Appraisal of hepatic lipase and lipoprotein lipase activities in mice

G. M. Dallinga-Thie,[1,*] A. J. Zonneveld-de Boer,[†] L. C. van Vark-van der Zee,[§] R. van Haperen,[**] T. van Gent,[**] H. Jansen,[†] R. De Crom,[**] and A. van Tol[**]

Department of Vascular Medicine,* Academic Medical Center, Amsterdam, The Netherlands; and Department of Clinical Chemistry,[†] Department of Vascular Medicine,[§] and Department of Cell Biology and Genetics,** Erasmus University Medical Center, Rotterdam, The Netherlands

Abstract **A variety of methods are currently used to analyze HL and LPL activities in mice. In search of a simple methodology, we analyzed mouse preheparin and postheparin plasma LPL and HL activities using specific polyclonal antibodies raised in rabbit against rat HL (anti-HL) and in goat against rat LPL (anti-LPL). As an alternative, we analyzed HL activity in the presence of 1 M NaCl, a condition known to inhibit LPL activity in humans. The assays were validated using plasma samples from wild-type and HL-deficient C57BL/6 mice. We now show that the use of 1 M NaCl for the inhibition of plasma LPL activity in mice may generate incorrect measurements of both LPL and HL activities. Our data indicate that HL can be measured directly, without heparin injection, in preheparin plasma, because virtually all HL is present in an unbound form circulating in plasma. In contrast, measurable LPL activity is present only in postheparin plasma. Both HL and LPL can be measured using the same assay conditions (low salt and the presence of apolipoprotein C-II as an LPL activator). Total lipase activity in postheparin plasma minus preheparin HL activity reflects LPL activity. Specific antibodies are not required.**—Dallinga-Thie, G. M., A. J. Zonneveld-de Boer, L. C. van Vark-van der Zee, R. van Haperen, T. van Gent, H. Jansen, R. De Crom, and A. van Tol. **Appraisal of hepatic lipase and lipoprotein lipase activities in mice.** ***J. Lipid Res.*** **2007.** 48: **2788–2791.**

Supplementary key words high salt • preheparin plasma • postheparin plasma

LPL and HL play an essential role in lipoprotein metabolism. Hepatic lipase is a lipolytic enzyme synthesized and secreted by hepatocytes that binds extracellularly to parenchymal and endothelial cell surfaces in the space of Disse (1, 2). HL affects the lipid composition of all lipoprotein classes by hydrolyzing phospholipids and triglycerides. It has been demonstrated that mice have HL circulating in preheparin plasma (3), suggesting that the affinity between mouse HL and its cellular binding sites is low. In contrast, rat and human preheparin plasma do not contain any measurable HL activity (3).

LPL is synthesized by parenchymal cells in a variety of tissues, including adipose tissue, skeletal muscle, and heart, and is subsequently translocated to its site of action, the endothelium (4). The major target lipoprotein is the triglyceride-rich apolipoprotein B containing VLDL and chylomicrons. LPL hydrolyzes triglycerides into free fatty acids for direct use in muscle and adipose tissue (5, 6). As a result of the action of LPL, VLDL, and chylomicrons will become triglyceride-depleted, cholesterol-enriched lipoprotein particles, which are potentially atherogenic. LPL may act as a bridging molecule between these atherogenic lipoproteins and hepatic receptors (7, 8).

The mouse is a frequently used animal model in atherosclerosis research, even though large differences exist in lipoprotein metabolism between mice and humans. However, the possibilities to create specific genetic models by overexpression, deletion, specific inducible gene expression, and specific tissue expression of a target gene have led to a spectacular increase in the use of transgenic mice. Recently, newly identified proteins turned out to have LPL-regulating properties [e.g., apolipoprotein A-V (9), the angiopoetin-like proteins (10, 11), and the recently discovered glycosylphosphatidylinositol-anchored HDL binding protein 1 (12)]. This further emphasizes the need for accurate and relatively simple assays of LPL and HL activities.

In humans, LPL and HL are bound to the vascular endothelium, and their activities have been analyzed in plasma obtained from blood samples collected after intravenous injection of heparin, a procedure to release LPL and HL from cellular proteoglycans. Total lipase activity is then analyzed in the presence of the LPL activator apolipoprotein C-II and at low salt concentrations. LPL activity may be inhibited by high salt (1 M NaCl)

Manuscript received 27 July 2007 and in revised form 17 August 2007 and in re-revised form 4 September 2007.

Published, JLR Papers in Press, September 13, 2007.
DOI 10.1194/jlr.D700021-JLR200

[1] To whom correspondence should be addressed.
e-mail: g.m.dallinga@amc.uva.nl

in the assay mixture, thereby enabling the measurement of HL activity (13). In addition, specific antibodies have been used to inhibit either LPL activity or HL activity in human postheparin plasma (14, 15).

In the present study, we compare two approaches to analyze HL and LPL activity in mouse plasma. In the first approach, we use specific antibodies to inhibit either LPL or HL activity. In the second approach, we test the use of 1 M NaCl to inhibit LPL activity, thereby enabling the assay of HL activity.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice were housed in a temperature-controlled room operating under a 12 h/12 h light/dark cycle. Animals were fed a regular chow diet and obtained water ad libitum. HL-deficient mice with a C57BL/6 background were obtained from the Jackson Laboratories. Preheparin blood was obtained by orbital puncture under isoflurane anesthesia. Postheparin blood was obtained at 5 min after heparin injection (200 U/kg body weight injected into a tail vein). Plasma was isolated by centrifugation at 3,000 rpm for 15 min and frozen at −80°C before analysis of lipase activities. Plasma LPL activity was determined in both preheparin and postheparin plasma obtained from three individual mice with hepatic lipase deficiency. Ten control C57BL/6 mice were used to create a pool of both preheparin and postheparin plasma. All animal experiments were performed in accordance with institutional and national guidelines.

Antibodies

Rat HL and LPL were isolated as described (16–18). Polyclonal antibodies against rat HL and LPL were generated in rabbit (anti-HL) and goat (anti-LPL). The IgG fractions of both antibodies were isolated by protein G affinity chromatography according to the manufacturer's procedure. The IgG fraction was dialyzed against 5 mM $(NH_4)HCO_3$ and lyophilized. The obtained pellets were resuspended in 0.15 M NaCl and stored at −80°C (17). Total protein concentration was 18.7 g/l in the anti-HL preparation and 95.3 g/l in the anti-LPL preparation.

Treatment of plasma with antibodies

Immunoinhibition of HL and LPL was accomplished by mixing a mouse plasma sample with anti-LPL and anti-HL and subsequent incubation at 4°C for 2.5 h. As a control, plasma was incubated with a saline solution. For LPL analyses, 25 μl of plasma was incubated with 6.25 or 25 μl of anti-HL in a total volume of 125 μl. For HL analyses, 25 μl of plasma was incubated with 25 or 50 μl of anti-LPL in a total volume of 125 μl. Subsequently, after the preincubation, 50 μl of the mixture was used in the lipase assay.

Lipase assay

Preheparin and postheparin total lipase activities were assayed using an artificial glycerol-[1-^{14}C]trioleate (Sigma T-7140)-containing lipid emulsion (13, 19). A mixture of 20 μCi of [1-^{14}C]trioleylglycerol and 35 mg of unlabeled trioleylglycerol was dried under nitrogen and emulsified by sonification in 2.5 ml of 5% (w/v) gum acacia (BDH Chemicals) containing 1% $NaHCO_3$ (pH 7). The emulsion was mixed with 3 ml of 10% (w/v) fat-free BSA (Fluka BioChemika 05490), 0.5 ml of 1 M Tris-HCl (pH 8.5), and 0.5 ml of 2.5 M NaCl + 4.5 ml of water (low-salt assay) or 5 ml of 2.5 M NaCl (high-salt assay). Lipase activity was analyzed in the presence of heat-inactivated normolipidemic human plasma as a source of apolipoprotein C-II (10%, v/v) (20). A substrate mixture (200 μl), a 10 μl serum sample, and 40 μl of 0.9% NaCl were incubated for 30 min at 30°C. The reaction was stopped by adding 3.25 ml of a mixture of chloroform-methanol-heptane (33:40:27) and 1.05 ml of 0.1 M K_2CO_3 and 0.1 M H_3BO_3, pH 10.5, and putting the tubes on ice. Free fatty acids were measured in the water phase. Lipase activities are given in mU/ml, where 1 mU represents 1 nmol of free fatty acid released from the triglyceride substrate per minute at 30°C.

Statistical analyses

Lipase values are expressed as means ± SD and were compared using *t*-test statistics.

RESULTS AND DISCUSSION

It is generally accepted that lipase activity in murine preheparin plasma represents HL activity (3), reflecting its low affinity for heparin sulfate proteoglycans. Virtually zero lipase activity was measured in preheparin plasma from HL-deficient mice (data not shown). Subsequently, we analyzed preheparin lipase activity in the presence of different antibodies. First, it was observed that incubation with the anti-LPL antibody does not affect lipase activity in preheparin plasma from wild-type mice (**Fig. 1**). Hence, this antibody does not interfere with mouse HL activity, illustrating its specificity. The efficacy of the antibody directed against HL (anti-HL) to inhibit its activity was tested using the same batch of preheparin plasma. Figure 1 shows that the total lipase activity in preheparin plasma of wild-type mice was 92 ± 2 mU/ml. Treatment with anti-LPL did not result in a significant change in total lipase activity (86 ± 3 mU/ml), whereas treatment with anti-HL inhibited lipase activity by 97% (remaining activity, 3 ± 2 mU/ml). Different antibody dilutions were tested. These experiments illustrate the specificity of the antibody and dem-

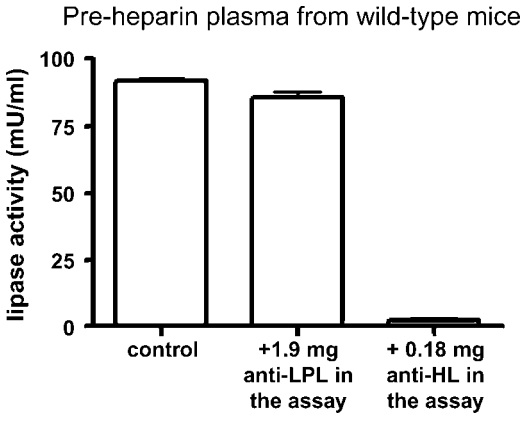

Fig. 1. Effects of anti-LPL and anti-HL antibodies on lipase activities in preheparin plasma from wild-type mice. Experiments were performed in triplicate. The indicated amounts of IgG (mg) were present in the lipase assay. Values are expressed as means ± SD.

onstrate that virtually all lipase circulating in preheparin plasma in mice is indeed HL.

In the experiments described above, all assays were performed at low NaCl concentration. In humans, both HL and LPL activities are present in postheparin plasma, and the contribution of HL can be measured by blocking LPL activity with 1 M NaCl (21). However, in our experiments with mouse plasma, increasing the final salt concentration in the assay mixture to 1 M NaCl led to a significant 61% increase in HL activity in preheparin mouse plasma (from 92 ± 2 to 148 ± 3 mU/ml). Total lipase activity in postheparin plasma of wild-type mice was 717 ± 10 mU/ml (**Fig. 2**). Surprisingly, in the presence of 1 M NaCl, the residual lipase activity remained 579 ± 30 mU/ml. This suggests that either most of the total lipase activity is derived from HL in postheparin mouse plasma or that 1 M NaCl does not completely inhibit mouse LPL. We also have to keep in mind that HL activity is increased in the presence of high salt (see above).

To obtain conclusive evidence regarding whether 1 M NaCl inhibits LPL activity in mouse plasma, we analyzed total lipase activity in postheparin plasma of the HL-deficient mouse with and without 1 M NaCl. In these knockout mice, HL was not present in postheparin plasma. Total lipase activity was 755 ± 17 mU/ml when measured in low NaCl, whereas plasma lipase activity in 1 M NaCl was 456 ± 14 mU/ml (**Fig. 3**). Thus, 1 M NaCl only partly inhibited postheparin plasma lipase activity in HL-deficient mice, suggesting that 1 M NaCl does not completely inhibit mouse LPL. Alternatively, another lipase could contribute to total lipase activity in these animals, although no evidence for an additional lipase in mice could be found (3). Recently, endothelial lipase (EL) was described as a new member of the lipase family (22). Despite the fact that a significant degree of sequence homology exists between EL and LPL, its substrate specificity is quite different (23). In contrast to LPL, which has the capacity to hydrolyze triglycerides, EL is active as a

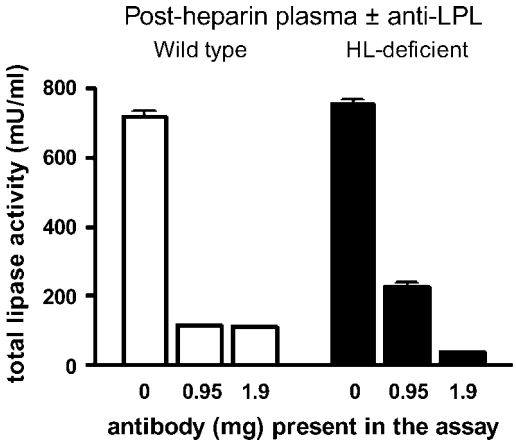

Fig. 2. Effects of incubation with anti-LPL antibody on lipase activities in postheparin plasma from wild-type mice and HL-deficient mice. Experiments were performed in triplicate. The indicated amounts of IgG (mg) were present in the lipase assay. Values are expressed as means ± SD.

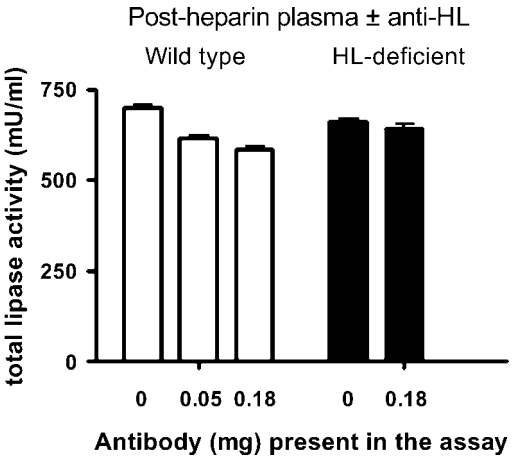

Fig. 3. Effects of incubation with anti-HL antibody on lipase activities in postheparin plasma from wild-type mice and HL-deficient mice. Experiments were performed in triplicate. The indicated amounts of IgG (mg) were present in the lipase assay. Values are expressed as means ± SD.

phospholipase. Because our assay measures the capacity of the enzymes to hydrolyze triglycerides, it is unlikely that we measured EL activity.

In another series of experiments, we used anti-LPL and anti-HL in postheparin mouse plasma of both wild-type animals and HL-deficient animals. All experiments were again performed under low-salt conditions and in the presence of heat-inactivated plasma as a source of apolipoprotein C-II. Incubation of postheparin plasma from HL-deficient mice with anti-LPL resulted in a 95% inhibition of lipase activity, as shown in Fig. 2. Total lipase activity decreased from 755 ± 17 to 37 ± 2 mU/ml. Therefore, it can be concluded that there is no alternative lipase that significantly contributes to postheparin lipase activity other than LPL. When using plasma from wild-type animals, plasma total lipase activity in postheparin plasma after treatment with anti-LPL was inhibited from 717 ± 10 to 110 ± 2 mU/ml. As shown in Fig. 2, no further inhibition can be achieved by increasing the antibody concentration. Figure 3 shows the results of similar experiments with postheparin plasma from wild-type mice, but after treatment with anti-HL. We already showed (Fig. 1) that the decrease in HL activity in preheparin plasma was almost complete under these conditions. In postheparin plasma from wild-type mice, treatment with anti-HL resulted in a decrease of the lipase activity of only ~10% (from 657 ± 10 to 590 ± 8 mU/ml). Therefore, HL activity in postheparin plasma was 67 mU/ml. This value is similar to the preheparin plasma HL activity shown in Fig. 1. Figure 3 shows that little if any effect of anti-HL treatment was evident in postheparin plasma from HL-deficient mice.

It can be concluded that virtually all active HL is circulating in wild-type mouse plasma, even before heparin injection, and that liver-bound HL is hardly present. Based on these results, we propose to measure LPL activity in postheparin plasma by analyzing total lipase activity in the presence of apolipoprotein C-II as activator and under

low-salt concentration. Of course, this activity measured in postheparin plasma has to be corrected for the small amount of HL activity already present in preheparin plasma to obtain the actual postheparin LPL activity. Thus, total postheparin plasma activity minus preheparin plasma activity equals postheparin plasma LPL activity.

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