Lipoprotein structure in male subjects during in vivo lipolysis: effect of an anti-lipolytic treatment with acipimox

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Abstract Plasma free fatty acid (FFA) levels were raised in healthy volunteers by the administration of a fatty meal and epinephrine infusion (0.15 mg/kg per min), to test the hypothesis that enhanced lipolysis might lead to changes in lipoprotein distribution and to the formation of lipoprotein complexes, also impairing the interconversion of high density lipoproteins (HDL). The study was carried out in double-blind conditions in volunteers pre-treated with either placebo or with acipimox, a nicotinic acid analogue with a long-lasting activity. Lipolysis was effectively induced; the treatment with acipimox prevented the rise of free fatty acids (FFA), and it also blunted the triglyceride (TG) increase occurring during the test. Whereas the mean low density lipoprotein (LDL) particle size did not change, the HDL particle distribution showed a progressive shift to smaller particles, both after placebo and after acipimox, the changes in size being maximal 3–7 h after the meal. Evaluation of HDL interconversion in plasma samples incubated at 37°C for 6 h showed the expected accumulation of HDL_{2a} particles, with a parallel decrease of HDL_{3a}; however, this conversion was not affected by the presence of elevated FFA levels and no difference was noted in subjects taking either placebo or acipimox. These clinical data fail to confirm the hypothesis that enhanced lipolysis may lead to dramatic changes in plasma lipoprotein distribution and/or in aggregation or fusion of lipoprotein particles, as reported from in vitro experiments. This study, however, successfully achieved a useful model of exaggerated lipolysis and confirmed the important activity of a low dose nicotinic acid analogue in inhibiting lipolysis.—**Pazzucconi, F., G. Franceschini, G. Gianfranceschi, E. Brambilla, and C. R. Sirtori.** Lipoprotein structure in male subjects during in vivo lipolysis: effect of an anti-lipolytic treatment with acipimox. *J. Lipid Res.* 1993. **34:** 1465–1472.

Supplementary key words lipolysis • epinephrine • fatty meal • lipoprotein conversion

The toxic effect of elevated levels of free fatty acids (FFA) in the circulation, be they due to their detergent activity and/or to specific alterations in membrane permeability (1), are well known. In particular, elevated FFA levels may alter intermediary metabolism and cell levels of high energy phosphates in muscular tissues, such as the heart (resulting in arrhythmias) (2, 3). Less well known are the effects of elevated FFA levels on lipid/lipoprotein metabolism; FFA may, in fact, act as triglyceride (TG) precursors, thus favoring hypertriglyceridemia (4).

Recently the possibility of a direct interaction of circulating FFA with lipoproteins, promoting the formation of complexes between lipoproteins themselves (5) as well as impairing the activity of transport proteins, in particular the cholesteryl ester transfer protein (CETP), has been suggested (6). In vitro incubations of very low and low density lipoproteins (VLDL and LDL) with lipoprotein lipase, inducing elevated FFA in the environment, may result in aggregation or fusion of lipoprotein particles (5). This phenomenon is prevented by the addition of albumin, thus underlining the importance of the accumulation of lipolytic products for the formation of the lipoprotein complexes (5), and also by the addition of high density lipoproteins (HDL), either whole or delipidated, to the system (7). HDL themselves, however, are as a consequence markedly modified, with increased particle diameter, buoyancy, and relative glycerol content (7).

Albumin, in the presence of the low FFA concentration usually found in normolipidemic individuals, should be able to fully prevent this complexing phenomenon (5). However, during fasting or in pathological conditions, e.g., uncontrolled diabetes or severe hypertriglyceridemia, FFA levels may be exceedingly elevated (8), so as not to allow this preventive effect to occur in vivo (5).

Acipimox, a synthetic lipid-lowering drug chemically related to nicotinic acid (NA), has shown a significant

Abbreviations: CETP, cholesteryl ester transfer protein; FFA, free fatty acid; GGE, gradient gel electrophoresis; HDL, high density lipoprotein; LDL, low density lipoprotein; NA, nicotinic acid; TC, total cholesterol; TG, triglyceride; TGPLP, TG-rich lipoproteins; VLDL, very low density lipoprotein; AUC, area under the curve.

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hypotriglyceridemic activity (9), associated with a good tolerance. The mechanism of action of acipimox is similar to that of NA; it can effectively inhibit hormone-stimulated lipolysis, even after submaximal stimuli. In vitro studies on human adipose tissue in the presence of acipimox have shown a marked inhibition of FFA and glycerol release in the medium, after isoprenaline (10, 11); cyclic AMP levels in adipocytes are concomitantly reduced (11). In vivo acipimox is about 20 times more potent that NA against fasting-induced lipolysis (11, 12). Moreover, whereas a single dose of NA can inhibit FFA release from the adipose tissue for only 90 min, followed by a marked rebound, under the same conditions acipimox inhibits FFA release for 5 h or more, without a significant rebound (12). This different dynamic behavior may be related to the lack of significant metabolism of the drug versus the considerable presystemic transformation of NA (13).

In view of the current interest in understanding the mechanisms of lipolysis-induced lipoprotein changes in man, lipolysis was promoted in healthy volunteers by concomitant administration of a fatty meal + short epinephrine infusion, or by prolonged epinephrine infusion without a fatty meal. In the former experimental protocol, the activity of acipimox was tested under double-blind conditions.

METHODS

Volunteers and study design

The study was carried out according to a double-blind cross-over protocol, in eight healthy male volunteers ages 21 to 30 years. Each subject received detailed information on the study and on the drug and signed a consent form according to the declarations of Helsinki. The study was approved by the Hospital Internal Review Board. Each volunteer underwent a complete clinical examination, with a standard battery of hemato-chemical tests. All subjects were nonsmokers and none were following any chronic drug treatment. All were moderate consumers of wine (< 250 ml/day) and were asked to refrain from wine intake during the 2 days of the test.

The protocol required administration of a tablet either of a sustained release formulation of acipimox (500 mg) or of placebo at 8 AM and 8 PM, the day preceding the experiment, and at 8 AM the day of the lipolysis test. Subjects were randomized according to the chronological admission to the study and received either active drug or placebo in a sequence drawn from a table of random numbers. Each subject underwent two tests 4 weeks apart, the alternate treatment being given in the second test.

The day of the experiment, at 8 AM, each volunteer ingested the last dose of the drug in the presence of the investigator, together with fresh cream. The amount of fat administration to each individual was calculated from the body surface and corresponded to 60 g lipid/m^2. This type of fat load, in our hands, constantly leads to a near doubling of triglyceride levels (14). Two h after the fatty meal (T = 0 h) the volunteers underwent a slow infusion of epinephrine (0.15 μg/kg per min) for 15 min (15), with constant electrocardiographic and blood pressure monitoring. In three volunteers, an additional experiment was performed, with a prolonged (60 min) infusion of epinephrine (0.04 μg/kg per min), without a fatty meal.

In both protocols, blood samples were collected at times −2 h, 0 h (start of the infusion), 5 min, 10 min, 15 min (end of the infusion), 30 min, 45 min, 60 min, 90 min, 2 h, 3 h, 5 h, 7 h, and 9 h. Samples were drawn in EDTA (1 mg/ml)-containing tubes and kept on ice, and plasma was separated by low speed centrifugation at 4°C.

Laboratory methods

Plasma concentrations of FFA (16), total cholesterol (TC) (17), and TG (18) were determined by enzymatic methods; HDL-cholesterol (HDL-C) was measured after the selective precipitation of apoB-containing lipoproteins by dextran-$MgCl_2$ (19).

In samples collected at times −2 h, 0 h, 15 min, 30 min, 60 min, 3 h, 5 h, and 9 h, TGRLP (d < 1.006 g/ml) and LDL (d 1.006–1.063 g/ml) were separated by preparative ultracentrifugation in a Beckman TL-100 centrifuge equipped with a TL 100.3 rotor (20). TC contents were determined in both fractions. At the same time intervals the d <1.21 g/ml ultracentrifugal fraction was isolated for particle size evaluation; lipoprotein particles were then separated by nondenaturing polyacrylamide gradient gel electrophoresis (GGE) using either 2–16% (LDL) or 4–30% (HDL) gradient gels (21).

The gels were scanned with an LKB Ultroscan XL laser densitometer and particle size was calculated with the LKB 2400 Gelscan XL software using calibrated latex spheres (38.0 nm in diameter), thyroglobulin, apoferritin, lactate dehydrogenase, and bovine serum albumin as standards. A single major LDL subpopulation was detected in each sample, and the corresponding particle diameter was calculated as previously described (21). Five HDL subpopulations were identified (21): HDL_{2b}: 9.7–12.9 nm; HDL_{2a}: 8.8–9.7 nm; HDL_{3a}: 8.2–8.8 nm; HDL_{3b}: 7.8–8.2 nm; and HDL_{3c}: 7.2–7.8 nm. To calculate the percentage distribution of HDL subpopulations, areas under the scanning curves were integrated by dropping vertical lines corresponding to subpopulation size limits. We then assigned each sample an "HDL particle score," i.e., the sum of values calculated by multiplying the area of each subpopulation by the band number (5 for HDL_{2b}, 4 for HDL_{2a}, 3 for HDL_{3a}, 2 for HDL_{3b}, 1 for HDL_{3c}); a large score thus represents a particle distribution shifted toward larger sizes (22).

Statistical analyses

The differences between or within experiments were tested for statistical significance by one-way ANOVA, or by a Bonferroni "t" test, with similar results; a probability value of less than 0.05 (two-tailed) was considered as significant. When specified, the TG levels during the experiment were normalized to the baseline level by dividing each subsequent value by the fasting level. The areas under the curve (AUCs), described by the normalized TG concentrations plotted against time, were calculated according to the trapezoidal rule, by connecting data points with the line originating from the baseline value, parallel to the "X" axis. The obtained values were tested for statistical significance between experiments by one-way ANOVA.

RESULTS

The monitoring of blood pressure and heart rate during epinephrine infusion reflected the expected cardiovascular modifications, with no difference between the two treatments. Drug administration was not associated with any specific clinical symptoms (flushing, nausea, etc).

Highly significant differences were noted in the behavior of FFA between the placebo and post-acipimox tests (**Table 1** and **Table 2**). After placebo an early FFA peak was evident immediately after the start of the short epinephrine infusion, gradually waning within 45–60 min (Table 1, **Fig. 1**). After acipimox there was essentially no rise of plasma FFA and differences at all time intervals, from 5 min up to 45 min (i.e., 30 min after the end of the infusion) were significant (Fig. 1, Table 2). The FFA-AUC between 0 and 90 min was significantly lower during acipimox versus placebo (37,366 ± 3,493 μEq/l • 90 min vs. 64,460 ± 7,021 μEq/l • 90 min). At about 90 min from the start of the infusion there was a second shallow peak of FFA, with no difference between the two treatments, both in absolute values and AUCs. The rise of plasma FFA after a prolonged epinephrine infusion was similar to that achieved by fatty meal + short infusion, but elevated FFA levels were maintained for a longer period (up to 60 min) (**Fig. 2**).

TG concentrations rose twice after fat mal + short epinephrine infusion (**Fig. 3**). The first peak, i.e., at 15 min, was essentially unaffected by treatment (placebo: +19%; acipimox: +15%). Interestingly, however, the TG rise observed after about 1 h from the start of the epinephrine infusion was significantly depressed by acipimox. In fact, after placebo, TG levels remained elevated (with a maximum of 185.0 ± 82.5 mg/dl) for about 6 h. With acipimox, this second peak reached a maximum of 145.8 ± 58.2 mg/dl and the return to baseline was faster (Fig. 3). In view of the different baseline TG levels in experiments with/without acipimox, the individual data were adjusted to account for the lower fasting TG levels after acipimox, so that the two groups began the test with identical fasting triglyceridemia (see Methods). Again, after this normalization, the second rise of plasma TG levels (i.e., that occurring after 90 min from the start of infusion) was significantly blunted by acipimox, as demonstrated by the lower TG-AUCs in the 180–420 min time interval (268 ± 24 AU vs. 315 ± 27 AU); the first (0–45 min) TG peak was instead nearly identical after the two treatments (49.0 ± 2.7 AU vs. 51.5 ± 2.8 AU).

TABLE 1. Plasma lipid and lipoprotein levels in eight volunteers during the placebo treatment

	Plasma			TGRLP		LDL	HDL
Time	Cholesterol	Triglycerides	FFA	Cholesterol	Triglycerides	Cholesterol	Cholesterol
	mg/dl	*mg/dl*	*μEq/l*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
−2 h	184.3 ± 11.7	103.6 ± 12.0	291.4 ± 44.2	21.1 ± 3.3	56.3 ± 11.3	117.1 ± 10.8	45.8 ± 2.9
0 h	171.3 ± 12.3	132.3 ± 23.7	412.3 ± 42.5	18.1 ± 2.9	80.4 ± 17.7	111.3 ± 11.7	41.8 ± 2.8
5 min	161.5 ± 11.8	134.3 ± 22.0	542.1 ± 30.1[a]				39.8 ± 3.1
10 min	162.4 ± 12.3	143.6 ± 23.5	805.0 ± 81.0[a]				39.0 ± 2.9
15 min	170.6 ± 12.4	157.8 ± 24.0	1085.4 ± 119.3[a]	18.5 ± 3.4	116.4 ± 15.5	110.0 ± 11.2	40.9 ± 3.1
30 min	169.3 ± 12.0	153.9 ± 23.2	1134.6 ± 164.7[a]	17.1 ± 2.5	112.6 ± 14.6	111.3 ± 10.8	40.6 ± 3.1
45 min	162.3 ± 11.3	136.4 ± 23.8	682.4 ± 99.3[a]				39.6 ± 3.7
60 min	176.0 ± 13.4	140.6 ± 24.9	561.6 ± 85.2	18.8 ± 3.5	97.5 ± 21.2	116.0 ± 11.3	41.5 ± 2.8
90 min	162.5 ± 11.5	150.3 ± 23.7	415.3 ± 44.1				39.1 ± 3.1
2 h	166.1 ± 10.4	155.4 ± 22.0	547.9 ± 65.5				39.8 ± 3.1
3 h	176.9 ± 11.7	185.0 ± 29.2[a]	622.6 ± 61.1	22.8 ± 3.4	128.3 ± 20.6	113.5 ± 10.1	40.5 ± 3.1
5 h	182.9 ± 14.0	173.0 ± 26.5[a]	675.3 ± 103.1	22.6 ± 3.6	114.3 ± 18.2[a]	115.6 ± 11.7	44.0 ± 2.9
7 h	188.5 ± 13.5	128.9 ± 24.3	597.6 ± 61.0	18.5 ± 4.1	76.1 ± 15.9[a]	122.5 ± 9.6	45.5 ± 3.0
9 h	186.4 ± 12.7	83.9 ± 12.6	576.1 ± 62.6	13.1 ± 2.8	37.8 ± 6.3	127.0 ± 9.7	46.3 ± 3.4

Data are expressed as mean ± SEM.
[a]Statistically different from 0 h.

TABLE 2. Plasma lipid and lipoprotein levels in eight volunteers during acipimox treatment

Time	Plasma			TGRLP		LDL	HDL
	Cholesterol	Triglycerides	FFA	Cholesterol	Triglycerides	Cholesterol	Cholesterol
	mg/dl	*mg/dl*	*μEq/l*	mg/dl	mg/dl	mg/dl	mg/dl
-2 h	185.8 ± 16.0	70.9 ± 7.9	266.2 ± 74.0	16.8 ± 3.3	39.0 ± 3.5	123.4 ± 14.8	46.8 ± 2.6
0 hr	164.8 ± 15.7	102.1 ± 12.2	342.3 ± 56.9	13.0 ± 1.7	44.3 ± 5.0	109.1 ± 13.9	42.6 ± 3.6
5 min	155.6 ± 14.0	101.3 ± 13.5	373.9 ± 63.9				40.4 ± 3.4
10 min	162.3 ± 17.1	122.6 ± 14.6	433.4 ± 68.5				40.4 ± 3.3
15 min	171.0 ± 19.3	117.9 ± 13.7	510.6 ± 63.6	14.1 ± 3.4	72.9 ± 8.6	114.5 ± 16.9	42.9 ± 3.5
30 min	164.4 ± 16.7	113.6 ± 13.8	464.6 ± 48.3	14.0 ± 1.7	72.3 ± 8.4	109.6 ± 42.9	39.6 ± 3.4
45 min	153.5 ± 14.9	102.0 ± 13.4	370.8 ± 41.1				38.6 ± 3.3
60 min	163.9 ± 17.0	112.3 ± 12.2	357.9 ± 26.9	13.9 ± 1.8	63.6 ± 8.2	109.4 ± 42.3	40.8 ± 3.4
90 min	163.3 ± 15.9	123.1 ± 14.1	461.1 ± 51.7				40.8 ± 3.9
2 h	169.0 ± 16.8	131.0 ± 18.9	584.4 ± 58.6				38.9 ± 3.6
3 h	162.4 ± 16.2	145.8 ± 20.6	765.6 ± 77.5	16.0 ± 2.2	89.0 ± 10.8[a]	106.0 ± 15.2	40.3 ± 3.6
5 h	175.8 ± 16.4	109.5 ± 17.9	572.4 ± 98.0	14.8 ± 2.4	58.9 ± 10.0	115.5 ± 15.0	44.9 ± 3.8
7 h	177.8 ± 17.7	77.1 ± 13.1	559.9 ± 108.0	10.6 ± 1.8	44.3 ± 9.2	117.9 ± 13.3	47.4 ± 4.2
9 h	172.5 ± 14.4	55.3 ± 7.5	651.4 ± 234.7	6.9 ± 0.8	28.0 ± 3.5	115.4 ± 13.1	47.9 ± 3.6

Data are expressed as mean ± SEM.
[a]Statistically different from 0 h.

The cholesterol and TG concentrations in the TGRLP fraction followed in part the behavior of plasma TG (Tables 1, 2). In this case, however, only one peak, corresponding to the second, wider plasma TG peak, was noted. Again, the two treatments showed a clearly different activity versus the TGRLP peak, with a far lesser rise of TGRLP-C and TGRLP-TG after acipimox (Tables 1, 2). No significant differences were noted for other study parameters: TC, HDL-C, and LDL-C (Tables 1, 2) were minimally influenced by the meal + epinephrine-induced lipolysis.

The distribution of LDL and HDL particles was modified to a minor extent in all tested experimental conditions: epinephrine + fatty meal with/without acipimox pretreatment and also after prolonged epinephrine infusion. In none of these were lipoprotein complexes larger than LDL evident; moreover, the mean LDL particle size (**Table 3**), as well as the height and shape of the LDL peak, did not change. In contrast, the HDL particle size distribution showed a progressive shift towards smaller particles after the fatty mean + epinephrine (**Table 4**), as also indicated by the significant decrease of the HDL particle score, a number expressing the whole distribution of HDL particles (22). The formation of small HDL was detected both after placebo and acipimox, but not after prolonged epinephrine infusion without a fatty meal, thus in-

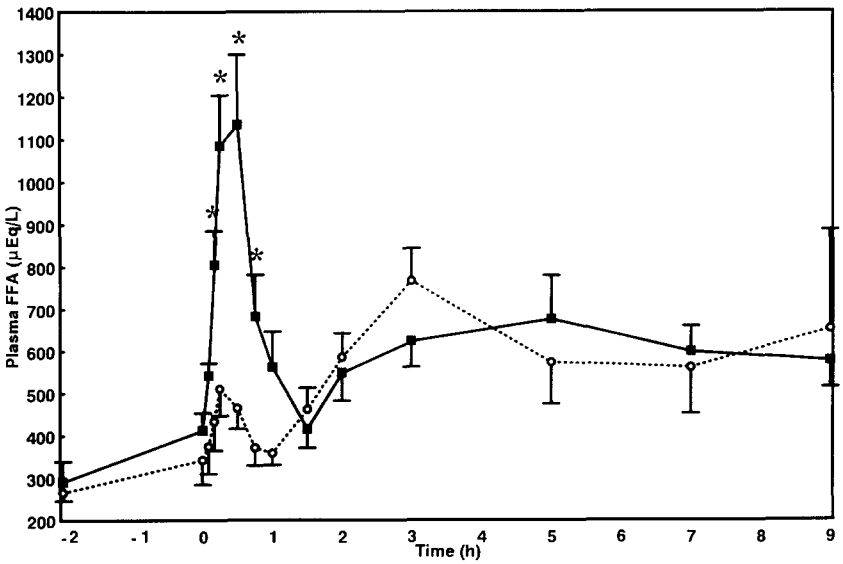

Fig. 1. Plasma FFA levels during placebo (-■-) and acipimox (--O--) treatment in eight volunteers; * statistically significant difference between the two treatments.

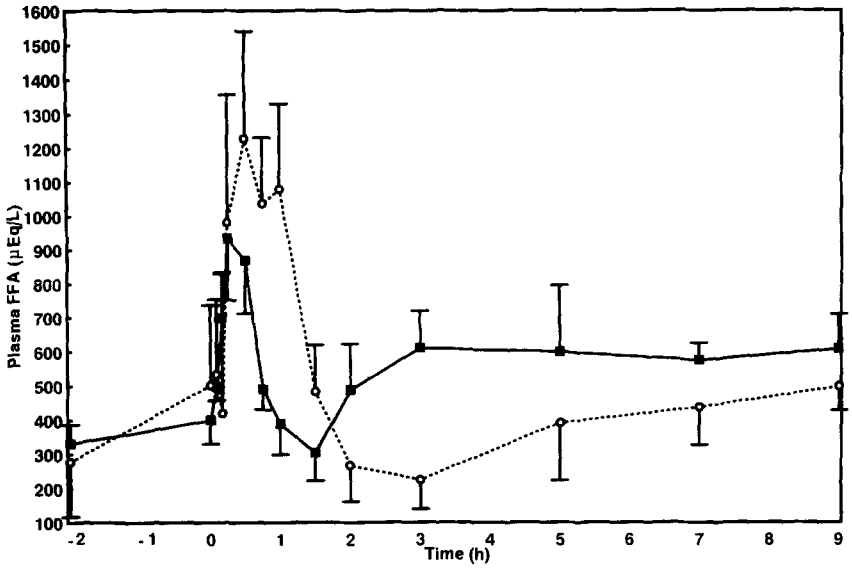

Fig. 2. Plasma FFA levels during prolonged epinephrine infusion (--O--) and short infusion + fat load (-■-) in three volunteers.

dicating that this finding is most likely a direct effect of the fat load.

In order to further investigate the effect of elevated plasma FFA levels on HDL interconversion (23), plasma samples collected before the epinephrine infusion, 30 min after the short, and 60 min after the prolonged epinephrine infusion were incubated at 37°C for 6 h and the HDL particle size distribution was analyzed by GGE. Incubation of pre-infusion plasma resulted in the accumulation of particles migrating within the HDL_{2a} size interval, with a parallel decrease of HDL_{3a} (24), as documented by a significant increase of the HDL particle score from 3.378 ± 0.135 to 3.669 ± 0.127 (**Fig. 4**). The mean particle size of the major HDL subpopulation increased from 8.7 ± 0.1 nm to 9.4 ± 0.1 nm. The elevated plasma FFA levels in post-infusion samples did not affect this conversion (Fig. 4): the HDL particle score similarly increased from 3.312 ± 0.132 to 3.610 ± 0.124 after short infusion, and from 3.421 ± 0.144 to 3.721 ± 0.143 after prolonged epinephrine infusion. Changes in the particle size of the major HDL subpopulation were almost identical to those recorded after incubation of pre-infusion samples. Moreover, no difference on the entity of HDL conversion was noted in subjects taking either placebo or acipimox.

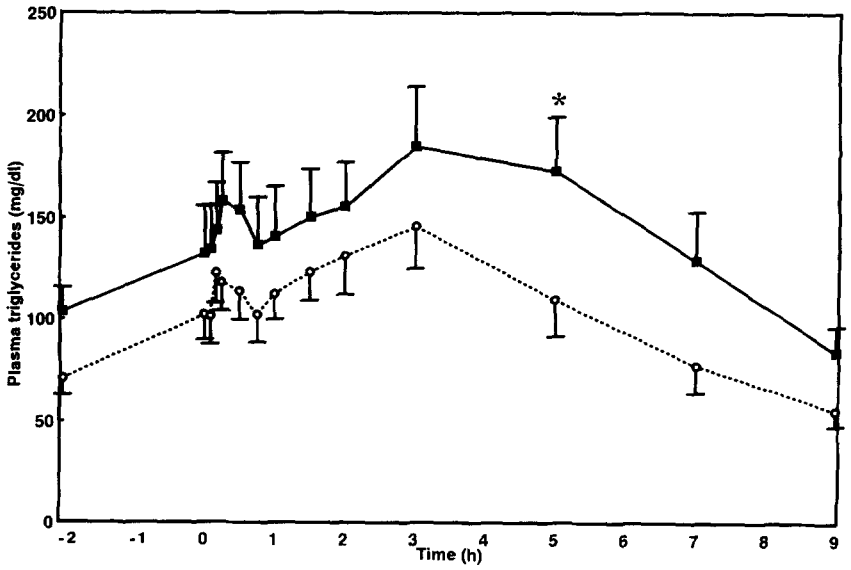

Fig. 3. Plasma triglycerides during placebo (-■-) and acipimox (--O--) treatment in eight volunteers; * statistically significant difference between the two treatments.

TABLE 3. LDL particle size after acipimox or placebo treatment

Time	LDL Diameter	
	Acipimox	Placebo
	nm	
−2 h	25.29 ± 0.25	25.29 ± 0.23
0 h	25.29 ± 0.25	25.28 ± 0.25
15 min	25.35 ± 0.28	25.26 ± 0.14
30 min	25.34 ± 0.30	25.24 ± 0.14
60 min	25.35 ± 0.28	25.26 ± 0.23
3 h	25.34 ± 0.23	25.27 ± 0.31
5 h	25.42 ± 0.27	25.46 ± 0.14
7 h	25.40 ± 0.28	25.35 ± 0.21
9 h	25.29 ± 0.19	25.36 ± 0.15

Data are expressed as mean ± SEM.

DISCUSSION

The present study had the objective of establishing in humans whether enhanced or reduced epinephrine-induced lipolysis can modify lipoprotein particle distribution. At present, based on in vitro data, it is believed that in the course of lipolysis surface lipid products may accumulate in VLDL, followed by transfer and complex formation with LDL and HDL (5–7). In addition to the formation of VLDL-LDL aggregates, lipolysis may also promote the association of apoA-I with these complexes, thus theoretically reducing the cholesterol removal capacity of HDL (7). These findings have been more recently complemented by other in vitro studies showing that increased concentrations of FFA affect the cholesteryl ester/triglyceride exchange between lipoproteins (6), resulting in a reduction of HDL particle size. These findings again support an involvement of FFA in lipoprotein remodeling.

Of course, a complete in vivo reproducibility of this type of findings is difficult in humans. It should be noted that fibric acids, potent stimulators of LPL activity, reduce VLDL levels by promoting their catabolism (25), but the presence of sufficient albumin in plasma never leads to complex formation of VLDL or of their remnants with either LDL or apoA-I.

In the present study, healthy, normotriglyceridemic volunteers were given a fat load, followed by intravenous epinephrine administration. This type of stimulus has, in a previous study by our group (15), resulted in elevated plasma FFA levels for a prolonged period of time, with negligible cardiovascular effects in a healthy human sample. The plasma FFA concentrations achieved during this stimulated lipolysis are in a range that should lead to a significant formation of complexes between VLDL and LDL (5). Obviously, complex formation may be inhibited by the binding of FFA to albumin. However, as shown by these same authors (5), when the FFA/albumin molar ratio exceeds 2–3, then complexes should appear in plasma. According to available data collected under various physiological or pathological conditions (5, 26, 27), this ratio may be reached during a markedly enhanced lipolysis.

In the present study, in spite of a careful design and successful induction of lipolysis (reaching an FFA/albumin ratio > 3 for about 20 min during the prolonged epinephrine infusion) in the volunteers, as well as of a

TABLE 4. Distribution of HDL subpopulations and HDL particle score after acipimox or placebo treatment in eight volunteers

Time	HDL Subpopulations					HDL Particle Score
	2b	2a	3a	3b	3c	
			%			
Acipimox						
−2 h	19.2 ± 2.3	32.4 ± 2.0	30.0 ± 1.5	13.5 ± 2.1	6.3 ± 1.8	3.421 ± 0.144
0 h	18.0 ± 2.4	30.9 ± 2.0	29.5 ± 1.5	13.6 ± 2.1	8.0 ± 1.8	3.375 ± 0.148
15 min	17.7 ± 2.3	29.3 ± 2.0	30.0 ± 1.3	13.7 ± 2.0	9.3 ± 2.0	3.324 ± 0.148
30 min	17.0 ± 2.2	29.2 ± 2.0	30.9 ± 1.1	13.8 ± 1.9	9.1 ± 2.0	3.311 ± 0.138
60 min	16.3 ± 2.3	29.4 ± 2.0	31.1 ± 1.3	14.0 ± 2.0	9.2 ± 2.0	3.295 ± 0.146
3 h	15.1 ± 2.1	30.9 ± 2.0	30.5 ± 1.1	14.8 ± 1.8	8.7 ± 1.9	3.288 ± 0.117
5 h	14.1 ± 2.3	30.9 ± 2.1	30.4 ± 1.3	16.2 ± 2.2	8.1 ± 1.9	3.271 ± 0.146
7 h	14.7 ± 2.2	32.5 ± 2.5	29.7 ± 1.7	15.8 ± 2.3	7.2 ± 2.0	3.311 ± 0.162
Placebo						
− 2 h	19.3 ± 2.5	32.8 ± 2.8	29.8 ± 1.5	12.8 ± 2.0	6.2 ± 1.9	3.425 ± 0.132
0 h	18.9 ± 2.4	31.4 ± 2.5	29.6 ± 1.5	13.3 ± 2.2	8.7 ± 1.9	3.378 ± 0.135
15 min	19.0 ± 2.2	30.4 ± 2.5	29.8 ± 1.1	13.5 ± 2.1	9.6 ± 1.9	3.342 ± 0.127
30 min	17.3 ± 2.1	30.0 ± 2.4	30.8 ± 1.0	14.0 ± 2.0	10.5 ± 2.0	3.312 ± 0.132
60 min	16.5 ± 2.3	29.8 ± 2.5	31.1 ± 1.3	14.1 ± 2.0	9.9 ± 2.0	3.267 ± 0.130
3 h	16.3 ± 2.3	31.7 ± 2.5	30.1 ± 1.3	14.9 ± 2.0	9.4 ± 2.0	3.314 ± 0.132
5 h	15.9 ± 2.4	32.4 ± 2.6	29.3 ± 1.3	15.6 ± 2.1	8.4 ± 2.0	3.302 ± 0.128
7 h	15.8 ± 2.5	34.4 ± 2.5	28.9 ± 1.6	14.9 ± 2.1	7.2 ± 2.1	3.333 ± 0.135

Data are expressed as mean ± SEM.

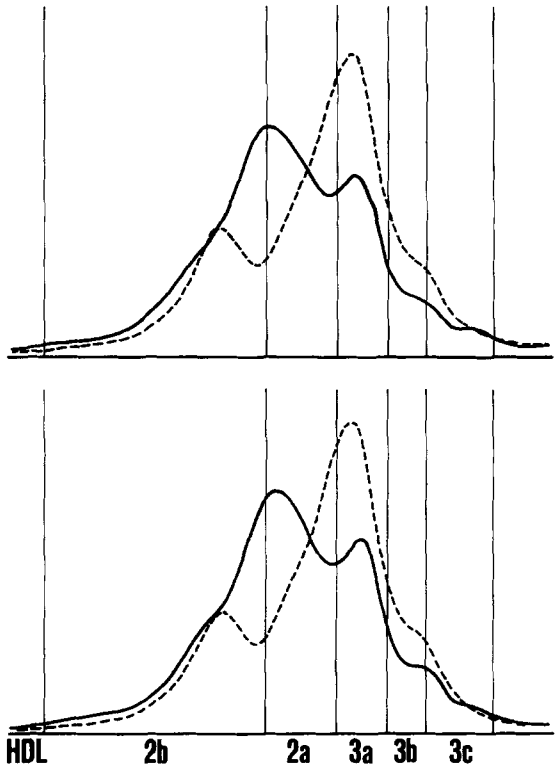

Fig. 4. GGE profiles of HDL before (·------) and after (——) 6 h incubation at 37°C of plasma samples collected at times 0 h (top) and 30 min (bottom) after short epinephrine + fatty meal infusion.

similarly successful inhibition by acipimox, there was no evidence by GGE of alterations in LDL particle distributions, similar to those found in vitro by Musliner et al. (5). There was also no clear evidence of a significant association of apoA-I to VLDL-LDL (7); the HDL particle distribution in vivo, as well as the formation of large HDL in vitro, were minimally affected either by the elevated post-lipolysis plasma FFA levels, or by the preventive action of acipimox. It may perhaps be noted, in this last case, that albumin addition to the in vitro system completely hinders the FFA-induced HDL modifications (7).

Failure to detect lipoprotein changes similar to those described in in vitro conditions does not, of course, rule out the possibility that in certain pathological states enhanced lipolysis may lead to the formation of lipoprotein complexes (28). This might be the case of patients with uncontrolled diabetes mellitus, severe hypertriglyceridemia, or reduced albuminemia (nephrosis, liver cirrhosis, etc.) (26, 28). The present findings do not, moreover, rule out a possible local effect of elevated FFA on lipoprotein structure. High FFA concentrations, with an elevated FFA/albumin ratio, can be achieved at the vascular surface, where TG are hydrolyzed; this may lead to local formation of lipoprotein complexes similar to those detected in vitro, but undetectable in plasma ex vivo.

While it seems at present that the toxicity of FFA elevations, if any, is likely to be linked to an impairment of tissue oxidative mechanisms, still some observations, particularly related to the effect of the tested drug, deserve comment. Acipimox SR, given a total of three times prior to a very potent lipolytic stimulation, showed a clear effect, maintained over many hours, i.e., different from the case of NA treatment, where the effect is generally poorly sustained (29). In addition, it blunted the secondary rise of triglyceridemia, possibly linked to the late release of VLDL following tissue lipolysis, i.e., consistent with the reduced transport rate of VLDL-TG (by an average of 21%) described after NA administration (30).

In conclusion, this study suggests that a brief, although intense, lipolysis does not seem to modify lipoprotein particle distribution to a great extent; these findings, however, cannot exclude the possibility that this phenomenon may occur after extremely prolonged lipolysis or in particularly susceptible patients. This clinical model of exaggerated lipolysis offers, however, an attractive system for investigating drug mechanisms. Treatment with acipimox, besides essentially canceling the lipolytic effect of fatty meal + epinephrine, reduced VLDL-TG levels at late intervals, thus suggesting that this type of agent may not only act by inhibiting lipolysis.

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