methods

# Determination of lipoprotein lipase activity using a novel fluorescent lipase assay[S]

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**Abstract A novel, real-time, homogeneous fluorogenic lipoprotein lipase (LPL) assay was developed using a commercially available substrate, the EnzChek lipase substrate, which is solubilized in Zwittergent. The triglyceride analog substrate does not fluoresce, owing to apposition of fluorescent and fluorescent quenching groups at the *sn*-1 and *sn*-2 positions, respectively, fluorescence becoming unquenched upon release of the *sn*-1 BODIPY FA derivative following hydrolysis. Increase in fluorescence intensity at 37°C was proportional to LPL concentration. The assay was more sensitive than a similar assay using 1,2-*O*-dilauryl-*rac*-glycero-3-glutaric acid-(6-methylresorufin ester) and was validated in biological samples, including determination of LPL-specific activity in postheparin mouse plasma. The simplicity and reproducibility of the assay make it ideal for in vitro, high-throughput screening for inhibitors and activators of LPL, thus expediting discovery of drugs of potential clinical value.**—Basu, D., J. Manjur, and W. Jin. **Determination of lipoprotein lipase activity using a novel fluorescent lipase assay. *J. Lipid Res.* 2011.** 52: **826–832.**

**Supplementary key words** Zwittergent • BODIPY • triglyceride

Lipoprotein lipase (LPL), which hydrolyzes triglycerides (TGs) to release free fatty acids (FFAs), is the rate-limiting enzyme controlling plasma TG levels. Hydrolysis of TGs is essential for energy storage and utilization, whereas variations in TG/FFA levels have a variety of clinical implications that have been extensively reviewed, (see, for example, Refs. 1–3). In evaluating LPL function in heparin-treated plasma, measurement of enzyme activity is more valuable than quantification of protein concentration by immunoassay. The latter does not differentiate between: active homodimer and inactive monomer (2, 3); active enzyme and inactive, proteolytically cleaved enzyme (4); the presence or absence of activity-modulating plasma lipid components (5) or proteins of clinical interest such as activators apolipoprotein C-II (apoC-II) (6) and apoA-V (7) and inhibitors apoC-I and apoC-III (8); angiopoietin-like protein-3 (9) and angiopoietin-like protein-4 (ANGPTL4) (10).

The available TG lipase assays have limitations for the determination of plasma LPL activity in one or another of the aspects of sensitivity, substrate stability, and environmental friendliness. For example, the conventional radiometric assay, using $^{3}$H- or $^{14}$C-labeled trioleoyl glycerol as substrate, requires separation of the released labeled FAs, is time-consuming with low throughput, and, particularly owing to the use of radiolabel, is limited to research applications (11). Fluorescence-based assays using coumarin derivatives are attractive because they fluoresce only after conversion to lipolytic products. However, the derivatives are unstable, requiring frequent substrate preparation, and are hydrolyzed nonspecifically by enzymes other than LPL (12). Another commonly used assay, the titrimetric method, is not very sensitive (12).

Heparin-treated plasma contains two additional members of the TG lipase family, hepatic lipase (HL) and endothelial lipase (EL), with biological roles different from that of LPL (13). These also contribute to TG hydrolysis, complicating data interpretation. Strategies that have been employed to dissect out LPL-specific activity have limitations. Under high-salt conditions, LPL activity is inhibited, suggesting that the difference between low- and high-salt measurements will yield LPL-specific activity. However, high salt also affects HL activity (14). Determination of activity in the presence and absence of apoC-II, a specific activator of LPL, is another approach. The limitation of this approach is that the interaction between these two proteins is affected by sphingomyelin (15). A third approach, inhibition of human LPL by the 5D2 neutralizing antibody, has documented complications (16).

Here, we report development of a novel, homogenous LPL TG lipase assay employing a commercially available

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Abbreviations: ANGPTL4, angiopoietin-like protein-4; apoC-II, apolipoprotein C-II; EL, endothelial lipase; FFA, free fatty acid; HIS, heat-inactivated rat serum; HL, hepatic lipase; LPL, lipoprotein lipase; TG, triglyceride; THL, tetrahydrolipstatin.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of three figures.

fluorogenic TG analog, the EnzChek lipase substrate. The kinetics of the assay were fully characterized, and the LPL-specific activity of mouse postheparin plasma was determined using lipase inhibitors.

## MATERIALS AND METHODS

### Materials and reagents

All reagents, including the detergent Zwittergent, which contains an 18-carbon side chain (Sigma), purified bovine LPL, apoC-II, and tetrahydrolipstatin (THL), were purchased from Sigma-Aldrich unless otherwise stated. The specific activity of the LPL used for the experiments was approximately 5,700 U/mg protein. One unit is defined as release of 1.0 nmol of *p*-nitrophenol per min, pH 7.2, 37°C using *p*-nitrophenyl butyrate as substrate. The BODIPY, Dabcyl-labeled TG analog, under the name EnzChek lipase substrate (Invitrogen), and the fluorescent standard 4,4-difluoro-5-methyl-4-bora-3a,4a-diaza-*s*-indacene-3-dodecanoic acid (BODIPY 500/510 C1, C12, abbreviation BODIPY-C12) were purchased from Invitrogen. Heat-inactivated rat serum (HIS) was generated according to standard procedures (11).

### Lipase assay

The SpectraMax M2 plate reader (hardware) and SoftMax Pro 5 (software) from MDS Analytical Technologies were used for fluorescent measurements. To calibrate the instrument for the LPL assay, both excitation and emission sweeps were performed on the BODIPY-C12 probe. The maximum excitation/emission wavelengths were determined to be 482 nm/515 nm with a 495 nm filter cutoff. The upper and lower detection limits for BODIPY-C12 using this assay and instrument are 2.4 μmol/well and 0.1 pmol/well, respectively. A typical assay was carried out at 37°C in a black 96-well plate (Costar) in the presence of 0.15 M NaCl, 20 mM Tris-HCl, pH 8.0, 0.0125% Zwittergent, and 1.5% FA-free BSA in a total volume of 100 μl. A 4× working solution was freshly prepared with 0.6 M NaCl, 80 mM Tris-HCl, pH 8.0, and 6% FA-free BSA. The EnzChek lipase substrate was stored in DMSO at −20°C. The EnzChek lipase substrate solution was freshly prepared in 0.05% Zwittergent for each use, although stable if left at room temperature for at least 10 days. The uncatalyzed hydrolysis rate of the EnzChek lipase substrate was approximately 0.1 pmol/min. The radiometric triolein assay was carried out as described by McCoy et al. (11).

### Conditioned media and mouse plasma

Conditioned media were generated by transfection of HEK293 cells with expression vectors pcDNA-(human LPL) and pcDNA-(human ANGPTL4) as previously described (4). Medium from cells transfected with an empty vector was always included as a control for the LPL assay. Postheparin mouse plasma was obtained from blood drawn 10 min after intravenous injection of 300 units heparin per kg body weight (a proper dose for mouse but very high for human) as described by Yagyu et al. (17).

### Recombinant N-terminal LPL-inhibitory domain of ANGPTL4

The DNA sequence encoding amino acids 26–159 of human ANGPTL4 and containing a cleavable His tag at the N-terminus was cloned into pTrc-His. The expression plasmid was transformed into Lemo21 (NEB) cells, and the protein was purified using a HisPur Ni-NTA column according to the manufacturer's instructions (Pierce).

### 1,2-*O*-dilauryl-*rac*-glycero-3-glutaric acid-(6-methylresorufin ester) assay

As a reference method for the measurement of LPL activity, the 1,2-*O*-dilauryl-*rac*-glycero-3-glutaric acid-(6-methylresorufin ester) (DGGR) assay was carried out as described by Panteghini, Bonora, and Pagani (19). Assay conditions are: 50 mM Tris-HCl (pH 7.4), 0.12 M NaCl, 0.5% Triton X-100, 10 mg/ml BSA.

### Data analysis

The relative fluorescence intensity (RFU/pmol) of the unquenched BODIPY-C12 FA standard was used to calculate the initial velocity of enzymatic reactions producing this molecule as pmol/ml/min. The rate of hydrolysis as determined from the continuous increase in fluorescence intensity was equal to the rate of separation of the *sn*-1 FA acid product from the EnzChek substrate. The enzymatic parameters $V_{max}$, $K_m$, Kcat, detection limit, Z factors, and correlation coefficient were calculated using the Graphpad Prism software. Individual points for kinetic data were presented as the mean ± SD of three determinations. Comparisons between two sets of conditions were analyzed by unpaired Student's *t*-tests; $P < 0.05$ was considered to be statistically significant.

## RESULTS AND DISCUSSION

### Development of a novel fluorescent lipase assay

*Principle.* We sought a commercially available, fluorescent TG derivative as the substrate because a TG-like molecule should be stable and soluble in the conditions under which LPL functions. The EnzChek lipase substrate (hence referred to as the EnzChek substrate) was a very attractive possibility, containing the fluorescent BODIPY-C12 FA derivative in ester linkage at the *sn*-1 position of glycerol, a BODIPY fluorescent quencher FA derivative, Dabcyl, at the *sn*-2 position, and a C-6 FA aliphatic chain in ether linkage at *sn*-3. The BODIPY dye had many fluorescent properties favorable to the assay. These included a narrow emission bandwidth around 515 nm with a high extinction coefficient ($\varepsilon > 80{,}000$ cm-1M-1) and a high quantum yield (often approaching 1, even in water), thus offering a higher sensitivity than pyrene-based fluorescent substrates (Molecular Probes) and a higher throughput than substrates for nonhomogenous assays (11). Its spectrum is relatively insensitive to solvent polarity and does not vary in the physiological pH range, both improvements over nitrobenzoxadiazole-labeled TGs (Molecular Probes). Both it and the Dabcyl quencher are photostable so do not require shielding from light.

Containing both the fluorescent dye and its quencher, the substrate is a stable and nonfluorescent compound. Lipase hydrolysis of the *sn*-1 ester severs the BODIPY-labeled FA, removing it from proximity to the Dabcyl quencher and resulting in bright green fluorescence (see supplementary Fig. I). LPL is known to attack preferentially at position 1 of TGs (19). Thus the EnzChek substrate can be used for the accurate and sensitive detection of LPL activity in solution. A similar type of fluorescence dequenching assay has been developed for LPL using pyrene-labeled TG substrates (20), whereas BODIPY-labeled nonfluorescent phospholipids have been produced

for assaying phospholipase activity (21). We decided to develop conditions using the EnzChek lipase substrate for LPL activity measurements.

*Conditions for using the EnzChek substrate to assay bovine LPL.* Because LPL acts preferentially at a water-lipid interface (22), we hypothesized that the physical state in aqueous solution of the EnzChek substrate would be critical for its use in an LPL assay. As expected, in the absence of detergent, the substrate could not be hydrolyzed by LPL (data not shown). Classically, the detergent Triton X-100 would be the first choice for formation of micelles containing TG that could act as substrate. However, when the EnzChek substrate was solubilized using Triton X-100, the fluorescence of the BODIPY dye was completely unquenched and the intrinsic fluorescent signal from the substrate was very high even in the absence of any lipase (data not shown). Attempts to use glycerol or other detergents, including bile acids and intralipid, to solubilize the substrate were not successful, but none of them unquenched the EnzChek substrate (data not shown). Eventually, the detergent Zwittergent was identified as an effective solvent. Although the exact nature of the interaction between Zwittergent and the substrate is not fully understood, a mixture of the detergent and the substrate would be expected to generate micelles of the type formed by TGs and Triton X-100 (23).

*Characterization of LPL activity with the EnzChek substrate.* Fluorescence increase with time was determined using a fixed amount of LPL (175 ng per well) and increasing amounts of the EnzChek substrate at 37°C in 100 μl total volume in the presence of 0.15 M NaCl, 20 mM Tris-HCl, pH 8.0, 0.0125% Zwittergent, and 1.5% FA-free BSA. Initial rates were then plotted against substrate concentrations and the curve followed Michaelis-Menten kinetics (**Fig. 1**). Nonlinear regression analysis was carried out to calculate the kinetic parameters: $K_m$ = 1.36 μM, $V_{max}$ = 0.89 μmol/ml/min, and Kcat = 0.0255 μmol/ml/min. The uncatalyzed background hydrolysis rate of the EnzChek substrate was very low, approximately 0.1 pmol/min.

To determine the upper limit of the enzyme concentration range where the initial rate was proportional to enzyme concentration, fluorescence increase with time was determined using 0.62 μM EnzChek substrate and increasing amounts of LPL (**Fig. 2**). With this substrate concentration, the time-dependent fluorescence increase was linear at each of the enzyme concentrations tested, including the highest, 1,109 ng/100 μl. Taking advantage of this linearity, the 540 s time point (9 min) in each assay was used to plot initial rate versus LPL concentration (**Fig. 3**, upper plot, the EnzChek substrate assay).

As just demonstrated, when using 0.62 μM EnzChek substrate concentration, the assay can easily be converted into a single-point assay by measuring the fluorescent signal as late as 10 min after the start of the reaction. Of note, the SpectraMax M2 plate reader settings for detection of BODIPY fluorescence are similar to those for measuring green fluorescent protein (Molecular Probes), which is very commonly used in research laboratories. Thus the assay can be easily used in many laboratories.

The effects of components that might be present in biological LPL samples and might affect the assay were evaluated (see supplementary Fig. II). Addition of heparin and calcium increased LPL activity minimally by about 6% over the concentration ranges tested (see supplementary Figs. IIA, B), whereas HIS, added from 0–10%, decreased the activity by 12% (see supplementary Fig. IIC). Addition of NaCl increased the reaction rate dramatically (see supplementary Fig. IID), with the highest rate, 46% higher than that without salt, occurring at 0.15 M. As NaCl was increased further, the reaction rate dropped, but even at 2.5 M was not reduced to that without NaCl. This result is very different from the effect of NaCl on LPL-catalyzed hydrolysis of radiolabeled triolein substrate, where 1 M NaCl inhibited the reaction by 80% (17). The original observation of salt inhibition of LPL is dependent on apoC-II (24). Because there is no apoC-II included in our assay, our results are consistent with the observation that there is no inhibition of LPL activity by NaCl if apoC-II is not used in the assay.

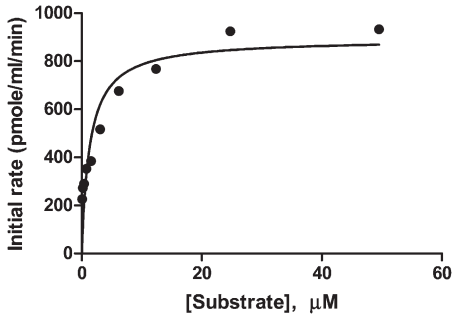


**Fig. 1.** Characterization of lipoprotein lipase (LPL) activity with the EnzChek substrate. Fluorescence increase with time was determined using a fixed amount of LPL (175 ng/100 μl) and increasing amounts of the EnzChek substrate at 37°C in 100 μl total volume in the presence of 0.15 M NaCl, 20 mM Tris-HCl, pH 8.0, 0.0125% Zwittergent, and 1.5% FA-free BSA. Background substrate hydrolysis was deducted from each measurement. Initial rate was plotted against substrate concentration. Each data point is the mean of triplicate determinations.

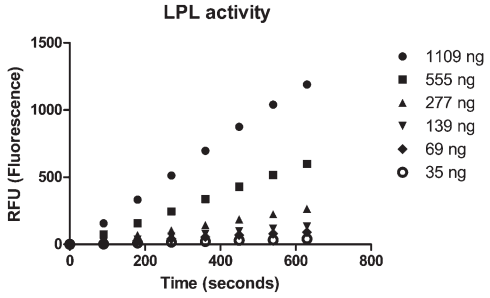


**Fig. 2.** Evaluation of the upper limit of the LPL concentration range that gives linear fluorescence versus time measurements. Reactions were carried out under the conditions described in Fig. 1 using a fixed EnzChek substrate concentration (0.62 μM) and increasing concentrations of LPL (35 ng to 1,109 ng). Background substrate hydrolysis was deducted from all measurements. Each data point is the mean of triplicate determinations.

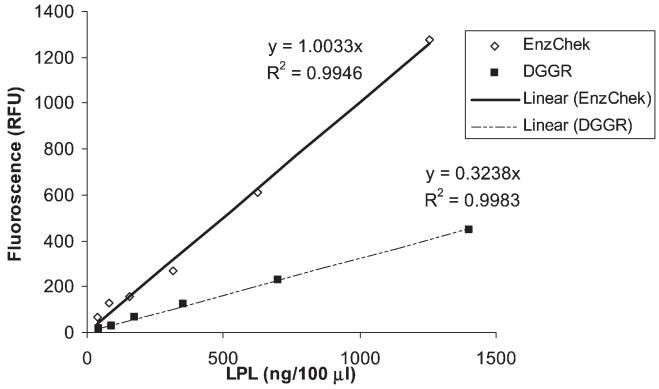


**Fig. 3.** Comparison of LPL activities using the EnzChek and 1,2-*O*-dilauryl-*rac*-glycero-3-glutaric acid-(6-methylresorufin ester) (DGGR) substrates. Initial rates were obtained using 9 min, single-point fluorescence measurements at increasing substrate concentrations. The EnzChek assay was carried out under the conditions described in Fig. 1, the DGGR assay as described by Panteghini, Bonora, and Pagani (19). Background substrate hydrolysis was deducted from all measurements. Data were analyzed by linear regression. The quantities y (y = zx, where y = relative fluorescent units (RFU), x = LPL concentration (ng/100 µl), z = slope of line) and $R^2$ ($R$ = the sample correlation coefficient) are given for each assay type. Each data point is the mean of triplicate determinations. The experiment was carried out three times with similar results.

On the basis of these results, we define the standard assay conditions for LPL as: 0.15 M NaCl, 20 mM Tris-HCl, pH 8.0, 0.62 µM EnzChek substrate, 0.0125% Zwittergent, and 1.5% FA-free BSA. When the reaction is carried out for 10 min at 37°C using 175 ng LPL/100 µl, about 7% of substrate is hydrolyzed.

*Comparison with the DGGR assay.* The DGGR assay was chosen to evaluate the EnzChek assay because this assay is often used for LPL activity measurements (25). The two assays were compared using the same batch of purified bovine LPL (Fig. 3). For both assay types, rate versus LPL concentration was linear to the highest enzyme concentration studied (1,250–1,400 ng). However, the slope of the EnzChek substrate assay was greater by a factor of approximately 3, indicating its greater sensitivity. Nevertheless, a correlation coefficient of 0.99 between these two assays was obtained. The detection limit for LPL (signal-to-noise ratio 3) was 53 ng for the DGGR assay compared with 0.53 ng for the EnzCheck assay. Based on determination of plasma LPL activity (Fig. 5), the latter was found to compare well with the detection limit of the radiometric assay (26). A comparison of the EnzChek and DGGR assays using medium generated by HEK293 cells transfected with a human LPL construct found that LPL activity was detectable using the EnzCheck assay but not the DGGR assay (data not shown). The DGGR substrate has limited solubility in its assay buffer and precipitated after 1 week. The EnzCheck substrate in 0.05% Zwittergent is very stable, giving reproducible results even when kept at room temperature for 1 week (data not shown). The LPL assay using the EnzChek substrate is thus valid, feasible from a practical standpoint, and has a sensitivity close to that of the conventional radiometric method, without the dangers associated with use of radiolabel.

*Determination of the effects of apoC-II and tetrahydrolipstatin on LPL activity using the EnzChek assay.* The hydrolysis of its by LPL requires its binding at the interface of the water/Zwittergent-substrate micelle prior to hydrolysis. ApoC-II, a protein cofactor, is known to enhance this interaction (5). To determine whether apoC-II functions in this way under the conditions of the EnzChek assay, the reaction was carried out at fixed substrate (0.62 µM) and LPL (175 ng/well) concentrations and increasing amounts of apoC-II (**Fig. 4A**). ApoC-II activated the enzyme in a saturable fashion. The maximal activation (3.5-fold) was similar to that displayed under the conditions of a radioassay (11) but less than that reported previously using another radioassay (6). The lower fold-activation of the EnzChek assay is due to the fact that it detects significant activity in the absence of apoC-II (~30% of maximal activity in the presence of this activator). It is also possible that additional factors may be required to facilitate full activation of LPL by apoC-II (5). Further studies are needed to test this.

The EnzChek substrate, presented to the enzyme in a detergent-facilitated micelle, is very different from the water-soluble substrates used in certain LPL assays (27) and provides opportunities for study of the interfacial activation property of apoC-II on the activity of LPL. It will also allow screening for small-molecule activators and/or

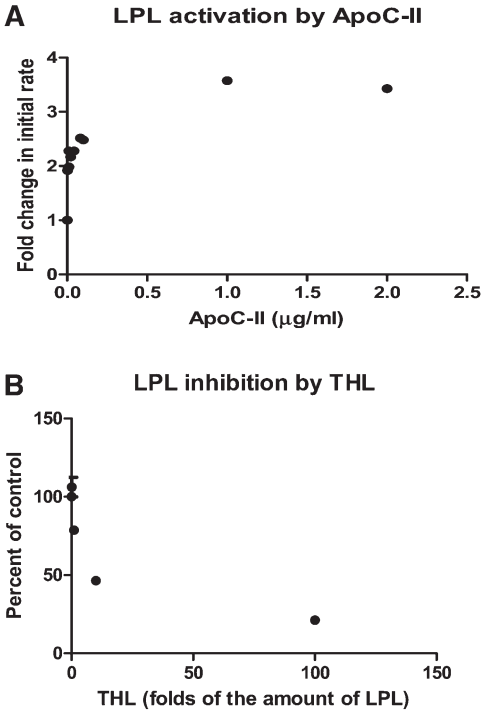


**Fig. 4.** The EnzChek substrate assay allows measurement of activation of LPL by apolipoprotein C-II (apoC-II) and inhibition by tetrahydrolipstatin (THL). The assay was carried out as described in Fig. 1 using 0.62 µM EnzChek substrate and 175 ng of bovine LPL, with human apoC-II (A) and THL (B) concentrations as indicated. Background substrate hydrolysis was deducted from all measurements. Each data point is the mean of triplicate determinations ± SD, the latter too small to be visible. The experiment was carried out three times with similar results.

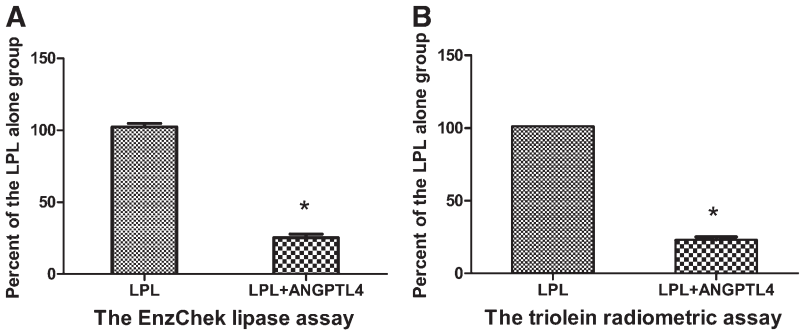


**Fig. 5.** Measurement of human LPL activity using the EnzChek assay. Conditioned human LPL medium (25 μl) was mixed with control medium (25 μl) or conditioned human angiopoietin-like protein-4 (ANGPTL4) medium (25 μl) and held on ice for 30 min prior to addition to LPL activity assays measured using: (A), the EnzChek assay as described in Fig. 1 with 0.62 μM EnzChek substrate, or (B), the radiometric triolein assay as described in Materials and Methods. Background substrate hydrolysis was deducted from each measurement. LPL activity is expressed as a percentage of the activity measured in the absence of ANGPTL4. Values are the mean ± SD of triplicate repeats.* $P < 0.05$.

inhibitors of LPL that may influence this process. HIS is often used as a source of apoC-II for activation of LPL. However, the purified apoC-II activation results shown in Fig. 4 differ from the minor decrease in activity found with increasing concentrations of HIS (see supplementary Fig. IIIC). HIS also contains other LPL-inhibitory factors such as angiopoietin-like protein-3, ANGPTL4, apoC-I, and apoC-III, and the effects of these may explain the lack of activation found with HIS.

THL is an active-site inhibitor of many lipases, including LPL (28). Analyses were carried out to determine whether this inhibition is apparent in the EnzChek assay (Fig. 4B). As expected, LPL was inactivated by THL in a concentration-dependent manner.

*Application of the EnzChek assay to human biological samples.* ANGPTL4, a recently reported endogenous inhibitor of LPL, irreversibly converts the active LPL homodimer into inactive monomers (29). To determine whether the conditions of the assay are such that the two forms of the enzyme are maintained and can be differentiated by the biological activity of the lipase, two conditioned media were generated containing human LPL and ANGPTL4, respectively. As shown in **Fig. 5A**, a mixture of the ANGPTL4-conditioned media with that containing LPL significantly inhibited the LPL activity ($P < 0.05$). A comparable result was obtained with the same samples using the conventional radiolabeled triolein assay (Fig. 5B). Thus, the EnzChek substrate assay is valid for measuring human LPL activity in conditioned media.

### Evaluation of LPL activity in mouse plasma

Significant TG lipase activity was detected using the EnzChek assay in both pre- and postheparin mouse plasma, with the postheparin activity being significantly higher (**Fig. 6**, PBS control groups). Because, in addition to LPL, the substrate is also hydrolyzed by EL and HL (data not shown), the contributions of these lipases to the control group results were evaluated using lipase-specific inhibitors. The pre- and postheparin samples incubated with a neutralizing antibody against murine EL [anti-mEL IgG (25)], or a nonspecific antibody (control IgG) showed no significant difference in TG lipase activity compared with the controls, indicating that EL contributes minimally to plasma TG lipase activity pre- or postheparin treatment. In the presence of the N-terminal domain of ANGPTL4, which inhibits the activity of LPL but not HL (30), the preheparin activity was not altered; however, the postheparin activity was reduced to a level similar to that of preheparin plasma.

The lack of evidence for EL activity and the finding of significant TG lipase activity in preheparin plasma are consistent with the observation that mouse HL circulates normally in the bloodstream (31). The preheparin plasma total activity represents HL activity, and postheparin plasma total activity minus preheparin plasma total activity represents postheparin plasma LPL activity, in agreement with the findings of Dallinga-Thie et al. (14).

Thus, the assay we have developed using the EnzChek substrate can be used to detect both plasma HL and LPL activity in as little as 1 μl of mouse plasma. Potential

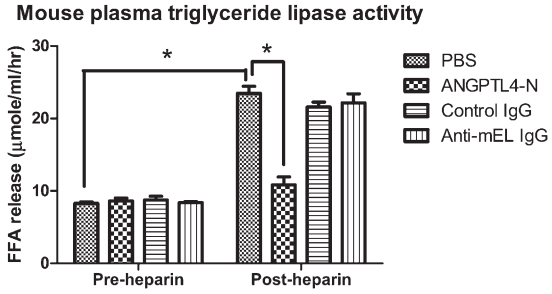


**Fig. 6.** Measurement of triglyceride lipase activities in mouse plasma. Pre- and postheparin plasma samples (1 μl) were incubated on ice for 30 min with one of the following: PBS, ANGPTL4-N, antibodies anti-mEL IgG or control IgG, prior to analysis, as described in Fig. 1 using 0.62 μM EnzChek substrate. The final concentrations in the assays of ANGPTL4-N and each antibody were 40 nM and 20 μg/ml, respectively. Background substrate hydrolysis was deducted from each measurement. Free FAs released are the mean ± SD of triplicate repeats. * $P < 0.001$. This experiment was repeated three times, and similar results were obtained.

contributions from all three lipases can be differentiated using the EL inhibitor anti-mEL IgG and the ANGPTL4-N. Because ANGPTL4 also inhibits human LPL, it is conceivable that human LPL activity can be measured using a similar strategy. This is currently being tested. Alternatively, in nonmurine models, this can also be done with the 5D2 antibody (16).

The EnzChek lipase substrate assay is similar to that developed by Hermetter's group using pyrene-labeled TG substrates (20). However, based on the different fluorescence properties of pyrene and BODIPY, the EnzChek assay will be at least 5-fold more sensitive. Hermetter et al. showed that HL, but not LPL, prefers long-chain (C16) over short-chain (C8) FAs at the *sn*-1 position. The EnzChek substrate contains a C12 chain at this position and therefore is suitable for measurement of both LPL and HL. Of note, the ratio of the TG hydrolysis activities of HL and LPL in postheparin plasma is dependent not only on the length of the FA chain, but also on the presence of apoC-II and other factors.

Another fluorescence quench assay, developed by Lund's group, uses BODIPY-TG substrates (23). Self-quenching is achieved when two BODIPY groups are in close proximity in micelles or lipoproteins. The fluorescent signal generated when the BODIPY product is released through lipolysis is complicated by the incomplete suppression of fluorescence between these two groups.

The LPL lipase assay described here has a simple "mix-and-go" format and does not require preparation of traditional substrate emulsions, which have limited stability. It fulfills many critical requirements for a high-throughput assay for LPL: the signal-to-background ratio is approximately 30, the coefficient of variation is below 5%, and the statistical parameter Z is 0.8. Although further tests are needed to validate these parameters, the assay is suited to high-throughput screening of both activators and catalytic/noncatalytic inhibitors of LPL, and, as demonstrated, can be used to assay other TG-hydrolyzing enzymes.

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