Immobilization Stress Alters Intermediate Metabolism and Circulating Lipoproteins in the Rat

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In humans, stress can increase the risk of cardiovascular disease by altering lipoprotein metabolism. Scarce experimental and clinical data are available on this effect. Therefore, we studied the metabolic response to acute and chronic stress following a model of immobilization (IMO) in rats and we evaluated the resulting circulating lipoprotein levels. Repeated IMO treatment (2 hours daily, always between 9:00 AM and 11:00 AM, for 2 periods of 5 and 4 consecutive days, separated by 2 days of rest) daily decreased body weight gain and food intake, increased adrenal weight, and slightly reduced liver glycogen and plasma insulin (without considerable variations of blood glucose), which is characteristic of chronic stress. A single IMO application (30 minutes of an unexpected IMO starting at 2:00 PM immediately before the animals were killed) significantly increased the circulating levels of corticosterone, glucose, insulin, glycerol, and ketone bodies, which is the typical response to acute stress. Both acute and chronic stress decreased the plasmatic triacylglycerol (TAG) concentration, as reflected by the reduction in the number of very–low-density lipoprotein (VLDL) particles. This may be due to an increase in the metabolization of TAG, as suggested by the slightly higher amounts of circulating LDLs. Chronic stress, but not acute stress, significantly increased both the number and the estimated size of circulating high-density lipoprotein (HDLs), as shown by the plasma cholesterol concentration. Acute stress did not have an additive effect over chronic stress on the lipoprotein parameters studied. The metabolic effects of these IMO-induced alterations on lipoprotein profiles are discussed, and future studies in lipidic metabolism are suggested.

THE EFFECT OF acute and chronic stress on total plasmatic cholesterol and triacylglycerol (TAG) in both humans and laboratory animals has been widely studied with contradictory results. This may be due to differences in the sex and age of the animals studied or in the stress models used.[1-7] Moreover, in some of these studies, a lipid-rich diet has been used in combination with stress.[2,3,5]

Lipoprotein composition has been modified by experimental and clinical stress[4,8-14] in a few studies, but only the high-density lipoprotein (HDL) fraction has been isolated from the rest of lipoproteins by precipitation. Even fewer reports describe the isolation of all lipoprotein fractions.[12-14]

Since alterations in circulating lipids may be linked to the risk of cardiovascular disease, the association between stress and lipoproteins is of great clinical interest. Thus, we first characterized the metabolic response to acute and chronic immobilization (IMO) following an experimental model of stress described by Kvetnansky and Mikulaj.[15] This schedule leads to fewer secondary alterations than others commonly used (eg, swimming stress with exercise and/or cold), and it strongly activates both components of the sympathoadrenal system.[16] Moreover, it is a potent and rapid stimulus of the expression of nuclear proteins, such as peroxisome proliferator-activated receptors (PPARs),[17] which are involved in the regulation of the extracellular transport of lipids.[18] The main aim of this study was to test the effects of acute and chronic stress on the amount, estimated size, and composition of major circulating lipoproteins in the rat, which may increase the risk of cardiovascular disease.

MATERIALS AND METHODS

IMO

The procedure used was similar to that described by Kvetnansky and Mikulaj.[15] Briefly, the rats were attached to wooden boards in the prone position by taping their forelimbs and hind limbs to metal mounts. Head motion was restricted by introducing the head into a transparent plastic, open-ended cylinder fixed over the neck area.

Experimental Groups

Male Wistar rats weighing 190 to 240 g upon arrival at the laboratory from Harlan Interfauna Ibèrica (Barcelona, Spain) were used. They were housed 2/cage in a controlled noise-free environment (lights on from 8:00 AM to 8:00 PM; temperature, 23 ± 2°C; humidity, 45% to 55%) and fed ad libitum on standard laboratory diet (water, 12%; protein, 17%; fat, 3%; carbohydrate, 58.7%; cellulose, 4.3%; and vitamins and minerals, 5% all by weight) (from Panlab, Barcelona, Spain) and water. After 6 days of acclimation, they were randomly assigned to 1 of 2 groups: control (nonstress) and chronic stress. Chronic stress consisted of 2 hours of IMO (always between 9:00 AM and 11:00 AM) for 2 periods of 5 and 4 consecutive days, separated by 2 days without stress treatment (Fig 1) to diminish habituation to stress stimulus.[19] Each 2 days in both groups, food intake was determined by measuring the difference between the amount of food put into the cages and that which remained the following morning. Body weight was also measured during the treatment. On day 16 (the last day of IMO), half of the animals of each group (control and chronic stress) were exposed to 30 minutes of an unexpected IMO (acute stress) starting at 2:00 PM and immediately killed. The other half were also killed at that time, but without any type of acute stress.

Therefore, we studied 4 experimental groups: (1) control (Co), intact animals (nonstressed); (2) Co + acute stress (Ac), animals immobilized only for 30 minutes immediately before they were killed; (3) chronic

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stress (Ch), animals immobilized for 2 hours daily during 2 periods (5 and 4 days) separated by 2 days of rest; and (4) chronic + acute stress (Ch + Ac), animals receiving the same schedule of IMO as group 3 and also immobilized 30 minutes immediately before they were killed.

To avoid the effect of uncontrolled stressors, such as noise, smells, and isolation in the cage, the 2 rats inhabiting each cage were immobilized and killed simultaneously and isolated from the rest of the animals in the colony.

Sacrifice and Sample Collection

The animals of all 4 groups were killed by decapitation. Blood was collected from the neck. Aliquots of whole blood were deproteinized[20] and supernatants were used for glucose determination using a commercial kit (GLU MPR 3; Roche Diagnostics, Mannheim, Germany). Other sets of blood aliquots were used for plasma separation using EDTA as anticoagulant. Insulin was determined by radioimmunoassay (RIA) (INSI-PR; CIS-bio International, Yvette, France), glycerol by the method of Garland and Randle[21] and ketone bodies by the method of Kientsch-Engel and Siess.[22] TAG, total cholesterol, and free fatty acids (FFA) were enzymatically analyzed by commercial kits (TRIG S1213 and CHOL S1211; Medical Analysis Systems, Camarillo, CA, and NEFA C Wako Chemicals, Neuss, Germany, respectively), and lipoproteins were isolated as described below. A third set of blood aliquots was used for serum separation and corticosterone determination[23] (slightly modified). Sections of liver and skeletal muscle (quadriceps) were rapidly removed, placed in liquid nitrogen (N_2), and maintained at -80°C until glycogen[24] determination. Adrenals were rapidly removed and weighed.

Lipoprotein Isolation

Lipoproteins were isolated from plasma samples by a sequential flotation ultracentrifugation micromethod (fixed-angle rotor RP100-AT4 in a Sorvall RC-M120EX ultracentrifuge, Du Pont, USA) following the method of Havel et al[25] after optimization in our laboratory.[26] Free cholesterol and phospholipids (PL) were enzymatically determined with F-CHOL MPR1 kit and Phospholipids MPR2 kit (Boehringer-Mannheim GmbH, Mannheim, Germany). For esterified cholesterol calculation, free cholesterol was subtracted from total cholesterol.

Statistics

Results are presented as mean ± SEM of 7 to 12 animals/group. Significance was assessed by analysis of variance (ANOVA) for 1 factor (stress) by the Graph Pad Software Prism program. Individual comparisons were performed by the Dunnet's *t* test. We compared the control with chronic and acute stress and chronic stress with chronic + acute stress.

RESULTS

Metabolic Characterization of the Stress Model

On the first days of IMO, body weight gain was arrested (Fig 1, arrows). During the next 2 days of rest (days 11 and 12), these animals showed the same body weight gain as controls, but in the second set of IMO treatment, body weight gain was again arrested and, on the day they were killed (day 16), chronically stressed animals weighed 83.8% of controls. Chronic IMO decreased food intake, especially during the first 2-hour IMO application (day 6, first arrow; intake 55% of controls) (Fig 1). On consecutive days, the decrease was of 60%, 62%, and 63%, respectively. After 2 days of rest (days 11 and 12), the effect of IMO was partially recovered, although it

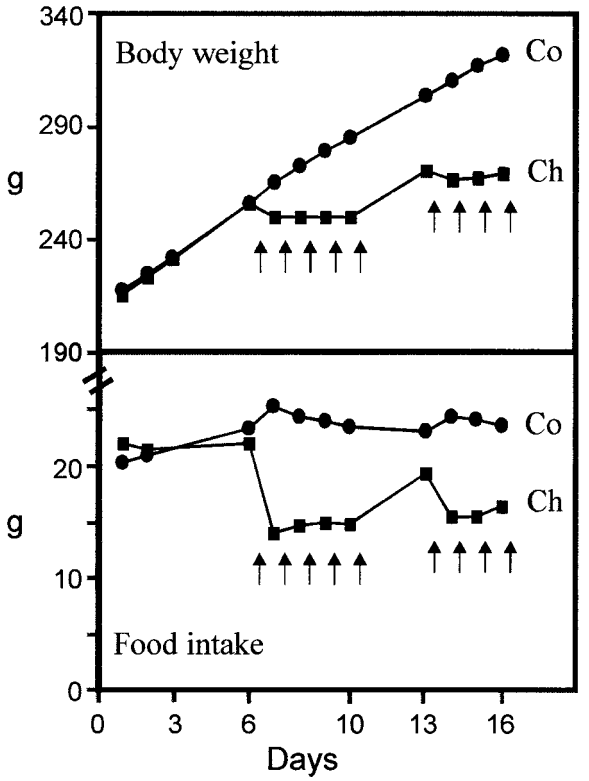

Fig 1. Body weight and food intake in control (Co) and chronically stressed (Ch) male rats by IMO. Arrows indicate 2-hour daily IMO period (starting at 9:00 PM, after collecting data) of Ch animals. Co were intact, nonstressed animals. Values are expressed as mean ± SE of n = 14 (for Co group) or n = 24 (for Ch group) animals (see Experimental Groups in Materials and Methods).

was lower than on day 6 (diminution to only 63% intake of controls).

Chronic stress increased the weight of the adrenal gland (170% of control value), especially when this increase was expressed as a percentage of body weight (209% of control) (Table 1). Unexpectedly, when animals were previously submitted to a repeated IMO schedule (chronically-stressed), acute stress significantly decreased (22%) adrenal weight, which is not observed in acutely stressed control animals. Serum corticosterone levels increased dramatically with acute stress both in control (22 times) and in chronically stressed (40 times) animals. Blood glucose and plasma insulin levels were normal in chronically stressed animals, whereas the glucose/insulin rate was higher (1.6 mmol glucose/ng insulin) than in controls (0.8). Both glycemia and insulinemia significantly increased with acute stress both in control and in chronically stressed animals. However, the response to acute stress decreased after the chronic stress treatment (1.35 times in chronically-stressed animals *v* 1.55 in controls for glycemia and 1.8 *v* 2.1 for insulinemia). No significant changes were observed in hepatic or muscular glycogen stores with acute or chronic stress treatments. Plasma glycerol and ketone bodies increased significantly after acute stress both in control and in chronically stressed animals, while chronically stressed animals presented

Table 1. Adrenal Weight, Tissue, Plasma Metabolites, and Hormones in Control and Chronically and/or Acutely Stressed Male Rats by IMO

	Control			Chronic Stress			
Acute stress	−	+		−		+	
Nomenclature	**Co**	**Co + Ac**		**Ch**		**Ch + Ac**	
Statistics versus			**Co**		**Co**		**Ch**
Adrenal weight (mg)	35 ± 1	37 ± 3	NS	59 ± 5	↑ ↑ ↑	46 ± 3	↓
(mg/100 g BW)	11 ± 0.5	12 ± 1.2	NS	23 ± 1.5	↑ ↑ ↑	18 ± 0.9	↓ ↓
Serum corticosterone (ng/mL)	20 ± 4	442 ± 22	↑ ↑ ↑	12 ± 1	NS	485 ± 17	↑ ↑ ↑
Blood glucose (mmol/L)	5.9 ± 0.2	9.3 ± 0.5	↑ ↑ ↑	5.7 ± 0.1	NS	7.7 ± 0.2	↑ ↑ ↑
Plasma insulin (ng/mL)	7.2 ± 1.5	14.8 ± 2.4	↑ ↑	3.5 ± 0.5	NS	6.2 ± 0.7	↑
Liver glycogen (g glc/100 g)	5.9 ± 0.3	5.5 ± 0.3	NS	5.2 ± 0.2	NS	4.3 ± 0.2	NS
Muscle glycogen (g glc/100 g)	0.35 ± 0.02	0.28 ± 0.04	NS	0.42 ± 0.05	NS	0.44 ± 0.02	NS
Plasma glycerol (μmol/L)	113 ± 23	177 ± 15	↑	115 ± 19	NS	190 ± 17	↑
Plasma NEFA (mmol/L)	0.25 ± 0.02	0.25 ± 0.02	NS	0.33 ± 0.03	↑	0.33 ± 0.03	↑
Plasma ketone bodies (μmol/L)	3.9 ± 0.5	8.4 ± 0.5	↑	4.1 ± 0.6	NS	10.4 ± 1.5	↑ ↑ ↑

NOTE. Adrenal weight is expressed as absolute value and as percentage of BW of each animal. Glycogen is expressed as gram of glucose-of-glycogen/100 g of tissue. Values are expressed as mean ± SE of n = 7 for control groups [with (+) or without (−) acute stress] or n = 12 for chronic stress groups [with (+) or without (−) acute stress]. Statistics by ANOVA and Dunnet *t* test *v* indicated groups are shown by ↑ (higher than) and ↓ (lower than). One arrow, $P < .05$; 2 arrows, $P < .01$; 3 arrows, $P < .001$.

Abbreviations: BW, body weight; NS, not significant.

normal values for these parameters. In contrast, acute stress did not affect nonesterified fatty acid (NEFA) levels. Plasma NEFA increased significantly with chronic stress treatment.

Effect of Stress on Circulating Lipoproteins

Plasma TAG diminished significantly after both acute and chronic stress, whereas acute stress had no additive effect on chronically stressed animals, and plasma total cholesterol increased significantly after chronic stress, but not after acute stress (Fig 2).

The TAG values of the very–low-density lipoprotein (VLDL), LDL, and HDL fractions (isolated from plasma samples by a sequential flotation ultracentrifugation, see Materials and Methods) were measured in the 4 experimental groups, showing that TAG in the VLDL fraction (VLDL-TAG) decreased with acute and chronic stress, while acute stress did not produce any additional effect over chronic stress (Fig 3). The TAG values in the LDL fraction (LDL-TAG) did not change, whereas those of the HDL fraction (HDL-TAG) significantly increased with acute stress, but not with chronic stress. Acute stress had no additive effects on chronic stress.

VLDL-cholesterol decreased with acute stress, but did not change with chronic stress (Fig 4). The effect of acute stress is not reproduced in chronically stressed animals. LDL-cholesterol was unchanged, while HDL-cholesterol increased significantly in the chronically stressed group.

Phospholipid levels decreased in the VLDL fraction with stress, although the decrease was not significant (Fig 5). In the LDL fraction, they did not vary. In the HDL fraction, they increased significantly with chronic stress, as cholesterol did (Fig 4).

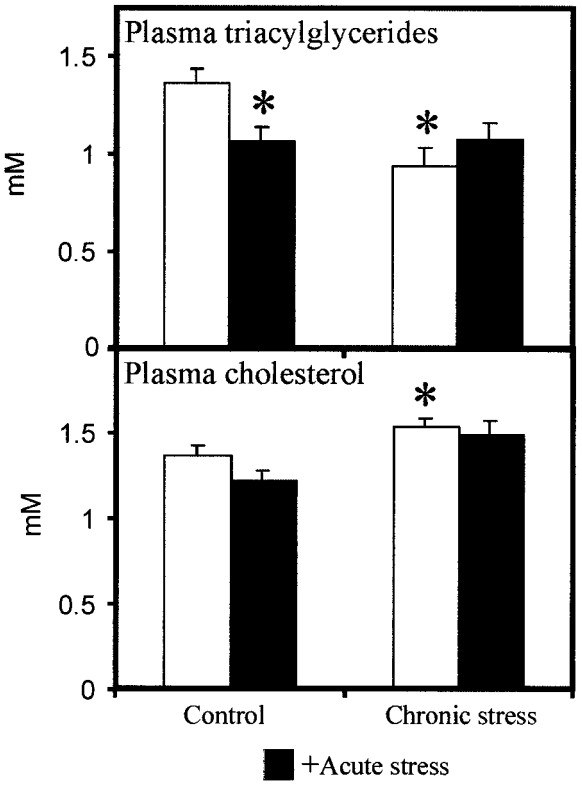

Fig 2. Plasma TAG and total cholesterol in control and chronically and/or acutely stressed rats by IMO. On day 16 of the chronic IMO schedule (see Fig 1.), half of the animals in each group (control and chronically-stressed) were exposed to 30 minutes of IMO starting at 2:00 PM (acute stress) and immediately killed (■). The other half of the animals, nonacutely stressed, were also killed at that time (□). Values are expressed as mean ± SE of n = 7 for control groups (with or without acute stress) or n = 12 for chronic stress groups (with or without acute stress). Statistics by ANOVA and Dunnet *t* test *v* controls (without acute stress): *$P < .05$. F value for plasma TAG = 3.618 and plasma cholesterol = 4.353.

DISCUSSION

Metabolic Characterization of the Stress Model

First, we studied the effects of acute and repeated IMO schedules on glucose and lipid intermediate metabolism in the rat.

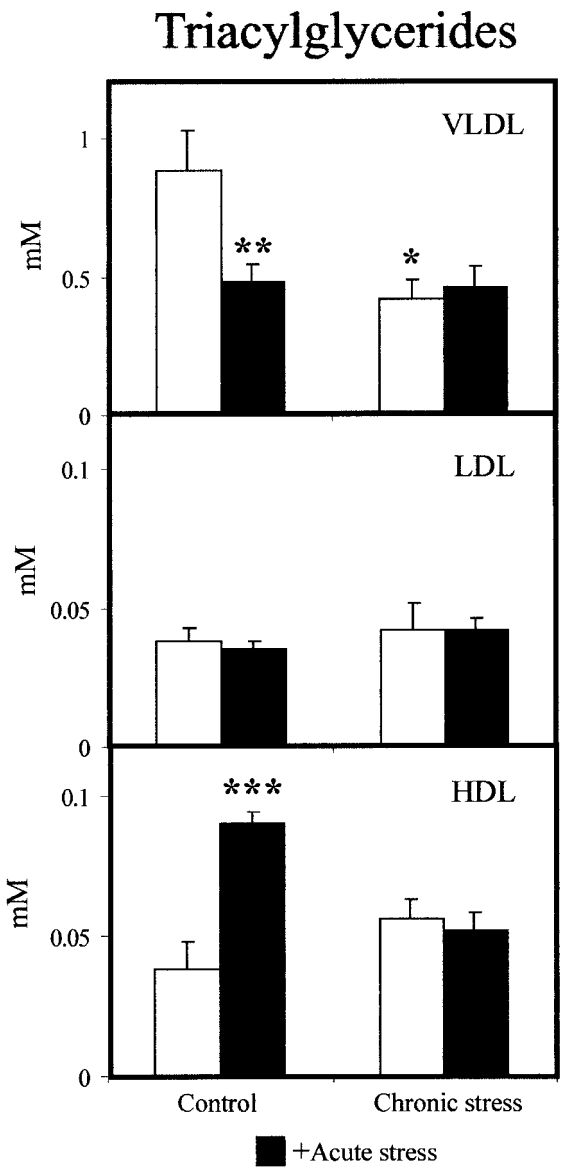

Fig 3. Composition of TAG of plasmatic lipoprotein fractions in control and chronically and/or acutely stressed rats by IMO. Same legend as in Fig 2. VLDL, very–low-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins. Statistics by ANOVA and Dunnet *t* test *v* controls (without acute stress): **P* < .05, ***P* < .01, ****P* < .001. F value for VLDL = 5.449, LDL = 0.202, and HDL = 7.356.

Daily repeated IMO (Ch group) can be regarded as chronic stress, since the animals present a decrease in body weight gain and a clear increase in adrenal gland weight, both of which are typical markers of this stress situation.[27,28] The decrease in body weight gain is probably due to a general reduction in animal growth, because the proportional weight of tissues measured, referred to total body weight, is unaltered (data not shown), and this would not have happened if the animals had been lean. The decrease in growth may be due to the lower food intake induced by stress (Fig 1), as reported elsewhere.[29,30] On the other hand, under chronic stress, hypophysis adrenocorticotropin (ACTH) continuously stimulates adrenal glands, leading to their hypertrophy[28,31] and ultrastructural modifications.[32] All the metabolic parameters studied were normal in the chronically stressed animals (Table 1), although they were immobilized for 2 hours (between 9:00 AM and 11:00 AM) before death (2:00 PM). This may be due to (1) the rapid recovery of basal levels after this IMO and/or (2) the fact that the metabolic response to a daily repeated stress is lower (owing to habituation or metabolic tolerance) than the response to unexpected acute stress just before sacrifice (from 2:00 PM to 2:30 PM).

Acute IMO of nonpreviously immobilized animals (Co + Ac group) significantly increases circulating corticosterone, and

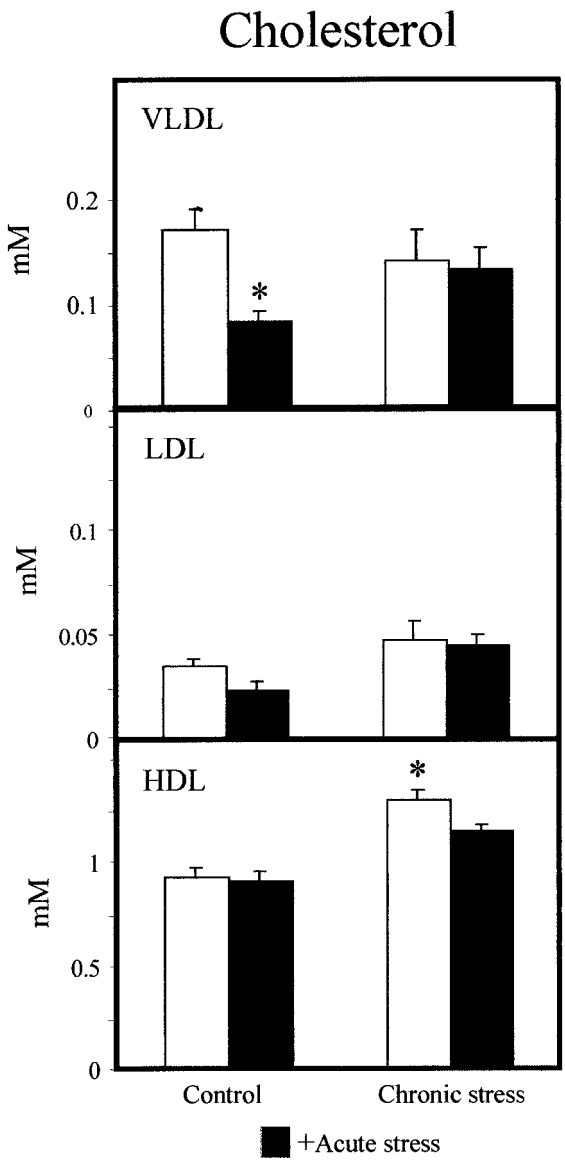

Fig 4. Composition of total cholesterol of plasmatic lipoprotein fractions in control and chronically and/or acutely stressed rats by IMO. Same legend as in Fig 3. F value for VLDL = 1.628, LDL = 0.071, and HDL = 5.254.

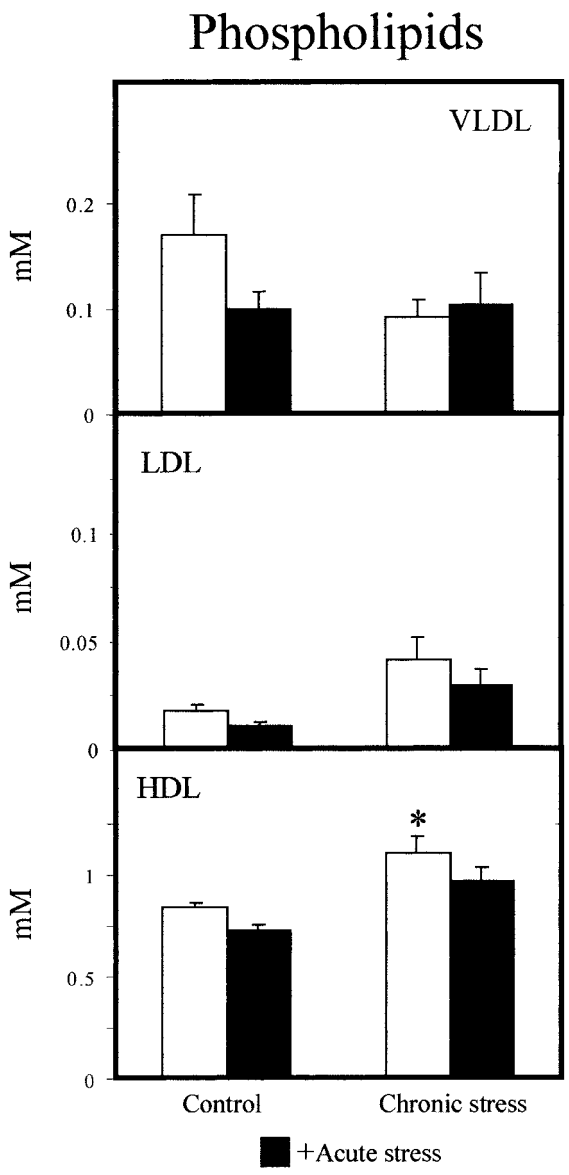

Fig 5. Composition of PL of plasmatic lipoprotein fractions in control and chronically and/or acutely stressed rats by IMO. Same legend as in Fig 3. F value for VLDL = 1.524, LDL = 2.872, and HDL = 5.254.

blood glucose, which are markers of acute stress,[27,33] plasma insulin, glycerol, and ketone bodies, showing that acute IMO triggers acute stress.

As expected,[34,35] acute stress increased glycemia in both control and chronically stressed animals. This may be due, in part, to hepatic glycogen mobilization induced by epinephrine, although no significant diminution was observed. Another hypothesis is that stress induces gluconeogenesis through glucocorticoids, as described elsewhere,[7,30] but this is not probable in our model after only 30 minutes of IMO. Muscular glycogen does not decrease, probably because IMO prevents muscular contraction and thus glucose consumption decreases, as reported in stressed mice with reduced clearance of radiolabeled glucose.[35] Moreover, glycogen degradation is inhibited by glucose-6-phosphate and adenosine triphosphate (ATP) intracellular levels. The increase in glycemia after acute IMO is accompanied by an increase in plasma insulin levels (Table 1), as described elsewhere for the same model of IMO,[36] although the opposite effect has also been reported.[34] In humans, severe stress is accompanied by marked increases in plasma glucagon, catecholamines, and corticosterone and decreases in plasma insulin.[34] Some investigators have reported that chronic stress does not affect insulinemia,[1] whereas others have reported increases in chronically stressed animals fed with fat-rich diets.[37] The decrease in insulin levels in chronically stressed animals may be due to the effect of stress hormones on pancreatic insulin secretion, which decreases by epinephrine infusion in humans.[38] Moreover, stress induces parasympathetic (acetylcholine) and/or sympathetic (norepinephrine) stimulation.[39] Thus, the balance between the 2 neurotransmitters may explain the differences in the bibliography on insulin secretion since acetylcholine stimulates insulin secretion and norepinephrine inhibits it.[16] Finally, although the correlation between stress and diabetes is unclear, it has been suggested that stress affects the onset of this disease.[40]

With regard to lipid metabolism, acute stress increases the circulating levels of glycerol and ketone bodies both in control and in chronically stressed animals, probably because catecholamines stimulate the hormone-sensitive lipase in white adipose tissue, leading to TAG mobilization. Increased hydrolysis raises the circulating levels of both glycerol and NEFA, and its oxidation in the liver leads to the observed increase in ketone body levels. It must be noted that no apparent changes in NEFA levels were found after acute stress. Nevertheless, some investigators[17] described that glucocorticoids released during acute IMO were directly involved in the fast stimulation of liver PPARα expression. This may be associated with the hasty NEFA oxidation by the liver suggested in our stress situation, since PPARα regulates genes involved in the activation and oxidation of fatty acids.[18] Thus, the metabolic response to acute stress is characterized by energy mobilization of substrates (glucose, glycerol, NEFA, and ketones) that occurs in tissues in the "fight or flight" response, as reported in this study in both control and chronically stressed animals.

Effect of Stress on Circulating Lipoproteins

The decrease in TAG plasma levels observed after both acute and chronic stress has been described elsewhere,[2,6] but once again, with contradictory results, ranging from no variation[1] to increases.[3] Nevertheless, in some of these studies, a lipid-rich diet was used, which may condition the results. The decrease observed in this study may be due to an increase in circulating TAG utilization by enhanced activity of vascular lipoprotein lipase (LPL) in some tissues, as proposed elsewhere for another stress situation.[41] With regard to plasma cholesterol, both acute and chronic stress increase plasmatic cholesterol levels in humans,[4,7,42] but in animals, this response is more variable, and the main effects are found in chronic stress, again with increments,[5] no changes,[2] or reductions.[3] In these studies, a cholesterol-rich diet and various types of stress are often used, but in our model, the animals were fed with standard chow, and total

circulating cholesterol increased significantly under chronic stress. Once again, stress hormones may account for this response, as repeated infusion of epinephrine in vivo stimulates hepatic cholesterol synthesis in rats.[11]

The increase in cholesterol and NEFA in chronic stress is of great interest, because it is involved in cardiovascular disease, and the effects are only due to stress treatment, not to diet. Thus, we studied the effect of stress on plasmatic lipoprotein profiles in the same experimental model.

The decrease in plasmatic TAG with both acute and chronic stress is reflected in the VLDL fraction, since VLDLs are the main (82% to 92%) carriers of total TAG. The unexpected increase in HDL-TAG with acute stress is high in the fraction, but slight when the total TAG amount in plasma is considered, because HDL-TAG is only 5% to 10% of the total TAG in plasma. Stress does not affect LDL-TAG. In this regard, the available data on lipoprotein TAG and stress are scarce. In rats submitted to 10 hours of acute IMO stress, VLDL does not change, whereas the levels in the LDL and HDL_1 fractions increase.[14]

While VLDL-TAG levels are modified, changes in plasma total cholesterol are reflected in the HDL fraction, the main lipoprotein carrying cholesterol (83% to 90%) in the rat. The decrease in the cholesterol content of the VLDL fraction (VLDL-cholesterol) with acute stress is probably due to lipoprotein metabolization, which reduces the particle number in the fraction (see below). No significant changes in LDL-cholesterol are observed after stress.

Here, the animals were fed on a standard diet. In contrast, when a lipid-rich diet is combined with repeated IMO,[10] cholesterol levels increase in the VLDL-LDL fractions and decrease in the HDL fraction. With regard to acute stress, a 10-hour IMO in the rat increases cholesterol in VLDL, LDL, and HDL.[14] Distinct results are obtained when other types of stress are studied. Thus, acute electric shock decreases cholesterol levels in VLDL, LDL, and HDL, and acute water immersion has no effect on rats.[13]

Stress increases circulating catecholamines. Administration of adrenaline to rats in vivo increases both cholesterol synthesis, by stimulating the activity of hepatic 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase,[11,43] and the levels of plasmatic FFA.[4] Furthermore, FFA induces cholesterol synthesis in perfused rat livers.[43]

PL decrease in the VLDL fraction with acute and chronic stress, as does TAG. These decreases observed in the VLDL fraction are probably due to a reduction in the number of these particles. The decreased amount of VLDLs may be due to an increased lipoprotein metabolization by LPL. This endothelial enzyme hydrolyzes the TAG of VLDL, which yields LDL particles. In stress conditions, such as trauma, the elevated levels of catecholamines and glucocorticoids may account for the decrease in the adipose tissue LPL activity and the increase in muscle.[44] Enhanced VLDL metabolization may explain the slight, but not significant, increase in LDL estimated by means of its PL content. We aim to explore this in further studies.

In chronic stress, the amount of HDL, estimated by means of its PL concentration, is significantly higher. Therefore, the increased levels of HDL-cholesterol may be due to an increase in the particle amount rather than to changes in its composition. In contrast, other investigators do not report changes in the HDL-PL of rats injected with epinephrine[11] or an association between lipid changes and lipoprotein amount.[10]

To examine the changes in lipoprotein fractions, we estimated the size of the lipoprotein particles under study as the ratio between core (TAG plus esterified cholesterol) and surface elements (PL plus free cholesterol) (data not shown).

No significant changes were observed in VLDL estimated size in any group of animals. LDL particles were significantly smaller in chronically stressed animals (1.7 ± 0.2 *v* 4.2 ± 0.7 in controls, $P < .05$), and their amount increased, as indicated by the PL amount. As mentioned above, this may be due to an increase in VLDL metabolization. Note that LDLs are minor rat lipoproteins, which hinders their isolation and analysis, as they are easily contaminated by other fractions. Moreover, their contribution to the rat lipoprotein profile is not quantitatively relevant.

HDL particles were significantly ($P < .05$) larger in both the acute (1.0 ± 0.04) and chronic (0.8 ± 0.01) stress groups than in controls (0.7 ± 0.04). Hepatic lipase (HL) activity in the rat liver decreased with acute stress in our model (unpublished data). This enzyme hydrolyzes HDL-TAG, and the decrease in its activity may partially explain the accumulation of TAG in these lipoproteins under acute stress. In chronically stressed animals, the HDL amount is higher, probably as a result of increased VLDL metabolization by LPL, which produces membrane discoids that convert into HDL.

In summary, stress by IMO decreases plasmatic TAG levels probably increasing VLDL metabolization, and thus reduces the amount of these lipoproteins. Such VLDL clearance in chronic stress may slightly increase the LDL amount and significantly increase the number of HDL. Only HDL particles reflect the increase in total plasmatic cholesterol observed in chronically stressed animals. On the other hand, acute stress does not have an additive effect on the lipoprotein profile over chronic stress, although chronically stressed animals respond to acute stress. These changes in lipoproteins may be strongly linked to the activity and regulation of LPL and HL. Therefore, given the association between stress, the risk of atherosclerotic disease, and the changes in circulating lipoproteins,[42] we aim to examine the regulation of LPL and HL activities in our experimental stress model in further studies.

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