

# Increased plasma triglycerides, cholesterol and apolipoprotein E during prolonged fasting in normal subjects

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**Summary:** Plasma lipid and high density lipoprotein (HDL) levels were studied in 20 normal, healthy, non-obese males while fasting (150 kcal/d with free intake of water) for 6 d in a hunger strike. Plasma triglyceride and cholesterol levels were increased by 18% after 6 d of fasting. HDL-cholesterol concentration was not significantly changed for 4 d, but decreased by 22% after 6 d. Platelet aggregation induced by adenosine diphosphate (ADP) or collagen after 6 d of fasting was in the normal range. In 3 subjects fasted for 9 d, a complete plasma lipoprotein analysis was done. Very low and low density lipoprotein (VLDL and LDL) levels were elevated, whereas HDL was reduced after 9 d of fasting. On isoelectric focusing analysis, a marked reduction in apolipoprotein (apo) E concentration in both VLDL and HDL was noted. Liver function tests showed a reduction in hepatic enzyme activity; and since apo E is of hepatic origin also, we suggest that long fasting inhibits liver function in normal subjects.

## Introduction

The effect of fasting or low-calorie intake on plasma lipid levels is different in obese and non-obese subjects. In obese subjects, fasting results in a reduction in plasma triglyceride and cholesterol concentrations (Jackson, 1969; Sorbis *et al.*, 1981; Wolf & Grundy, 1983; Olefsky *et al.*, 1974; Zimmerman *et al.*, 1984; Yeshurun *et al.*, 1982). The results of high density lipoprotein (HDL)-cholesterol changes are inconsistent (Streja *et al.*, 1980; Wechsler *et al.*, 1981; Brownell & Stunkard, 1981). In normal non-obese subjects, however, fasting results in an elevation of plasma lipid levels (Stout *et al.*, 1976; Ende, 1962; Consolazio *et al.*, 1967; Fainaro & Schaffer, 1984). The changes in plasma lipids are reflected by similar changes in very low density and low density lipoprotein (VLDL and LDL) levels (Sorbis *et al.*, 1981; Wolf & Grundy, 1983; Zimmerman *et al.*, 1984; Yeshurun *et al.*, 1982; Streja *et al.*, 1980; Brownell & Stunkard, 1981; Fainaro & Schaffer, 1985), while the results for HDL levels after fasting in non-obese subjects are inconsistent (Zimmerman *et al.*, 1984; Yeshurun *et al.*, 1982; Streja *et al.*, 1980; Wechsler *et al.*, 1981; Brownell & Stunkard, 1981).

The present study was undertaken during a national physicians' hunger strike, in which they consumed 0–150 kcal/d derived from sweetened water for up to

nine days. We investigated plasma lipid and lipoprotein concentration and composition in 20 normal males during the fasting period. Since plasma lipoproteins affect platelet function (Aviram *et al.*, 1981; Aviram & Brook, 1982, 1983a, 1983b; Brook *et al.*, 1983), we also determined platelet aggregation after long fasting.

## Subjects and methods

Twenty male physicians starting a hunger strike volunteered for this study. All subjects were normolipidaemic, non-obese, healthy and taking no medications. Diabetes mellitus, thyroid, kidney and liver disease were excluded by appropriate laboratory tests. Insulin and thyroid hormones were found to be in the normal range both before and after fasting. All subjects were drinking plain water or sweetened water freely with a maximum of 150 kcal/d for six days, except for 3 subjects who continued fasting over 9 d. All subjects continued to attend to daily activities.

Blood was drawn at 08.00 h into disodium EDTA (1 mg/ml final concentration), and plasma was separated by centrifugation at  $2000 \times g$  for 10 min at 4°C. For platelet analysis, blood was drawn into 3.8% sodium citrate (v/v = 9/1). HDL-cholesterol concentration was analysed after precipitation of VLDL and LDL from the plasma by heparin and manganous chloride (Warnick & Albers, 1978).

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### Preparation of lipoproteins

Plasma lipoproteins were separated by discontinuous density gradient ultracentrifugation (Aviram, 1983). The density of 4 ml samples of plasma was raised to 1.250 g/ml with potassium bromide. Four ml of a solution of density 1.084 g/ml were then carefully laid over the plasma sample, followed by 4 ml of sodium chloride ( $\rho = 1.006$  g/ml). Ultracentrifugation was performed in an SW-41 rotor in a Beckman L2-65B preparative ultracentrifuge at  $35,000 \times g$  for 48 h at 4°C. Lipoproteins (VLDL, LDL and HDL) and lipoprotein-deficient plasma (LPDP) were then carefully removed and dialysed extensively against 150 mmol/l sodium chloride in 1 mmol/EDTA, pH 8.6. The purity of each fraction was analysed by cellulose acetate electrophoresis, Ouchterlony double immunodiffusion and immunoelectrophoresis for all the experiments. The lipoproteins were pure and free of other plasma proteins. Lipoprotein-protein concentration was determined using the procedure of Lowry *et al.* (1951). The cholesterol and triglyceride content of plasma and of each lipoprotein was determined by enzymatic methods (Zak, 1977; Ziegenhorn, 1975) on a centrifugal fast analyser (Electro-Nucleonics, Gemsac, Fairfield, New Jersey).

### Polyacrylamide gel isoelectric focusing (PAGE)

Plasma lipoproteins were delipidated using 4.2 mol/l of 1,1,3,3-tetramethylurea, and 100  $\mu$ g of protein was applied to each gel ( $8 \times 0.6$  cm). Each sample was treated with 50 mmol/l dithiothreitol, 0.05% Triton X-

100 and 5% sucrose solution (final concentration). Gels for PAGE were prepared with 6 mol/l urea, 7.5% acrylamide and 0.2% bisacrylamide, using 3% ampholine (Bio-Lyte 4/6 for HDL and 3/10 for VLDL, Bio-Rad Laboratories, Richmond, California), 0.06% ammonium persulphate and 0.006% tetramethylethylenediamine.

The anode solution was 0.01 mol/l phosphoric acid and the cathode solution, 0.2 mol/l sodium chloride. The sample (200  $\mu$ l) was applied onto the gel through the electrolyte solution, and PAGE was performed at 250 V for 18 h at 4°C (Warnick *et al.*, 1979). To determine the pH gradient, 2 mm segments of unfixed and unstained gels were transferred into a glass tube containing 2 ml of distilled water, and the pH of each segment was determined after 24 h at room temperature. The gels were simultaneously fixed and stained for 2 h at 45°C with 0.25% Coomassie blue G in 50% methanol and 10% acetic acid. Destaining was performed using volumes of acetic acid:methanol:water of 1:3:5, respectively.

### Rocket electro-immunophoresis of plasma apolipoproteins

Plasma and lipoprotein levels of apolipoproteins B and A-I were determined by rocket electro-immunoassay, adapting the technique of Laurell (1966). Appropriate standards for plasma and lipoproteins were included in each run.

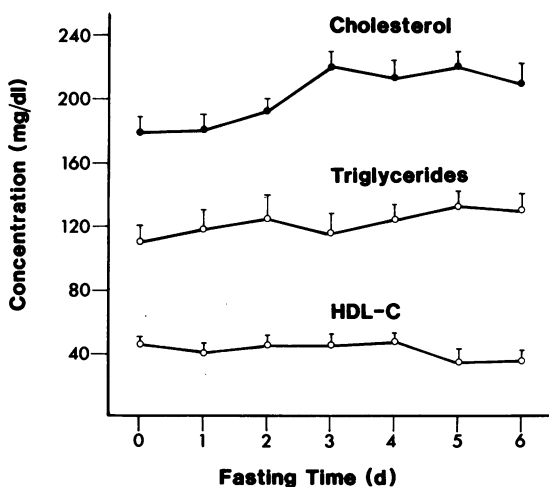
### Platelet aggregation

Platelet-rich plasma (PRP) was prepared from citrated whole blood by low-speed centrifugation at  $200 \times g$  for 10 min at 23°C. Platelet aggregation was studied in a dual aggregometer (Chrono-Log Corporation, Havertown, Pennsylvania) according to the method of Born (1969). Adenosine diphosphate (ADP; 5  $\mu$ mol/l) and collagen (1  $\mu$ g/ml) were used as the aggregating agents. The per cent transmittance of PRP was recorded as 0 and that of its appropriate blank (platelet-poor plasma) as 100. Results of platelet aggregation were expressed as the percentage of aggregation amplitude.

### Results

Plasma cholesterol and triglyceride concentrations were found to increase during fasting by 18% after 6 d (Figure 1). The main increment in plasma cholesterol was between the first and third days of fasting, whereas plasma triglyceride concentration was elevated along the fasting period.

HDL-cholesterol did not change significantly for 4 d and then dropped by 22% on days 5 and 6 of fasting (Figure 1).



**Figure 1** Time-course study of plasma cholesterol, triglyceride, and HDL-cholesterol concentration during fasting in normal, healthy subjects. Each point represents mean  $\pm$  s.e.mean of 20 subjects. HDL-C = high density lipoprotein-cholesterol.

**Table I** Effect of long fasting on plasma lipoprotein concentration in three normal, healthy, non-obese male subjects

Subject Plasma VLDL LDL HDL					
Cholesterol (mg/dl)					
Basal	1	178	20	101	57
	2	168	23	100	45
	3	169	22	102	45
Fasted	1	200	38	113	49
	2	195	39	111	37
	3	197	38	127	32
Triglycerides (mg/dl)					
Basal	1	108	70	21	17
	2	109	67	27	15
	3	103	60	29	14
Fasted	1	129	83	33	13
	2	133	81	41	11
	3	131	74	47	10
Protein (mg/dl)					
Basal	1		17	86	162
	2		20	80	190
	3		21	73	216
Fasted	1		21	91	148
	2		23	90	164
	3		27	81	152

Results are shown on day 1 (basal) and day 9 (fasted) of fast.

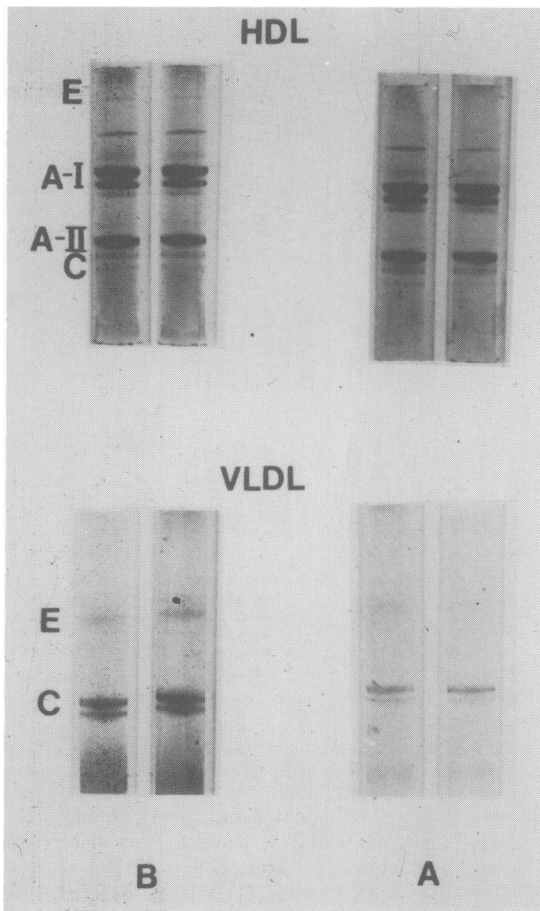
Three subjects continued fasting for 9 d, and we analysed their plasma lipoprotein pattern after 1 and 9 days of fasting. Table 1 shows that the elevation in both plasma cholesterol and triglyceride levels was the result of increased levels of VLDL and LDL. Similar elevation was found for VLDL and LDL protein concentration. HDL levels, on the other hand, markedly reduced after 9 d of fasting (Table I). Analysis of the apolipoproteins from HDL and VLDL revealed that after 9 d of fasting there was a marked reduction in the apolipoprotein (apo) E concentration in both lipoproteins in comparison to the situation before beginning the fast (Figure 2). Plasma apo A-I reduced in the three subjects by 18, 10 and 17%, whereas plasma apo B concentration increased by 21, 39 and 17% after 9 d of fasting.

Analysis of compliance to fasting revealed a significant reduction in body weight, with elevated uric acid concentration (Table II). The levels of the liver enzymes glutamic-oxaloacetic transaminase (GOT) and alkaline phosphatase, however, decreased during the fasting. No significant changes were observed in blood electrolyte, urea, glucose and creatinine concentrations. Platelet aggregation induced by ADP (5 mmol/l) and collagen (1 µg/ml) after 6 d of fasting was 48 ± 6 and 77 ± 9%, respectively, which is in the normal range as found before fasting (42 ± 7 and 72 ± 10%, respectively, n = 10).

**Table II** Compliance to fasting of 20 subjects studied

Fasting days	Weight kg	Glucose mmol/l	BUN mmol/l	Creatinine mmol/l	Uric acid mg/dl	Sodium mmol/l	Potassium mmol/l	OSM mOsm/kg water	SGOT U/ml	AP U/ml
0	80.3 ± 1.8	4.81 ± 0.19	3.92 ± 0.60	93.70 ± 6.19	7.2 ± 0.2	137 ± 0.4	4.3 ± 0.07	271 ± 1.2	17.6 ± 1.7	175.2 ± 7.4
2	78.9 ± 1.8*	4.63 ± 0.20	4.14 ± 0.71	86.63 ± 3.54	7.9 ± 0.03*	136 ± 0.6	4.1 ± 0.07	272 ± 1.7	16.5 ± 1.8	174.2 ± 7.0
4	77.5 ± 2.0*	4.51 ± 0.22	3.71 ± 0.78	91.05 ± 2.65	8.4 ± 0.5*	137 ± 0.6	3.9 ± 0.09	266 ± 0.8	15.7 ± 1.9*	146.5 ± 6.2*
6	77.1 ± 2.1*	4.49 ± 0.17	3.49 ± 0.96	84.86 ± 3.54	8.7 ± 0.3*	136 ± 0.7	3.9 ± 0.9	272 ± 1.7	14.1 ± 1.6*	149.7 ± 7.4*

BUN = blood urea nitrogen, OSM = osmolarity, SGOT = serum glutamic-oxaloacetic transaminase; AP = alkaline phosphatase. \* P < 0.01 (vs zero time).



**Figure 2** Polacrylamide gel isoelectric focusing analysis of HDL and VLDL apolipoproteins. Seventy-five  $\mu\text{g}$  of lipoprotein-protein were loaded onto each gel. Lipoproteins from 2 subjects are shown. B = before fasting (after 12 h of fast); A = after fasting (9 days of fast); E, A-I, A-II, and C = apolipoproteins.

## Discussion

This study demonstrates that fasting for up to 9 d induces increments in plasma triglyceride and cholesterol concentrations, as well as enhanced levels of VLDL and LDL, in normal, non-obese, healthy male subjects (Figure 1 and Table I). This is in contrast to the hypolipidaemic effect observed in obese subjects (Jackson, 1969; Sorbis *et al.*, 1981; Wolf & Grundy, 1983; Olefsky *et al.*, 1974; Zimmerman *et al.*, 1984; Yeshurun *et al.*, 1982).

A similar pattern of plasma triglyceride elevation in non-obese subjects has been observed by others, where it was followed by an increased availability of fatty acids to the liver, decreased plasma insulin, increased

plasma glucagon and peripheral insulin resistance (Fainaro & Schaffer, 1984; Rubin & Aladjem, 1954; Newman & Brodows, 1983). Increased free fatty acid availability to the liver results in increased VLDL synthesis, and thus all VLDL components increased after fasting (Table I). The increased plasma cholesterol level after fasting is a result of elevation in both VLDL and LDL concentrations. Increased cholesterol biosynthesis upon fasting or cholesterol release from cell breakdown (Swaner & Connor, 1975; Dietchy & Gamel, 1971) may thus contribute to the observed plasma cholesterol elevation (Figure 1).

HDL-cholesterol concentration was unchanged 4 d into the fast, but dropped on the fifth day (Figure 1). These results are in accordance with those found during a 10 d modified fast in man (Wallentin & Sköldstam, 1980).

The increase in apo B and the reduction in apo A-I levels in plasma correlate with the changes observed for LDL- and HDL-cholesterol, respectively. The normal values obtained for platelet aggregation after 6 d of fasting suggest that fasting does not affect platelet function and that the relatively small changes in plasma lipid levels cannot affect platelet aggregation.

A marked reduction in VLDL and HDL apo E was found after 9 d of fasting (Figure 2). Since apo E is mainly of liver origin (Schaefer *et al.*, 1978), these results may represent changes in the lipoproteins induced by reduction in some of the liver's activities. Our observation (Table II) of decreased alkaline phosphatase as well as of GOT (which are enzymes of hepatic origin) supports this hypothesis of reduced liver function during fasting in normal subjects.

The elevated plasma lipid levels in non-obese fasted subjects, as opposed to the reduced plasma lipid levels in obese fasted patients, are probably a result of the difference in their tissue lipid content. It might be that in obese normo-lipidaemic subjects the mobilization rate of free fatty acids is lower than in non-obese normo-lipidaemic subjects. Thus, it is suggested that the increased free fatty acid availability to the circulation in the non-obese subjects contributes to enhanced VLDL synthesis and elevated plasma triglyceride levels. In obese subjects, however, reduced free fatty acid mobilization may result in its plasma VLDL utilization and, hence, reduced plasma triglyceride concentration.

The increased plasma apo B levels are probably related to the fact that LDL catabolism is significantly decreased, as has been shown in fasted rabbits (Klauda & Zilversmit, 1978). In fasted dogs, however, the apo B and apo E receptors have been induced by prolonged fasting (Mahley *et al.*, 1981), and in fasted rats liver triglyceride accumulation had been observed when perfused with apo E-containing triglyceride emulsions (Quarfordt *et al.*, 1981). It might be that the resistance

of these animals to atherosclerosis in comparison to rabbits and humans is related to their ability to metabolize the LDL molecules.

In anorexia nervosa, hypercholesterolaemia resulting from elevated plasma LDL levels has also been found (Mordasini *et al.*, 1978), and this probably partially resulted from a hypocaloric metabolic state.

Our study demonstrated plasma lipid elevation after fasting of normal, healthy, non-obese subjects. This resulted from increased plasma concentration of both the triglyceride and the cholesterol carrier lipoproteins.

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