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Low molecular weight heparin-induced increase in chylomicron-remnants clearance, is associated with decreased plasma TNF-α level and increased hepatic lipase activity

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ABSTRACT

Objective: The acute effect of heparin on lipoprotein clearance is well characterized. Yet, the effect of prolonged low-molecular-weight-heparin (LMWH) administration on post-prandial lipemia has remained so far unexplored. Recent reports suggest that LMWH could modify lipid and carbohydrate metabolism by diminishing TNFα-mediated inflammatory response. This, together with the known negative effect of TNF-α on insulin sensitivity, prompted us to hypothesize that LMWH would favorably affect post-prandial lipoprotein disposal.
Methods: Twenty four patients were given a vitamin A-fat loading meal at the end of 6-week enoxaparin treatment and after 3-month washout period. Post-prandial lipemia was assessed by measuring retinyl-palmitate (RP) during 8 hours following the meal. Insulin sensitivity index (ISI), plasma lipolytic activity and plasma TNF-α were measured.
Results: Enoxaparin did not impact fasting plasma lipids and lipoproteins levels. Enoxaparin increased RP clearance in the chylomicron remnant (CMR) fraction by 32% ($P < 0.01$). Additionally, enoxaparin decreased plasma TNF-α by 22% ($P < 0.01$), increased hepatic lipase (HL) activity by 81% ($P < 0.01$), along with a 2-fold increase in ISI ($P < 0.01$). The decrease in CMR correlated with the reduction in TNFα and the increase in ISI and HL activity ($R = 0.48, -0.68, -0.56$, respectively, $p < 0.05$). Significant correlations were also found between the reduction in TNFα and both the increase in ISI and increase in HL activity ($R = -0.43, -0.54$, respectively, $P < 0.05$).
Conclusions: The association of the effect on post-prandial metabolism, plasma TNFα level and HL activity during prolonged enoxaparin treatment may support the hypothesis that the beneficial outcome of enoxaparin may possibly be linked to anti-inflammatory and lipase-potentiating impact.
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Introduction

A substantial proportion of patients with coronary artery disease (CAD) manifest an array of metabolic abnormalities, including glucose intolerance, low high-density lipoprotein cholesterol, increased small dense low-density lipoprotein (LDL) particles, and exaggerated post-prandial lipemia. Insulin resistance was implicated as the pivotal player in the emergence of this cluster [1]. Intestinally-derived triglyceride-rich lipoproteins (TRL) i.e. chylomicrons (CM) and chylomicron remnants (CMR) make up a significant part of post-prandial lipemia. Unlike CM and large very-low-density lipoprotein (VLDL) particles, CMR are able to migrate through the endothelial layer and enter the subintimal space [2,3] where they promote the formation of atherosclerotic plaques [4]. One remnant particle that enters the intima, delivers from 5- to 20-fold more cholesterol than each LDL particle, and is therefore considered more atherogenic [5]. During the past 3 decades, a substantial body of evidence has accumulated supporting the role of intestinally-derived TRL in the pathogenesis of atherosclerosis [6,7]. Indeed, several studies recently demonstrated the association of post-prandial lipemia with increased risk for CAD [8–10].

In many cases, heparin is included in the standard treatment protocol for CAD and a plethora of other surgical and medical conditions [11]. The lipolytic effect of acute intravenous administration of heparin was established some 50 years ago when it was shown that heparin displaces lipoprotein lipase from the endothelium [12]. Evidence from recent trials strongly support the preferential use of low molecular weight heparin (LMWH) in a variety of clinical settings including

Abbreviations: AUC, area under the curve; CAD, coronary artery disease; CM, chylomicron; CMR, chylomicron remnants; HL, hepatic lipase; hsCRP, high sensitive C-reactive protein; ISI, insulin sensitivity index; LDL, low density lipoprotein; LMWH, low molecular weight heparin; LPL, lipoprotein lipase; RP, retinyl palmitate; TNF-α, tumor necrosis factor alpha; TRL, triglyceride rich lipoprotein; VLDL, very low density lipoprotein.

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acute coronary syndrome [13–16]. The few works that investigated the effect of LMWH on lipolysis were carried out uniquely on patients undergoing hemodialysis. This is exceedingly remote from the vast majority of clinical settings in which LMWH is administered for preventive and therapeutic purposes. Hence, characterization the effect of LMWH on plasma lipids and lipoproteins in setting other than hemodialysis may add important information on the action of this commonly used medication. Additionally, since prolonged circulatory residence time of intestinally-derived TRL was shown to be associated with CAD even if fasting lipids were within normal limits [8] we reasoned that exploring the role of LMWH in metabolism of post-prandial lipemia may provide some clinically relevant novel data.

Recently, LMWH was reported to attenuate inflammation [17] and a few *in vitro* and *in vivo* studies showed that it decreased plasma level of the proinflammatory cytokine TNFα [18–21]. The crucial role of TNFα in deterioration of insulin resistance is well documented [22]. Notably, Skoog et al. showed that plasma TNFα level were associated with a higher degree of alimentary lipemia, increased plasma VLDL triglycerides, and insulin resistance [23]. The recent reports on the favorable effects of LMWH on inflammation and particularly on TNFα, led us to hypothesize that by decreasing TNFα level, LMWH may expedite postprandial lipoprotein catabolism. The scarce data on the effect of LMWH on plasma lipids/lipoproteins prompted us to test this hypothesis by challenging patients with a fatty meal during and subsequent to enoxaparin treatment in order to monitor the kinetics of intestinally-derived TRL clearance and TNFα level.

Methods

Patients

The institutional ethical committee approved the study protocol, and participants gave written informed consent before entering the study. Consecutive patients undergoing coronary catheterization were asked to participate in the current study if their angiogram was normal, i.e. there was no indication for revascularization. Specifically, up to 7 days after patients had first presented with clinical manifestation suggestive of anginal syndrome and were started on subcutaneous enoxaparin, they underwent coronary catheterization. Patients with normal coronary arteries or non-significant CAD were asked to continue subcutaneous enoxaparin 1 mg/kg/day for 6 additional weeks while all medications but enoxaparin were discontinued.

Patients were included in the study if their fasting blood glucose was less than 6 mmol/l, fasting total cholesterol and triglyceride levels were less than 6.2 and 2.3 mmol/l, respectively, and if they had no clinical and biochemical evidence of liver, kidney or endocrine disease. Patients were excluded if they had acute coronary event, overt heart failure, bleeding diathesis, were treated with statins, calcium channel blockers or ACE inhibitors. At the end of a 6-week treatment period with subcutaneous enoxaparin, participants underwent laboratory tests, including fasting glucose, fasting lipid profile and a standard glucose tolerance test followed by a vitamin A-fat loading test. A second set of laboratory tests, an oral glucose tolerance test and vitamin A-fat loading was performed 3 months after the end of enoxaparin treatment. Participants were discharged from the hospital within 48 hours of initiation of enoxaparin and were instructed to resume their normal daily activity and regular nutrition within the following 3 days.

Vitamin A-fat Loading Test

Vitamin A-fat loading test was performed as previously described [24]. Briefly, after a 12-h overnight fasting, the subjects ate a fatty meal plus 60,000 U of aqueous vitamin A/m^2 body surface. The fatty meal contained 50 g of fat/m^2 body surface, consisting of 65% calories as fat, 20% as carbohydrate and 15% as protein. It contained 600 mg cholesterol/1000 calories, and the P/S ratio was 0.3. This was given as a milkshake, scrambled eggs, bread, and cheese, and was consumed in 10 min. The vitamin A was added to the milk shake. After the meal the subjects fasted for 8 h, but drinking water as desired was allowed. Blood samples were drawn before the meal, 1, 2, 3, 4, 5, 6 and 8 h after the meal. Vitamin A is known to specifically label intestinally derived TRL with retinyl palmitate (RP).

Analysis of Samples

Isolation of lipoproteins and the RP assay were performed as described elsewhere [24].

Lipid and Lipoprotein Determinations

Cholesterol and triglycerides were measured enzymatically using the reagents cholesterol 236991 and triglyceride 126012 (Boehringer Mannheim, Indianapolis, IN, USA). HDL-cholesterol was determined after precipitation of whole plasma with dextran sulfate magnesium. LDL-cholesterol concentration was calculated using the Friedewald's equation.

Determination of Lipase Activity

Lipase activity was measured prior to glucose tolerance test according to Nilsson-Ehle and Schotz [25]. Briefly, [9,10-^{3}H]-Triolein (American Radiolabeled Chemicals, Inc. Saint Louis, MO, USA) was mixed with unlabeled triolein (600 mg; 2500 X 10^6 cpm) and lecithin (36 mg) was added. After evaporation of the solvents emulsification in glycerol (10 mL) was carried out by homogenization on ice. Immediately before the assay substrate was diluted in 4 volumes of 0.2 M Tris–HCl buffer (pH 8.0) containing 3% bovine serum albumin (essentially free from fatty acid). Incubation at 37 °C was started by addition of 0.1 mL of pre- or post-heparin plasma to 0.1 mL substrate. After 20 minutes the reactions were stopped by addition of methanol-chloroform-heptane 1.41: 1.25: 1 (3.25 ml) followed by 1.0 mL of 0.1 M potassium carbonate-borate buffer (pH 10.5). After vigorous mixing, the tubes were centrifuged for 15 min at 3000 g. A 1-mL aliquot of the methanol–water upper phase was counted to determine the [^{3}H]-oleic acid released. This assay measured total plasma lipolytic activity. Preincubation of plasma samples with anti-hepatic lipase monoclonal antibody (XHL1-1C, cat # sc-21741, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) diluted 1:500 (10 μL/sample) at 0 °C for 1 hour to inactivate HL, selectively measured LPL activity. HL activity was calculated as the difference between total plasma activity and activity measured in the presence of the antibody. To validate the inhibitory effect of XHL1-1C antibody, HL activity was measured in normal plasma after inactivation of LPL either by removal of apoCII from the assay mixture or by incubation of plasma samples in 1 M NaCl (for 10 minutes at 0 °C). Preincubation of samples with XHL1-1C anti-hepatic lipase antibody decreased HL activity in these assays to less than 3% of initial activity. We considered this to be complete inactivation of HL by the antibody. Assays with XHL1-1C antibody had intra-assay coefficient of variation of 2%-3% and inter-assay coefficient of variation of 5%-8%.

Determination of Blood Glucose and Insulin

Glucose levels were measured using an enzymatic kit (number 315–100; Sigma-Aldrich). Plasma insulin concentration was measured by radioimmunoassay using a commercial kit (SRI-13 K) obtained from Linco Research (St. Charles, MO, USA). The insulin sensitivity index (ISI) was calculated according to Matsuda and DeFronzo [26], i.e., 10,000/square root of (fasting glucose x fasting insulin) x (mean glucose x mean insulin during the oral glucose tolerance test).

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