Sensitivity of Plasma Pre-Heparin Lipases and Cholesterol to Surgical Stress in Humans

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Abstract

While lipolytic activities (lipoprotein lipase, LPL, and hepatic lipase, HL) in post-heparin plasma have been widely studied, the plasma pre-heparin activities of these enzymes have attracted less interest, possibly due to the difficulty in determining their levels.

We studied the effects of surgical stress on the activities of pre-heparin lipases (LPL and HL) and the levels of different lipids in plasma. Patients were studied three days prior to (basal conditions) and immediately before the onset of gastrointestinal surgery (stressed). LPL and HL activities, triacylglycerides (TAG), total cholesterol (TC), LDL cholesterol (cLDL), HDL cholesterol (cHDL), cortisol, insulin, and glucose were measured in plasma. Both glucose and cortisol levels in plasma increased, characteristic of a stress situation before the onset of surgery. Plasma pre-heparin LPL and HL activities increased, and TC, cLDL, and cHDL decreased, although total TAG did not change.

Conclusions

In humans, acute stress from the immediacy of a surgical procedure increases LPL and HL activities in pre-heparin plasma. These changes are consistent with the effects of stress described in animals, which include the release of LPL and HL into the bloodstream. Changes in lipid metabolism in these situations are discussed.

Keywords: Pre-Heparin Lipolytic Activities; Lipoprotein Lipase; Hepatic Lipase; Cholesterol; Surgical Stress

Introduction

Two major lipolytic activities can be distinguished in human plasma, hepatic lipase (HL) and lipoprotein lipase (LPL) [1]. HL is selectively expressed in the liver, while LPL is produced in several extra-hepatic tissues, including adipose tissue, heart and striated muscle, mammary glands, and macrophages. HL and LPL play pivotal roles in lipoprotein metabolism since they are involved in the metabolism of triacylglycerol-rich lipoproteins, such as chylomicrons and very low-density lipoproteins (VLDL), and in the metabolism of the reverse transport of cholesterol (high-density lipoproteins, HDL) [1].

The amount of LPL is regulated by the rate of its synthesis (transcription, translation, and post-translational processing) [2,3]. However, we hypothesized that LPL may also be regulated in situations such as stress by catecholamine-mediated release into the bloodstream from the endothelium in white adipose tissue (WAT), as occurs in rats [4,5]. These hormones are also responsible for a rapid decrease in hepatic HL activity, which may be caused by a decrease in its secretion from hepatocytes [6]. Stress can be defined as a response by an organism to agents that pose a threat to its overall well-being [7]. This individual response is activated when the effect of a stimulus is greater than the capacity of the organism’s homeostatic mechanisms to deal with changes in the internal medium. Stress involves activation of both the sympatho-medullo-adrenal axis (with the secretion of catecholamines) and the hypothalamus-hypophyseal-adrenal axis (which leads to glucocorticoid release). Catecholamines and glucocorticoids act on their target tissues and provoke a metabolic response characterized by an increase in available energy substrates [7].

Measurement of post-heparin LPL activity is a standard method for evaluating this enzyme, but it requires a heparin injection and a fairly long waiting time (15 min) before being able to harvest the post-heparin sample. The activity in plasma without previous administration of heparin (pre-heparin plasma) is very low, but it can be measured in the presence of an HL antibody. It is known that pre-heparin plasma LPL activity is not always correlated with post-heparin plasma LPL activity and its correlation with triglyceride and cholesterol levels in lipoproteins are controversial [8-10]. However, the unclear metabolic significance of pre-heparin plasma lipase activity has led us to study the role of stress in regulating the release of LPL and/or HL, in contrast to post-heparin plasma lipase activities.

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The aims of this study were to determine variations in pre-heparin plasma of lipolytic activities (HL and LPL) and lipid components in surgical patients from three days before (basal conditions) and immediately before gastrointestinal surgery (stressed).

Subjects and Methods

Patients

This study included 24 patients (30-80 years old) who were undergoing elective abdominal surgery in the Vall d’Hebron Hospital (Barcelona, Spain) (15 colon cancer, 5 gastric neoplasia, 3 diverticulosis, and 1 angiodyplasia). The exclusion criteria were: kidney or liver disease, peritoneal carcinomatosis or known metastasis, malnutrition (abnormal albumin and transthyretin, abnormal BMI, weight loss greater than 10% in the previous three months), and metabolic disease such as metabolic syndrome, diabetes, or thyroid disease. Patients were informed by their surgeon of the value of the study, its design and risks, and the extra manipulations required. They were provided with written copies of explanations and signed an agreement of their participation in the study. The study was approved by the Ethics Committee of the Vall d’Hebron Hospital.

Design

Pre-operative values were taken in the morning after an overnight fast, and were determined three days prior to surgery (basal) and immediately before the onset of surgery (stressed).

LPL Activity Assay

LPL was assayed in plasma as described elsewhere [11]. The assay mixture included 0.6 mM glycerol tri [9,10(n)-³H]-oleate (12 Ci/mol), 50 mM MgCl₂, 0.05% FFA BSA, 3% (v/v) pre-heated serum as an apo C-II source (pre-heated to 52°C for 1 h to inactivate lipases), and 25 mM PIPES adjusted to pH 7.5. HL activity (plasma contains HL in addition to LPL) was inhibited before the LPL assay by incubating the sample for 2 h on ice with a non-commercial rabbit IgG against heparin-released hepatic lipase from perfused rat liver (1:32). This non-commercial antibody has been used successfully in different experiments [6-12]. Then, 0.007 mL of sample+antibody was used in a final volume of 0.067 mL. After incubation for 30 min at 25°C, the reaction was stopped, free FA was extracted, and (³H)-oleate was measured. The production of 1 µmol oleate per min is equivalent to 1 U LPL. Endothelial lipase (EL) has no TAG hydrolase activity when 3-5% serum is present in the assay [13].

HL Activity Assay

The assay mixture was an emulsion of 2.5 mM glycerol tri [9,10(n)-³H]-oleate (0.3 Ci/mol), 0.75 M NaCl, 3% free-FA BSA, 50 mM Tris, and 0.017 ml plasma at pH 8.5 and was used in a final volume of 0.067 mL. After incubation for 30 min at 25°C, the reaction was stopped, free FA was extracted, and (³H) oleate was measured [11]. The production of 1 µmol oleate per min is equivalent to 1 U HL activity. Both LPL and EL were inactive in this lipase assay due to the high NaCl concentration [13].

Other Determinations

Total cholesterol (TC), HDL cholesterol (cHDL), and triacylglyceride (TAG) measurements were made by enzymatic and colorimetric techniques using the CHOL-H L-type and Triacylglycerol L-Type kits, respectively (Wako Chemicals). The cLDL level was calculated by application of the Friedwald formula. Insulin and cortisol were determined by commercial immunoradiometric assays (Nichols Institute Diagnostics and Immunotech, respectively). Plasma glucose was measured with a Hitachi clinical analyzer (hexoquinase/GPDH method). The homeostasis model assessment of insulin resistance (HOMA-IR = (glucose (mM) x Insulin (µU/mL)) / 22.5) was also calculated.

Statistical Analysis

Sample size was calculated by a statistical package (Stat Graphics plus) from our previous data on protein synthesis and breakdown for an expected difference of 50% vs. basal levels in a two-tailed comparison with a power of 0.90 and a confidence interval of 95%. Results are given as means ± SE. Significance was assessed with the Graph Pad Prisma program (Graph Pad Software). Individual comparisons were made using the paired student’s t-test between the values (basal and stressed) of each patient. Asterisks denote the following: * = p<0.05; ** = p<0.01; *** = p<0.001. Correlations between independent variables, such as LPL, HL, and TAG, were determined by the Spearman correlation coefficient. Statistical comparisons were considered significant when p<0.05.

Results

Stress Parameters

Both cortisol and glucose in plasma (Figure 1) were significantly higher immediately before surgery (0.60±0.05 mM and 114.7±7.7 mg/dL, respectively) than three days earlier 0.97±0.07 mM and 153.0±12.4 mg/dL, respectively. These increases were taken to be indicators of stress for the patients [14].

Lipoprotein and Hepatic Lipase (LPL and HL)

Pre-heparin plasma LPL activity had increased stress (0.41±0.11 mU/mL) vs. basal 0.15±0.03 mU/mL, p<0.05) values (Figure 2, top panel).

Pre-heparin plasma HL activity showed the same pattern of LPL activity, but with the values doubled. HL was increased in the stress condition (0.76±0.29 mU/mL) vs. the basal condition 0.31±0.08 mU/mL, p<0.05) (Figure 2, bottom panel).

Metabolites

No statistically significant changes in TAG were observed between the basal (118±9 mg/dL) and stressed (129±16 mg/dL) conditions (Figure 3), but total cholesterol (198±9 vs. 127±7; p<0.001), cLDL (178±8 vs. 138±7 mg/dL, p<0.001), and cHDL (43±3 vs. 11±1 mg/dL, p<0.001) dropped significantly between the pre- and post-surgical periods.
Correlations

LPL activity correlated positively with cortisol ($r=0.3551$, $p<0.05$) and negatively with total cholesterol ($r= -0.3677$, $p<0.05$) and cHDL ($r= -0.3250$, $p<0.05$). HL activity correlated negatively with TAG ($r= -0.4344$, $p<0.01$). Cortisol correlated positively with glucose ($r= 0.4991$, $p<0.01$) and negatively with total cholesterol ($r= -0.3174$, $p<0.05$) and cHDL ($r= -0.3476$, $p<0.05$).

Discussion

There is little information in the literature on lipolytic activities in plasma without previous administration of heparin (pre-heparin plasma), and less data on surgical stress in humans. This may be due to the lack of sensitivity of the methods used to measure these lipases since they show low activity in pre-heparin plasma. The interassay reproducibility of preheparin lipase activity measurements was 11% for LPL (coefficient variation, CV) and 15% for HL and CV. Karpe et al [10]. reported that the interassay CV for LPL was 20-25%, and Watson et al [15]. reported values of 19.7% for LPL and 17.4% for HL. On the other hand, in this study
we show that the pre-heparin activities levels for LPL and HL with or without stress were similar to those values reported by others [8,15]. In addition, the method is sensitive enough to allow for the observation of changes in these enzymes as a result of stress.

Measurement of LPL and HL activities in non-heparinized plasma offers the potential for monitoring enzyme activities during metabolic studies, and could be used in situations where heparinization is not possible. Additionally, it may allow for the detection of changes during the dynamic release of LPL from tissues, which cannot be detected after a massive release as consequence of heparin administration.

In post-heparin plasma, the LPL released may come from heart muscle, skeletal muscle or adipose tissue, and could be contained in the capillaries of such tissues or inside cells. In pre-heparin plasma, LPL activity represents enzyme that, having fulfilled its role in the capillaries of the tissue, travels and binds to lipoproteins, usually LDL [16], until it is degraded in the liver. In this paper, we present the novel finding that pre-heparin plasma LPL activity in vivo in humans under surgical stress correlates with the plasma level of cortisol.

Therefore, LPL is probably released from white adipose tissue, which reduces its functional activity and thus the uptake of circulating TAG, although further studies in humans are needed to ascertain whether occurs. The TAG could then be taken up by other tissues, such as skeletal muscle or heart, as energy fuel. Some authors have reported that during short-term psychological stress in humans, TAG, TC, and cLDL rise [17]. Moreover, the clearance rate of fat emulsion decreases, possibly due to a reduction in the activity of LPL, which is the rate-limiting enzyme for the catabolism of triacylglycerol-rich lipoproteins [18] Brindley et al [19]. Point out that hypertriglyceridemia may occur during stress because of the decreased activity of LPL in adipose tissue. Nevertheless, Rosmond et al [20], reported that stress-related cortisol secretion in humans was positively associated with cLDL and glucose, but negatively associated with TAG, IGF-1, and HDL. However, other authors [21] have noted a decrease in plasma glucose during surgical stress, which contrasts not only with previous results but also with our own. Other authors have not seen any correlation between plasma pre-heparin lipolytic activities (LPL and HL) and TAG or cHDL activities; a correlation has been seen only with cLDL [8]. Watson et al [15], Observed a positive correlation between pre-heparin plasma LPL and VLDL cholesterol and FFA. Pre-heparin HL was correlated with body mass index, waist:hip ratio, and the concentrations of HDL cholesterol, HDL2, and the small dense LDL.

In our previous research, we saw that changes in the LPL enzyme in pre-heparin plasma reflect the response of tissues to the somewhat rapid changes that occur in response to physiological situations in rodents. Thus, we indicated that LPL increases in pre-heparin plasma in vivo during the surgical stress of a partial heptectomy in rats [22], while LPL decreases in adipose tissue. During stress induced by immobilization of rats [5,23], and during social stress induced in mice, we observed similar results [24]. We reported that in rats that were fed a standard diet, acute and chronic stress produced by body immobilization induced strong responses in both lipid (increases in plasma non-esterified fatty acid and glycerol) and lipoprotein (a decrease in plasma TAG and an increase in total cholesterol) metabolism [5,23]. These changes suggest that catecholamines and glucocorticoids, which are synthesized and segregated under such conditions, may alter LPL activity directly or indirectly, depending on the tissue [25].

HL levels also rise in plasma after surgery. Again, this increase may be due to release of the enzyme into the plasma, in this instance from the liver, as we have described in rats under surgical stress [26] or after the administration of catecholamines [6]. In these situations, the HL enzyme is transported to the adrenals (probably bound to HDL), which show high levels of activity even though they cannot synthesize the enzyme. This enables the adrenals to take up HDL cholesterol in order to synthesize glucocorticoids [12]. Other authors have suggested that during stress the increase in glucocorticoids reduces HL activity in the liver [24]. Some authors have reported that transgenic mice that over express the human HL gene have an increase of 25-50% in HL activity in post-heparin plasma, while a decrease in total plasma cholesterol of 80-85% was observed [28]. We observed a 59% increase and a 36% decrease in HL activity in pre-heparin and total plasma cholesterol levels, respectively. The pre-heparin HL probably reflects not only the lipolytic capacity of the enzyme, but also its actions on plasma lipoproteins.

Despite the absence of a consensus on the relationship between plasma pre-heparin lipolytic activities (although neither exists regarding the post-heparin) and lipoprotein parameters, it seems that there is evidence 1) that cholesterol metabolism in rodents and humans is significantly different [in rodents, cholesterol transport is carried out mainly through HDL, and LDL are virtually non-existent [24].]. Therefore, the effects of stress on lipoproteins may be different; 2) that in both species, increases in corticosterone (rodents) or cortisol (humans) are due to a release of the enzyme LPL (and also HL) in plasma; and 3) that the release of these enzymes from anchorage in their respective tissues causes notable changes in the composition of lipoproteins.

**Conclusion**

Surgical stress in humans releases into pre-heparin plasma the enzymes LPL and HL, which alter lipid metabolism, similarly to experimental animals. The method used to measure the activities of these enzymes in pre-heparin plasma was sensitive enough to observe metabolic changes.

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