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Heparin, lipoproteins, and oxygenated fatty acids in blood: A cautionary note ☆

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Abstract

We measured 16 nonesterified oxygenated fatty acid derivatives (oxylipids) in plasmas from seven human subjects. Two arterial samples from each subject were analyzed, drawn approximately 2 h apart. We observed a marked increase in levels of most oxylipids in the second sample, as high as 470-fold. Between the first and second samples, subjects received approximately 800–1000 IU of heparin to prevent clotting in intravascular catheters. We postulate that heparin activated lipoprotein lipases, which, in turn, released oxylipids from triglycerides and phospholipids in plasma lipoproteins. Some of that lipolysis may have occurred during sample storage. Measurements of nonesterified lipids in human plasma may be distorted if heparin is administered to subjects before blood is drawn and if lipase inhibitors are omitted from stored samples.
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1. Introduction

Oxygenated derivatives of long-chain fatty acids, including oxygenated linoleic and linolenic acids, eicosanoids, and docosanoids, (broadly referred to as oxylipids), are known to be incorporated into the complex lipids of blood cells and lipoproteins. Heparin is often administered to humans and animals to prevent clotting during treatments or experiments. Heparin activates lipases that enter the circulation from binding sites on endothelium. Those lipases, in sum, have a broad range of specificities, not restricted to simple fatty acid esters. We encountered a remarkable increase in apparent levels of some nonesterified oxylipids in plasma samples drawn from subjects who received heparin to maintain the patency of indwelling intravascular catheters. It was our intention to measure arteriovenous differences to estimate the contribution of kidneys and liver to the synthesis and degradation of selected oxylipids, but the experiment was confounded by the differences between pre- and post-heparin levels. Our observation can serve as a warning to those studying nonesterified lipids in the circulation: Do not administer heparin before drawing blood. Other implications of our results are discussed.

2. Subjects and methods

During an experiment to study catecholamine and palmitate turnover in obesity, lean and obese women

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were fasted overnight, then admitted to a clinical research unit where catheters were placed in one peripheral artery, a renal vein, and the hepatic vein. During the placement of the catheters and subsequent manipulations, boluses of heparin in saline were infused to maintain patency. All aspects of this experiment were approved by Institutional Review Boards at the relevant facilities.

The lipid measurements reported here were performed on two samples drawn from the peripheral arterial catheter; the first was collected after infusion of approximately 75 units of heparin into that catheter. The second sample was collected 90–160 min later, after a total of approximately 800–1000 units of unfractionated heparin had been infused, divided among the three catheters. No other drugs were administered to the subjects, except for two radiolabeled compounds, norepinephrine and palmitic acid, and the dose of norepinephrine was far below that required for an effect on adult humans. No physiologic effect of either tracer was noted.

Blood was collected in EDTA, centrifuged within 30 min, and the plasma stored at −80 °C for 3–6 years. Stored plasma samples were thawed periodically to remove aliquots, then re-frozen. For the lipid measurements reported here, plasma was thawed at 4 °C, and a mixture of surrogates and deuterated standards was added [1]. Aliquots of 0.25 ml were acidified with an equal volume of acetic acid (0.1%) in 5% methanol and applied to small Waters "Oasis" columns containing 60 mg of a reverse-phase adsorbent (Waters "HLB"). After a wash with the acetic acid/5% methanol mixture, lipids were eluted with pure methanol followed by ethyl acetate. The solvent was removed in a vacuum centrifuge. The residue was analyzed by liquid chromatography/mass spectroscopy as described, using the internal standards to calculate recovery [1].

3. Results

Concentrations of 16 lipids in the first and second arterial plasma samples are listed in Table 1 as averages of seven subjects. Also listed are ratios of second-to-first concentrations. All lipids from all seven subjects were higher in the second sample than their first. Increases in average values ranged from 1.22- to 470-fold. The magnitude of increase, expressed as a ratio, was independent of the concentration of the lipid in the first sample. Monohydroxy- and oxo- derivatives of linoleic and arachidonic acids exhibited the largest increases in the second samples. Within those classes, positional isomers differed markedly in their responses.

4. Discussion and conclusions

We observed marked increases in many nonesterified oxylipids in arterial plasma samples drawn from humans after they received infusions of heparin totaling 800–1000 units over approximately 2 h. Although these samples were drawn during an experiment whose aim was unrelated to oxylipids, the experimental interventions—apart from heparin—involved injection of only trace amounts of two radiolabeled compounds, norepinephrine and palmitic acid, and withdrawal of blood samples. During that time, the subjects were at rest in the supine position. Although there must have been some element of stress involved in the protocol, no physiologic manifestations of stress or drug were noted.

Table 1
Oxylipids in arterial plasma before and after heparin

Compound (abbreviation)	Art 1 (SEM) nM	Art 2 (SEM) nM	Ratio Art 2/Art 1
5-Hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE)	7.65 (1.59)	3619.1(183.4)	471
9-Hydroxy-5,7,11,14-eicosatetraenoic acid (9-HETE)	1.29 (0.20)	438.9(73.6)	341
11-Hydroxy-5,8,12,14-eicosatetraenoic acid (11-HETE)	1.51 (.020)	426.1(82.6)	283
12-Hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE)	6.47 (1.43)	285.8(60.1)	44
15-Hydroxy-5,8,11,13-eicosatetraenoic acid (15-HETE)	2.52 (.030)	325.5(62.8)	129
15-Oxo-5,8,11,13-eicosatetraenoic acid (15-oxo-ETE)	0.56 (0.15)	134.9(28.4)	240
5(6)-Epoxy-8,11,14,17-eicosatetraenoic acid (5(6)-EET)	6.06 (2.08)	17.4(4.7)	2.9
5,6-Dihydroxy-7,9,11,14-eicosatetraenoic acid (5,6-DHET)	0.88 (0.34)	7.1(1.0)	8.1
6-Oxo-9,11,15-trihydroxy-prost-13-enoic acid (6-keto-PGF1α)	2.16 (0.20)	11.3(1.0)	5.2
11,12-Dihydroxy-5,8,14-eicosatrienoic acid (11,12-DHET)	0.74 (0.08)	0.9(0.1)	1.2
14,15-Dihydroxy-5,8,11-eicosatrienoic acid (14,15-DHET)	0.75 (0.08)	1.3(0.1)	1.7
9-Hydroxy-10,12-octadecadienoic acid (9-HODE)	18.91 (1.78)	2636.3(294.8)	139
13-Hydroxy-9,11-octadecadienoic acid (13-HODE)	17.80 (2.22)	2468.6(261.2)	139
9-Oxo-10,12-octadecadienoic acid (9-KODE)	3.09 (0.73)	660.6(73.3)	214
13-Oxo-9,11-octadecadienoic acid (13-KODE)	22.09 (6.93)	1928.0(220.2)	87
12,13-Epoxy-9-keto-10(trans)-octadecenoic acid (EKODE)	7.02 (0.45)	14.9(1.1)	2.1

Our hypothesis is that it was the heparin, not the passage of 2 h or any other manipulation, that caused the increase in circulating nonesterified lipids.

The relationship of dose and route of administration of heparin to the rise in oxylipids was not explored in our study. All of the anticoagulant was administered intravenously or intra-arterially. Our subjects received about 75 units of heparin before the first sample was drawn. If that dose had an effect on nonesterified oxylipids, the first arterial value would be greater than basal, and the ratios of post-heparin to basal would be much greater than those listed. In subjects from another cohort, who received only 80 units of heparin, we observed a much smaller increase in the same oxylipids measured for this report, (data not shown). We conclude that the threshold dose for a significant increase in oxylipid de-esterification is between 100 and 1000 units.

We have no knowledge of the duration, in vivo, of the increases in oxylipids we have found, nor the extent to which repeated heparin injections would cause repeated increases in those lipids. Presumably, diet and age would impact on the loading of lipoproteins with oxylipids, as indicated by the work of Spiteller [2–4]. Body habitus probably plays a role, too. In the cohort we studied, three women were obese, and four were lean. The obese subjects showed, on average, a greater increase in oxylipids after heparin administration than did their lean counterparts (data not shown). Disease states undoubtedly alter quantities of oxylipids in lipoproteins. Kreil et al. [5] observed an increase in lipoprotein oxylipids after hemorrhagic shock in human patients. Newman et al. [6] have shown that experimental nephrosis in rats markedly increased the oxylipid content of lipoproteins.

The most likely mechanism by which heparin would increase circulating levels of nonesterified oxylipids is by activating lipases released from binding sites on vessel walls. If this postulated mechanism is correct, the results indicate the presence of large amounts of some oxylipids in glycerides circulating in lipoproteins. Many other investigators have found significant amounts of esterified oxylipids in lipoproteins, and their presence has been postulated to play a role in atherogenesis [7–9]. The susceptibility of various oxylipid esters to various lipases has not been published, and we do not know what proportion of total esterified oxylipids was liberated during our experiment.

Because the blood samples were not drawn specifically to study oxylipids, precautions were omitted that might have altered the results. For example, no antioxidants were added, and no attempt was made to exclude oxygen or light from the samples, so nonesterified fatty acids could have undergone autoxidation during storage. That would not explain the difference between levels in samples drawn after heparin administration and those drawn before unless heparin released large amounts of polyunsaturated fatty acids; however, when we measured total nonesterified fatty acids in the plasma samples, levels in the second set were only 15% higher than in the first, (data not shown).

Lipases could have acted upon plasma lipoproteins after the blood sample was drawn, releasing oxylipids as they do native fatty acids during handling and storage [10]. No lipase inhibitors were added to our samples to prevent this artifact, and the temperature of the plasma during repeated freeze-thaw cycles was not carefully controlled.

The fate and effects of circulating oxylipids, like those of their fatty acid precursors, are dependent on the degree to which they are esterified and released, processes that are tissue-specific. Our data indicate the large impact lipoprotein lipases must have on oxylipid biology. Furthermore, lipoproteins apparently carry a large amount of biologically active lipids into cells. This may account for some of the direct effects of lipoproteins. For example, we are interested in the effects of EKODE (see Table 1) and other oxylipids on steroidogenesis by adrenal cortical cells [11,12]. Lipoproteins are known to carry cholesterol to adrenal cells where it is converted to steroids; our observations suggest that the macromolecules may also carry regulators of that process.

If our proposed explanation for the increased oxylipid levels is correct, it may help explain some of the properties of heparin aside from its role as an anticoagulant [13]. It remains to be seen whether low-molecular-weight heparin has the same effect on oxylipid levels as the unfractionated heparin we used.

Our findings provide a warning against administering heparin before drawing blood for measurement of nonesterified oxylipids in plasma. We also strongly suggest that lipase inhibitors be added to all plasma samples drawn for these purposes; tetrahydrolipstatin (Orlistat) and paraoxon are available for that purpose, but the former is easier and safer to handle [14].

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