotein protein*				
Before diet								
C	59	41	1.4	33	4	29	11	22
H	56	44	1.2	37	5	32	14	26
Sunflower oil diet								
C	57.5	42.5	1.3	38	9	29	12	23
H_1	58	42	1.3	37	8	29	10	24
H_2	57	43	1.3	42	9	33	12	21
Palm oil diet								
C	60	40	1.5	31	3	28	6	28
H_1	60.5	39.5	1.5	28	3	25	8	28
H_2	56.5	43.5	1.3	35	3	32	8	32
Peanut oil diet								
C	65.5	34.5	1.9	16	10	6	7	19
H_1	62.5	37.5	1.6	26	9	17	11	22
H_2	62	38	1.6	28	9	19	12	20
Milk fat diet								
C	53.8	46.2	1.1	45	19	26	24	15
H_1	50	50	1	51	13	38	49	8
H_2	50	50	1	61	18	43	23	16

With the palm oil diet, LDL of groups C, H_1, and H_2 exhibited a decreased catabolism as compared with the ad libitum group. Binding of LDL was lowered by 10 to 15%, and internalization by 15 to 22%. Degradation was normal in groups C and H_1, but decreased by 10% in group H_2. There was no interaction between HDL of the tested subjects and fibroblasts.

With the peanut oil diet, the catabolism of LDL of the three groups was only slightly or not at all changed. There was a decrease in LDL binding in group C, a slight decrease in internalization of LDL in groups C and H_1, and no modification in degradation. The HDL of the three groups reacted normally with the fibroblasts.

With the milk fat diet, there were important modifications in the catabolism of LDL in groups C, H_1, and H_2. Binding was 18% lower, internalization 20 to 35% lower, and the proteolytic degradation 6 to 22% lower. On the other hand, there was a strong interaction of HDL with the fibroblasts in the three groups, 20% increase in binding, 25% increase in internalization, and 25% increase in degradation (Fig. 3).

*Regulation of the synthesis of intracellular cholesterol (**Table 10**).* With the sunflower oil, peanut oil, and milk fat diets, there was little or no change in the endogenous cholesterol content of the fibroblasts, whether the LDL used was from groups C, H_1, H_2, or from the control group on the ad libitum diet.

On the other hand, with the palm oil diet, a striking increase in the synthesis of intracellular cholesterol was observed in the three groups when compared with the control group. This suggests that regulation of the endogenous synthesis of cholesterol was perturbed in the presence of the LDL obtained from the subjects on the palm oil diet.

Intracellular esterification of the LDL cholesterol (Table 10). With the palm oil and peanut oil diets, but not with the milk fat diet, the esterification level was low in the presence of LDL from group C. In the presence of LDL from group H_1, a high esterification level was observed with the palm oil diet. With the peanut oil and milk fat diets, the esterification was normal.

In the presence of LDL from group H_2, the esterification level was normal with the palm oil diet and decreased with the milk fat diet.

Removal of the cellular free cholesterol by the HDL (Table 10). With the sunflower and peanut oil diets, the uptake of intracellular cholesterol by HDL was the same as that obtained with HDL from pooled control sera. With the palm oil diet, the uptake was higher in groups H_1 and H_2. With the milk fat diet, the uptake value was about twice that observed for the control sera in groups C, H_1, and H_2.

DISCUSSION

The purpose of this work was to study the qualitative and quantitative changes induced in the different lipoprotein classes by a long-term diet consumed by a feminine institutionalized population. In an earlier study (5) we

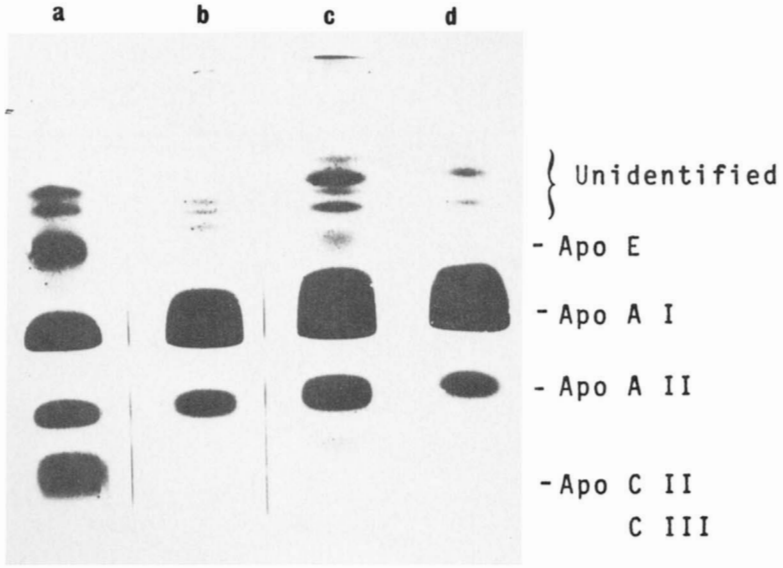

Fig. 1. SDS polyacrylamide gel electrophoresis of apoproteins of HDL from a pool of sera from ad libitum subjects, and from pools of sera from subjects after a milk fat diet. a, HDL from the pooled sera of ad libitum subjects; b, HDL from the pooled sera of group C after milk fat diet; c, HDL from the pooled sera of group H_1 after milk fat diet; d, HDL from the pooled sera of group H_2 after milk fat diet. About 50 μg of HDL delipidated with tetramethylurea was dissolved in phosphate buffer 0.01 M, pH 7, containing 1% SDS and then applied on 11% gels containing 0.05% SDS. The components of higher molecular weight are unidentified.

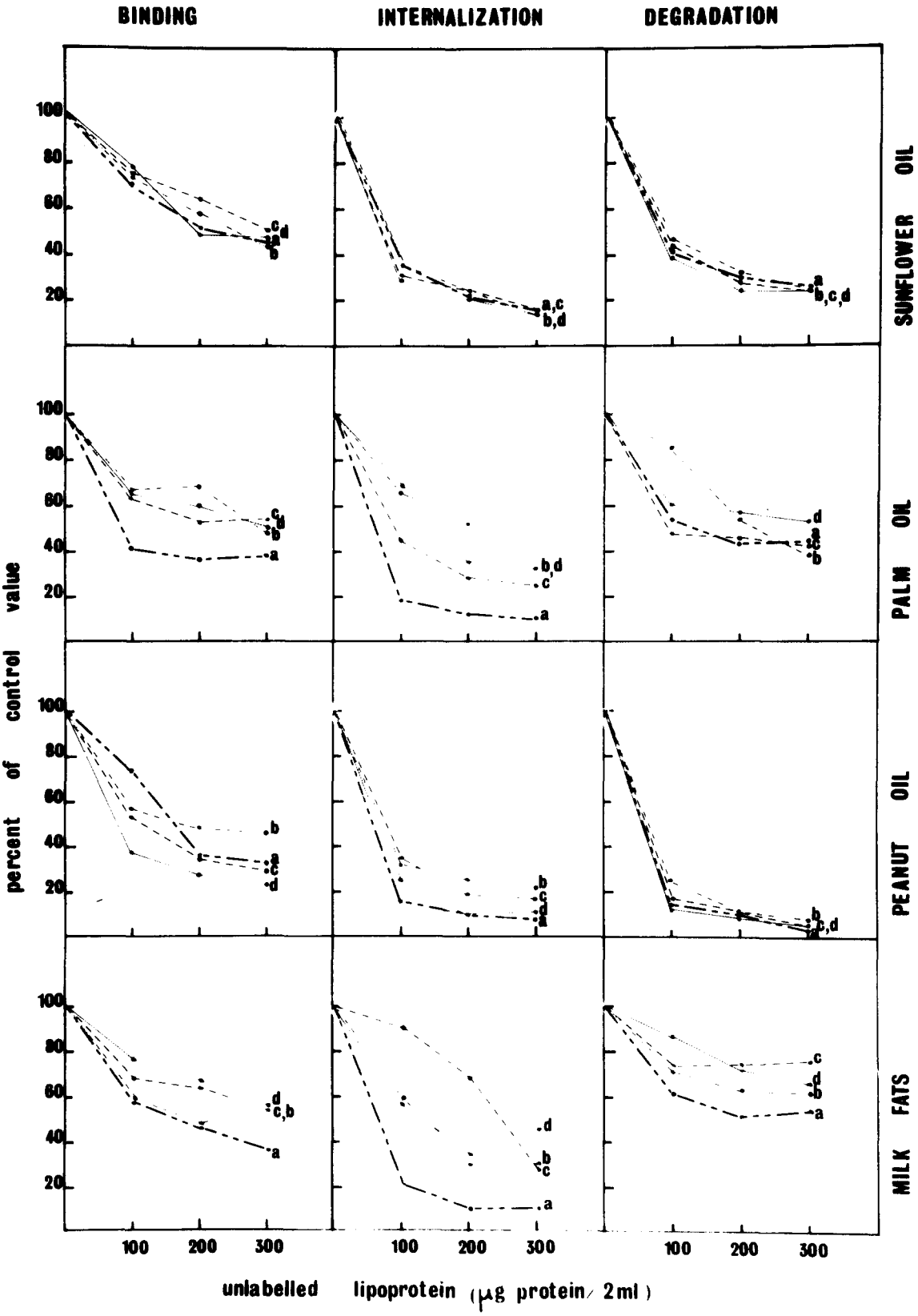

Fig. 2. Effect of the different LDL fractions on the binding, internalization, and protein degradation of ^{125}I-LDL by cultured fibroblasts. When cells were confluent, the medium was changed to medium containing LPDS (5% v/v) in place of fetal calf serum. After 48 hr, the medium was replaced by 2 ml of the same fresh medium containing 5 μg protein/ml ^{125}I-LDL (150–300 cpm/ng) plus the quoted quantities

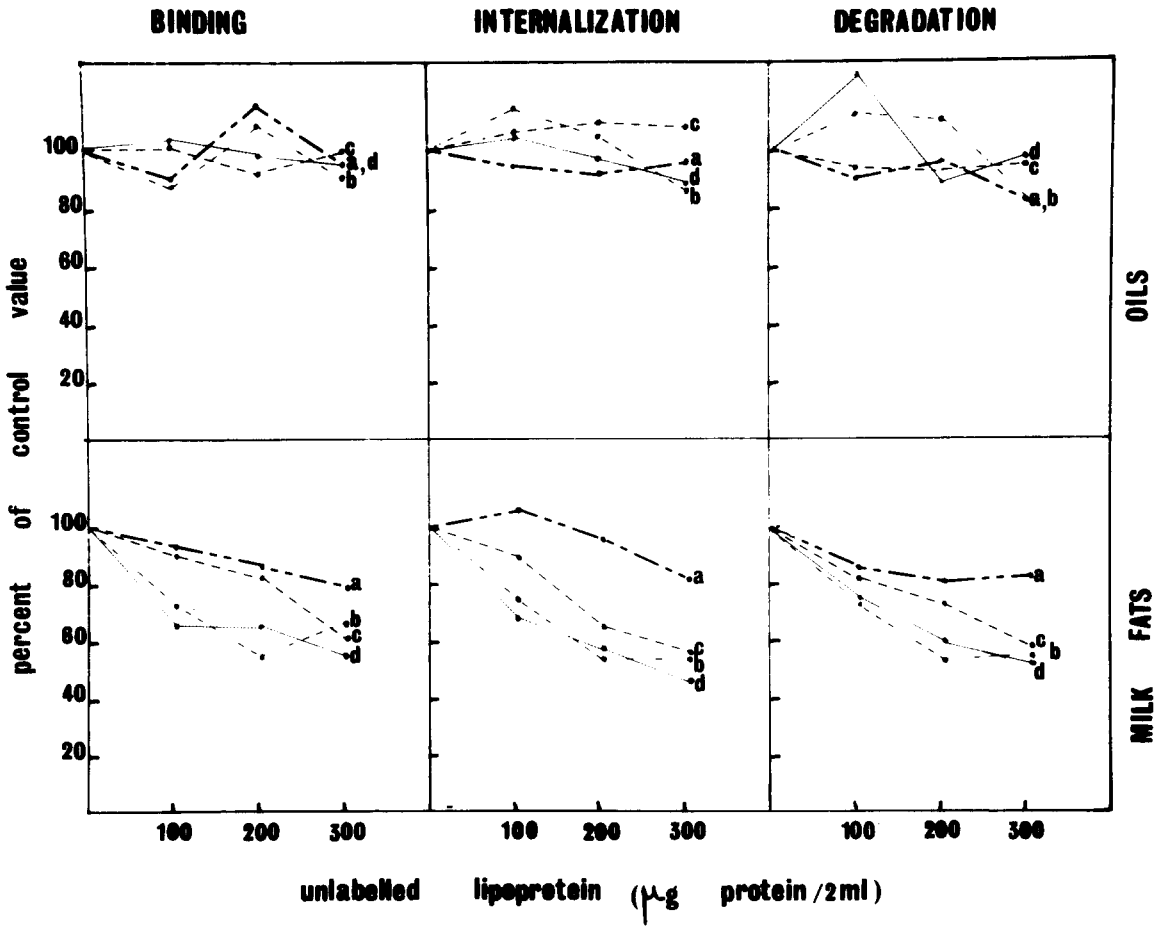

Fig. 3. Effect of the different HDL particles on the binding, internalization, and proteolytic degradation of the ^{125}I-LDL. When the fibroblasts were confluent, they were incubated for 48 hr in a 5% RPMI medium. This medium was then replaced with 2 ml of fresh medium containing 5 μg/ml of ^{125}I-LDL and different amounts of HDL were tested. a, HDL from the pool of ad libitum sera; b, HDL from the pool of group C sera; c, HDL from the pool of group H_1 sera; d, HDL from the pool of group H_2 sera. After 4 hr incubation at 37°C, the binding, internalization, and protein degradation were measured as described in Materials and Methods. The 100% value of the reaction represents the value obtained with only 5 μg/ml of ^{125}I-LDL in the medium. Each point corresponds to the average of three measurements. They varied by less than 5% of the mean values. The curves given for oils are those obtained with sunflower oil. The curves obtained with palm oil and peanut oil are superimposable to those obtained with sunflower oil.

had observed that after a short period a change in the P/S ratio of the ingested fats induced modifications in the composition of lipoproteins.

After a 5-month period on the diets, a comparison between the milk fat diet, very saturated fat diet, and the polyunsaturated diets revealed important differences in the serum concentrations of lipoproteins, as well as in their composition. Indeed, whereas we observed little or no modification in the concentration and composition of lipoproteins with the most polyunsaturated diets, a large increase in the serum level of all lipoproteins was obtained with the milk fat diet. This had been noted by numerous authors (1, 2, 4, 6, 7, 13, 30, 31, 33). We also observed a modification in the composition of lipoproteins after this diet. There was a large increase in serum VLDL due not only to an increase in the number of circulating particles, but also to an increase in the amount of apoprotein. Indeed the milk fat diet induced the appearance of VLDL particles having a higher protein-to-triglyceride ratio, because of an increase in the protein level and a decrease in the triglyceride level. This confirms what is known about the transport of saturated fats (32): they are carried

of the unlabeled tested LDL. a, LDL from the pool of ad libitum sera; b, LDL from the pool of group C sera; c, LDL from the pool of group H_1 sera; d, LDL from the pool of group H_2 sera. After incubation for 4 hr at 37°C, the binding, internalization, and protein degradation were measured as described in Materials and Methods. The 100% value of the reaction represents the value obtained in the absence of unlabeled LDL. All values are the average of triplicate incubations carried out at each LDL concentration. The variation is less than 5% of the mean value.

TABLE 10. Cellular metabolism of cholesterol in the presence of lipoproteins coming from the three groups of subjects on test diets

	[a]Synthesis of Intracellular Free Cholesterol N* = 38–54%	[b]Intracellular Esterification of the Cholesterol from LDL N* = 100%	[c]Removal of Cellular Free Cholesterol by HDL N* = 24–28%
Sunflower oil diet			
C	45		27
H_1	39		29
H_2	43		28
Palm oil diet			
C	70	50	27
H_1	70	207	32
H_2	66	111	33
Peanut oil diet			
C	39	62	23
H_1	48	111	27
H_2	53	119	27.5
Milk fat diet			
C	45	94	51
H_1	51	111	61
H_2	51	67	57

In all experiments, each value represents the average of three measurements; N* = the range value obtained from control lipoproteins.

[a] Synthesis of the intracellular free cholesterol from [^{14}C]acetate. The fibroblasts were incubated 24 hr in LPDS medium, then for 6 hr in the presence of the LDL fraction (2 μg/ml of LPDS medium). Forty μl of [^{14}C]acetate solution was then added (0.97 nM, 2800 cpm/nmol). After 20 hr incubation the cells were washed and dissolved in 2 ml of 0.1 N NaOH. The cellular proteins and the synthesized free cholesterol were determined as described in Materials and Methods.

[b] Intracellular esterification of LDL cholesterol. The fibroblasts were incubated for 24 hr in LPDS medium, then for 16 hr in the presence of the LDL fraction (20 μg/ml of LPSD medium). Forty μl of [^{14}C]oleate solution, prepared as described in Materials and Methods, was then added. AFter 2 hr incubation, the cells were washed and solubilized in 2 ml of 0.1 N NaOH. The cellular proteins and the amount of cholesteryl esters formed were determined as described in Materials and Methods.

[c] Removal of cellular cholesterol by HDL. After 48 hr incubation in ^{3}H-labeled RPMI fetal calf serum medium, the fibroblasts were washed; 2 ml of RPMI medium without fetal calf serum was then added to each Falcon flask with 250 μg/ml of the HDL fraction. After 24 hr incubation, the extra- and intracellular radioactivity was measured after extraction of the tritiated cholesterol as described in Materials and Methods.

on small particles having a high protein-to-triglyceride ratio, whereas unsaturated fats are carried on much larger particles with a low protein-to-triglyceride ratio. The concentration of LDL in the serum increased in spite of a marked decrease in the amount of apoprotein. Therefore there was an increase in the number of circulating particles enriched with cholesterol, mainly esterified. As for HDL, the increase in serum concentration was accompanied by a decrease in the amount of apoprotein. Therefore, there was also an increase in the number of these particles which were enriched with cholesterol and triglycerides.

The response of the three tested groups C, H_1, and H_2 to the different diets was qualitatively similar. Turner (7), who compared the influence of saturated and polyunsaturated diets in two groups of subjects with cholesterol levels either below or above 230 mg/dl, also found similar responses.

The modifications in the composition of lipoproteins induced by the milk fat diet, as compared to the three other diets, can be explained by the fact that the diets used in this study were very different from the diets generally used by other investigators (1, 4, 7, 30, 31). These investigators used diets having P/S ratios ranging between 0.2 and 0.25 for saturated diets, and between 4 and 8 for the polyunsaturated ones. The diets used in the present study included 30% calories as fat, 54% as carbohydrate, and 16% as protein, whereas the diets generally used by other authors included 40% as fat, 45% as carbohydrate, and 15% as protein.

The studies with fibroblast cultures show that the catabolism of LDL and HDL could change with the type of ingested fat. The catabolism of LDL which was normal with the sunflower oil diet, slightly decreased with the peanut oil diet, more so with the palm oil diet, and most markedly with milk fat diet. Thus, as suggested by several authors (3, 13, 34), modification of the structure, composition, and viscosity of LDL according to the degree of saturation of the diet alters its cellular metabolism. The palm oil diet could induce the appearance of LDL particles whose surface might be modified. This could explain the decrease in the binding, internalization, and degradation of LDL with this diet. Poor regulation of endogenous cholesterol synthesis would be the consequence of a decreased cholesterol supply by LDL.

St. Clair (35) has shown that, in monkeys fed hypercholesterolemic diets, the number of LDL particles bound on specific fibroblast receptors was reduced by half. These hypercholesterolemic diets induce formation of LDL particles enriched with esterified cholesterol, which are larger and consequently require more space on the cell surface. With the milk fat diet, we obtained LDL particles enriched with esterified cholesterol, which would explain the decreased binding, internalization, and protein degradation. The synthesis of endogenous cholesterol remained regulated, as the cholesterol supply was sufficient.

Moreover, with the milk fat diet, we observed that HDL reacted with cellular receptors that are specific for LDL. Mahley et al. (36) have shown that with some hypercholesterolemic diets, particles of HDL enriched with apoE appear. Studies with SDS-polyacrylamide gel electrophoresis do not allow us to measure the amount of this apoprotein in HDL with the milk fat diet.

With the milk fat and palm oil diets, we also observed an increase in the removal of cellular cholesterol by HDL. In the case of both diets, as also observed by Tan et al. (37) with a saturated diet, a marked increase in apoA-I was observed. Stein, Fainaru, and Stein (38) have shown

that apoA-I plays a very important role in the efflux of cholesterol. Thus we can think that the increase in apoA-I with the most polyunsaturated diets is responsible for the increased removal of cholesterol.

In conclusion, this study of the composition of lipoproteins and of their metabolism by normal cultured fibroblasts enables us to demonstrate important differences according to the nature of the ingested fat. However, the responses to the diets were the same for all subjects, with no measurable variation due to or predictable from their initial cholesterol levels.

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REFERENCES

1. Shepherd, J., C. J. Packard, S. M. Grundy, D. Yeshurun, A. M. Gotto, Jr., and O. D. Taunton. 1980. Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. *J. Lipid Res.* **21:** 91–99.
2. Schonfeld, G., W. Patsch, L. L. Rudel, C. Nelson, M. Epstein, and R. E. Olson. 1982. Effects of dietary cholesterol and fatty acids on plasma lipoproteins. *J. Clin. Invest.* **69:** 1072–1080.
3. Pownall, H. J., J. Shepherd, W. W. Mantulin, L. A. Skar, and A. M. Gotto, Jr. 1980. Effects of saturated and polyunsaturated fat diets on the composition and structure of human low density lipoproteins. *Atherosclerosis.* **36:** 299–314.
4. Shepherd, J., C. J. Packard, J. R. Patsch, A. M. Gotto, Jr., and O. D. Taunton. 1978. Effects of dietary polyunsaturated and saturated fats on the properties of high density lipoproteins and the metabolism of apolipoprotein A-I. *J. Clin. Invest.* **61:** 1582–1592.
5. Baudet, M. F., O. Esteva, B. Delplanque, N. Winchenne, and B. Jacotot. 1980. Effects of three dietary fats on plasma lipids and lipoproteins in fasting and postprandial humans after a short term diet. *Lipids* **15:** 216–223.
6. Spritz, N., and M. A. Mishkel. 1969. Effects of dietary fats on plasma lipids and lipoproteins: an hypothesis for the lipid-lowering effects of unsaturated fatty acids. *J. Clin. Invest.* **48:** 78–86.
7. Turner, J. D., N-A. Le, and W. V. Brown. 1981. Effect of changing dietary fat saturation on low-density lipoprotein metabolism in man. *Am. J. Physiol.* **241:** E57–63.
8. Engelberg, H. 1966. Mechanisms involved in the reduction of serum triglycerides in man upon adding unsaturated fats to the normal diet. *Metab. Clin. Exp.* **15:** 796–807.
9. Connor, W. E., D. T. Witiak, D. B. Stone, and M. L. Armstrong. 1969. Cholesterol balance and fecal neutral steroid and bile acid excretion in normal men fed dietary fats of different fatty acid composition. *J. Clin. Invest.* **48:** 1363–1375.
10. Gordon, D. T., R. E. Pitas, and R. G. Jensen. 1975. Effects of diet and type IIa hyperlipoproteinemia upon the structure of triacylglycerols and phosphatidylcholines from human plasma lipoproteins. *Lipids.* **10:** 270–283.
11. Bragdon, J. H., and A. Karmen. 1961. Effect of ingested fat on fatty acid composition of serum lipoproteins. *J. Lipid Res.* **2:** 400–402.
12. Skipski, V. P. 1972. Lipid composition of lipoproteins in normal and diseased states. *In* Blood Lipids and Lipoproteins: Quantification, Composition, and Metabolism. G. J. Nelson, editor. John Wiley & Sons, New York. 471–583.
13. Morrisett, J. D., H. J. Pownall, R. L. Jackson, R. Segura, A. M. Gotto, Jr., and O. D. Taunton. 1977. Effects of polyunsaturated and saturated fat diets on the chemical composition and thermotropic properties of human plasma lipoproteins. *In* Polyunsaturated Fatty Acids. R. T. Wolman and W. H. Kunan, editors. American Oil Chemists' Society monograph no. 4. A.O.C.S. Publishers, Champaign, IL. 139–161.
14. Thompson, G. R., A. Jadhav, M. Nava, and A. M. Gotto, Jr. 1976. Effects of intravenous phospholipid on low density lipoprotein turnover in man. *Eur. J. Clin. Invest.* **6:** 241–248.
15. Yeshurun, D., A. M. Gotto, and O. D. Taunton. 1976. Effect of polyunsaturated versus saturated fat on LDL metabolism in normal subjects. *Clin. Res.* **24:** 373 A (Abstract).
16. Jacotot, B., M. Lasserre, and M. F. Baudet. 1981. Note preliminaire sur l'enquête Jouarre. Effet des principales graisses alimentaires sur la lipidémie et les lipoprotéines dans une population institutionnalisée. *Cah. Nutr. Diét.* **16:** 119.
17. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1354.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
19. Van Gent, C. M., and O. J. Roseleur. 1974. Quantitative determination of phospholipids in blood, serum, or plasma by a nondestructive method. *Clin. Chim. Acta.* **57:** 197–205.
20. Weber, K., and M. Osborn. 1975. Protein and sodium dodecyl sulfate. Molecular weight determination on polyacrylamide gels and related procedures. *In* The Proteins. H. Neurath and R. L. Hill, editors. Academic Press, New York. 179–223.
21. MacFarlane, A. S. 1958. Efficient trace-labelling of proteins with iodine. *Nature.* **181:** 53.
22. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoproteins. I. Preliminary in vitro and in vivo observations. *Biophys. Biochim. Acta.* **260:** 212–221.
23. Baudet, M. F., C. Dachet, and J. L. Beaumont. 1978. In vitro interaction of LDL, antilipoprotein IgA and human fibroblasts. *Biomedicine.* **29:** 217–220.
24. Bierman, E. L., O. Stein, and Y. Stein. 1974. Lipoprotein uptake and metabolism by rat aortic smooth muscle cells in tissue culture. *Circ. Res.* **35:** 136–150.
25. Dachet, C., M. F. Baudet, and J. L. Beaumont. 1979. Cholesterol synthesis by human fibroblasts in the presence of LDL and anti-LDL IgA. *Biomedicine.* **31:** 80–82.
26. Van Harken, D. R., C. W. Dixon, and M. Heimberg. 1969.

Hepatic lipid metabolism in experimental diabetes. V. Effect of concentration of oleate on metabolism of triglycerides and on ketogenesis. *J. Biol. Chem.* **244:** 2278–2285.
27. Folch, J., I. Ascoli, M. Lees, J. A. Meath, and F. N. Le Baron. 1951. Preparation of lipid extracts from brain tissue. *J. Biol. Chem.* **191:** 833–841.
28. Stein, Y., M. C. Glangeaud, M. Fainaru, and O. Stein. 1975. The removal of cholesterol from aortic smooth muscle cells in culture and Landschutz ascites cells by fractions of human high-density apolipoprotein. *Biochim. Biophys. Acta.* **380:** 106–118.
29. Dole, V. P. 1955. A relation between nonesterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35:** 150–154.
30. Vega, G. L., E. Groszek, R. Wolf, and S. M. Grundy. 1982. Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins. *J. Lipid Res.* **23:** 811–822.
31. Kuksis, A., J. J. Myher, K. Geher, G. J. L. Jones, J. Shepherd, C. J. Packard, J. D. Morrisett, O. D. Taunton, and A. M. Gotto. 1982. Effect of saturated and unsaturated fat diets on lipid profiles of plasma lipoproteins. *Atherosclerosis.* **41:** 221–240.
32. Caselli, C., H. Carlier, and J. Bezard. 1979. Size of lipoprotein particles in the intestinal lymph of rats fed on corn oil, peanut oil, rapeseed oil or canbra oil. *Nutr. Metab.* **23:** 73–87.
33. Nicolosi, R. J., J. A. Marlett, A. M. Morello, S. A. Flanagan, and D. M. Hegsted. 1981. Influence of dietary unsaturated and saturated fats on the plasma lipoproteins of Mongolian gerbils. *Atherosclerosis.* **38:** 359–371.
34. Deckelbaum, R. J., G. G. Shipley, A. R. Tall, and D. M. Small. 1977. *In* Protides of the Biological Fluids. H. Peeters, editor. Pergamon Press, New York. 91–98.
35. St Clair, R. W., J. J. Mitschelen, and M. Leight. 1980. Metabolism by cells in culture of low-density lipoproteins of abnormal composition from nonhuman primate with diet-induced hypercholesterolemia. *Biochim. Biophys. Acta.* **618:** 63–79.
36. Mahley, R. W., R. W. Bersot, T. L. Innerarity, A. Lipson, and S. Margolis. 1978. Alterations in human high-density lipoproteins, with or without increased plasma cholesterol, induced by diets high in cholesterol. *Lancet.* **2:** 807–809.
37. Tan, M. H., M. A. Dickinson, J. J. Albers, R. J. Havel, M. C. Cheung, and J. L. Vigne. 1980. The effect of a high cholesterol and saturated fat diet on serum high-density lipoprotein-cholesterol, apoprotein A-I, and apoprotein E levels in normolipidemic humans. *Am. J. Clin. Nutr.* **33:** 2559–2565.
38. Stein, O., M. Fainaru, and Y. Stein. 1979. The role of lysophosphatidylcholine and apolipoprotein A-I in the cholesterol-removing capacity of lipoprotein-deficient serum in tissue culture. *Biochim. Biophys. Acta.* **574:** 495–504.