Chylomicron metabolism by the isolated perfused mouse heart

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Chylomicron metabolism by the isolated perfused mouse heart. Am J Physiol Endocrinol Metab 281: E357–E364, 2001. — The catabolism of rat chylomicrons, labeled in their triacylglycerol (TG) component, was investigated using perfused working mouse hearts. Perfusion of mouse hearts with heparin increased lipoprotein lipase (LPL) activity in the perfusate. This heparin-releasable LPL pool remained constant over a variety of experimental conditions, including workload and fatty acid concentrations, making the mouse heart a suitable model to study chylomicron catabolism. Endothelium-bound LPL hydrolyzed radiolabeled 3H-labeled chylomicrons (0.4 mM TG); the fate of LPL-derived 3H-labeled fatty acids was split evenly between oxidation (production of 3H2O) and esterification (incorporation into tissue lipids), mainly TG. In comparison, the oxidation of 0.4 mM [3H]palmitate complexed to albumin was fourfold greater than esterification into tissue lipids. Surprisingly, the addition of unlabeled palmitate (0.4 or 1.2 mM) to perfusions with 3H-chylomicrons did not affect the fate (either oxidation or esterification) of LPL-derived 3H-fatty acids. These results suggest that fatty acids produced from lipoprotein hydrolysis by the action of LPL and fatty acids from a fatty acid–albumin complex do not enter a common metabolic pool in the heart.

Although the heart can utilize a variety of substrates, the majority of ATP required for cardiac function is generated from fatty acid (FA) oxidation (31). The two sources of FA for cardiac metabolism are: 1) circulating FA bound to plasma albumin, derived from adipose tissue lipolysis, which can be taken up by the heart directly, and 2) hydrolysis of triacylglycerols (TG) in circulating lipoproteins (chylomicrons and very low density lipoproteins) by endothelium-bound lipoprotein lipase (LPL) to yield FA for cardiac uptake. In the heart, LPL is synthesized in cardiac myocytes and then is translocated to functional binding sites on the surface of capillary endothelial cells (6, 7, 9, 20). LPL is highly expressed in the heart (11), consistent with its catalytic role in providing an important oxidizable substrate for the heart. Cardiac muscle LPL plays an important role in catabolism of TG-rich lipoproteins in vivo; cardiac-specific LPL expression reduced plasma TG concentrations and rescued LPL knockout mice from neonatal death (17).

Although cardiac metabolism of albumin-bound FA has been studied extensively (18, 23), the precise contribution of LPL-derived FA from lipoprotein degradation to overall FA utilization by the isolated perfused heart has not been determined.

Early studies measuring cardiac catabolism of lipoproteins were conducted with Langendorff-perfused hearts (16, 22, 30), a nonworking model that does not reflect the energy demand of the heart in vivo. However, the catabolism of chylomicrons and very low density lipoproteins has been studied recently using perfused working rat hearts, a more physiological model, and the metabolic fate of LPL-derived FA has been compared with the metabolism of albumin-bound FA (4, 32, 34).

Mice provide an attractive experimental system because of the availability of naturally occurring genetic disease models and increasingly because of the creation of genetically engineered mice. For example, cardiac function can be evaluated in diabetic (db/db) mouse hearts and in hearts from transgenic db/db mice that overexpress GLUT-4 (1). Recently, we developed an isolated perfused working mouse heart preparation (2) that allowed the simultaneous assessment of contractile function and the utilization of two exogenous substrates, glucose and albumin-bound FA. The objective of the current investigation was to study the catabolism of a lipoprotein substrate, chylomicrons, by the working mouse heart preparation. Chylomicrons are the preferred lipoprotein substrate for LPL (8, 13, 15, 21). Also, the heart is one of the first organs to encounter newly synthesized chylomicrons after their entry into the circulation via the thoracic duct (14). The high intrinsic metabolic rate and the small size of a mouse heart allow for a reduction in the perfusate volume in the working heart model (1, 2), which is an advantage when studying the metabolism of a lipoprotein that must be isolated after radiolabeling in vivo. The metabolic fate of LPL-derived FA was measured in the absence and in the presence of FA bound to albu-
min to determine the relative importance of the LPL reaction as an FA source for the perfused mouse heart.

METHODS

Heart isolation and perfusion conditions. All experiments were approved by the University of Calgary Health Sciences Animal Welfare Committee in accordance with the guidelines of the Canadian Council on Animal Care. Mice were maintained under fed dietary conditions.

Metabolic experiments were conducted with an isolated perfused working mouse heart preparation, essentially as described by Belke and coworkers (1, 2). Swiss-Webster mice were used, because previous experiments measuring rates of FA oxidation used this mouse strain (2). Adult male mice were anesthetized with 10 mg pentobarbital sodium intraperitoneally. Because heparin treatment in vivo will displace LPL from endothelial binding sites, 0.3 ml of 10 mM EDTA was injected intravenously before excision of the heart to prevent blood coagulation. The excised heart was placed immediately in ice-cold Krebs-Henseleit-bicarbonate (KHB) solution, consisting of (in mM): 118.5 NaCl, 25 NaHCO3, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, 0.5 EDTA, and 11 glucose. The aorta was isolated and cannulated, and the heart was perfused in retrograde (Langendorff perfusion) with oxygenated KHB at 37°C, during which time the heart was perfused working mouse heart preparation, essentially as described by Belke and coworkers (1, 2).

For metabolic experiments, working hearts were perfused for ≤90 min with prefiltered KHB solution (gassed with 95% O2-5% CO2) supplemented with 3% (wt/vol) BSA and additional substrates (chylomicrons, palmitate), as indicated. The presence of 0.5 mM EDTA in the KHB solution provided a calcium buffer to counter calcium chelation by BSA. The BSA-containing working heart perfusion solution was diazoylized overnight against KHB (2) to stabilize the free calcium concentration before the addition of chylomicrons. The total recirculating perfusion volume was 35 ml. Buffer reservoirs and the preload line were water-jacketed and heated to maintain the heart temperature at 37°C.

Heart contractile function was monitored by a pressure transducer (Millar Micro-Tip, Millar Instruments, Houston, TX) in the afterload line. Pressure traces were recorded on a Grass Physiograph (model 79D) every 10 min to measure both developed pressure and heart rate. Hearts were not paced, because the sealed perfusion apparatus does not permit attachment of pacing electrodes to the right atrium (2). Aortic and coronary flow measurements were obtained by timing the collection of 2 ml of perfusion solution into graduated cylinders. Cardiac output was determined as the sum of aortic and coronary flow rates; cardiac power (milliwatts) was calculated from the product of cardiac output and developed pressure (2).

Isolation of rat intestinal chylomicrons. Because it was not feasible to collect chylomicrons from mice, rats were used as a source of chylomicrons, which were collected by thoracic duct cannulation (5). Male Sprague-Dawley rats (300–350 g) were fasted overnight and then fed 10 ml of a liquid meal replacement with high fat content (Ensure Plus; Abbott Laboratories, St. Laurent, QC, Canada) 2 h before surgery to facilitate visualization of the thoracic duct. Surgery was performed under halothane anesthesia to permit cannulation of the thoracic duct with Silastic tubing, which was externalized to collect chyle continuously. Upon recovery, rats were fed Ensure Plus and water ad libitum. Chyle was collected for ≤20 h in a tube containing 40 mg of EDTA, 19.6 mg of glutathione, and 6.1 mg of gentamycin. To collect radiolabeled chylomicrons [9,10-3H]palmitate (200–600 μCi; ICN) was mixed into the Ensure Plus.

After chyle collection, chylomicrons were isolated and purified by a series of centrifugations. The chyle was first mixed with an equal volume of saline and centrifuged at 25,000 rpm (55.2 Ti rotor) for 30 min. The chylomicron layer was removed from the top and resuspended in a solution of 4% (wt/vol) BSA to bind any free palmitate. Chylomicrons were isolated by a second centrifugation (30,000 rpm for 30 min) and then washed with saline (30,000 rpm for 40 min) to remove BSA. The TG content of washed chylomicrons was assayed with an enzymatic colorimetric kit (GPO-Trinder, Sigma Diagnostics) that measures glycerol content after TG hydrolysis and ranged from 14 to 30 mM. Thin-layer chromatography of radiolabeled 3H-chylomicrons revealed that >95% of the TG label was the 3H oil band (also quantitated; ranged from 496 to 1,376 dpm/nmol FA) with only 1.5% contamination with [3H]palmitate. To ensure quantitative measurement of radioactivity in the various lipid fractions, 0.5 ml of methanol was added to solubilize radioactive lipids in the silica scrapings before the addition of scintillation fluid (28).

Chylomicron metabolism by perfused working mouse hearts. 3H-chylomicrons were added to the KHB perfusion buffer containing 3% BSA to give a final fixed concentration of 0.4 mM TG in all experiments. The specific activity of the radiolabeled FA in 3H-chylomicrons at this final concentration (0.4 mM TG) was sufficient to permit metabolic measurements (oxidation and esterification). Because lymph chylomicrons are deficient in apolipoprotein CII, an essential cofactor for LPL activity, the KHB-BSA perfusate containing 3H-chylomicrons (0.4 mM TG) was further supplemented with 3% heat-inactivated rat serum to provide a source of apolipoprotein CII. Preliminary experiments measured the hydrolysis of chylomicrons by mouse heart homogenates in the presence of varying concentrations of chicken serum (0.5–5%). Serum concentrations >1% gave optimal stimulation of chylomicron metabolism; therefore, 3% rat serum was used routinely as the source of apolipoprotein CII. In some experiments, the metabolism of 3H-chylomicrons was measured in the presence of either 0.4 or 1.2 mM palmitate complexed to 3% BSA, as described by Belke et al. (2).

Oxidation of radiolabeled FA, produced by the action of LPL on 3H-chylomicrons, was determined by measuring the formation of 3H2O (2). Perfusate samples (2.5 ml) were collected at time 0 and after 30, 60, and 90 min of perfusion. The content of 3H2O was measured by mixing 0.5 ml of the perfusate solution with 1.88 ml of chloroform-methanol (1:2, vol/vol), followed by 0.625 ml of chloroform and 0.625 ml 1.1 M KCl dissolved in 0.9 M HCl. After mixing and phase separation (~1 h), the polar upper phase was pipetted into another tube and mixed sequentially with 1 ml of chloroform, 1 ml of methanol, and 0.9 ml of the KCl-HCl mixture. After phase separation, duplicate aliquots (0.5 ml) from the upper phase were removed and subjected to liquid scintillation counting.

After 90 min of working heart perfusion, hearts were perfused (retrograde) for 5 min with KHB solution to remove radioactivity from the coronaries. Hearts were then removed from the cannula, and the atria and vessels were removed. Ventricles were cut open, rinsed in KHB, blotted dry, and immediately frozen in metal clamps precooled to −80°C, weighed, and then stored at −80°C. The frozen heart was
used to determine dry heart mass and the incorporation of radioactivity into tissue lipids.

A portion of the frozen heart tissue (~20 mg wet wt) was dried to give dry weight. The ratio of this sample (dry to wet wt) was used to calculate the total dry weight of the heart so that metabolic rates could be normalized per gram dry weight to account for slight differences in heart size between individual mice. The remainder of the frozen heart tissue was minced and homogenized in 3 ml of ice-cold chloroform-methanol (2:1, vol/vol) with the use of a Polytron (setting 6, 2 x 30 s). This homogenate was diluted with ~1 ml of H2O, mixed, and centrifuged to achieve phase separation. The lower phase was dried under N2, resuspended in chloroform, and subjected to thin-layer chromatography with a solvent system consisting of heptane-diethyl ether-acetic acid (25:75:1). Bands corresponding to TG, FA, diacylglycerol (DG), monoacylglycerol (MG), and phospholipid (PL) were visualized by I2 and scraped into scintillation vials. After the addition of 0.5 ml of methanol and scintillation fluid (Ecolite,ized by I2) and scraped into scintillation vials. After the addition of 0.5 ml of methanol and scintillation fluid (Ecolite, ICN), the radioactivity in each lipid class was measured.

The presence of 3% BSA in the perfusate prevented 3H-FA adherence to glassware in the perfusion apparatus. Glassware and tubing were washed carefully between experiments.

**FA metabolism by perfused working mouse hearts.** The oxidation of 0.4 mM [3H]palmitate, complexed to 3% BSA, was determined as described by Belke et al. (2). Perfusate samples were removed at time 0 and after 20, 40, and 60 min of working heart perfusion, and the generation of 3H2O was measured. The incorporation of radioactivity into tissue lipids at the end of the 60-min perfusion was measured as described in the preceding section on 3H-chylomicron metabolism.

**Mouse heart LPL activity.** Functional endothelium-bound LPL activity was measured as heparin-releasable activity (HR-LPL) with the use of retrogradely perfused Langendorff hearts from fed mice. After aortic cannulation, hearts were perfused with KHB containing 3% BSA at a perfusion pressure of 50 mmHg with a flow rate of 1.2 ml/min. Perfusate was collected for 5 min before being switched to a perfusion solution (KHB-BSA) containing 5 U/ml heparin for an additional 20 min. After this heparin perfusion to displace HR-LPL into the perfusate, mouse hearts were homogenized in 2 ml of 25 mM NH4Cl, 5 mM EDTA, 0.8% (wt/vol) Triton X-100, 0.04% (wt/vol) sodium dodecyl sulfate, 33 μg/ml heparin, and 10 μg/ml leupeptin (pH 8.2). After low-speed centrifugation to remove unbroken cells and nuclei, the cell lysate was diluted 1:10 with an isotonic sucrose solution (0.25 M sucrose, 10 mM Hepes, 0.4 mM EDTA, pH 7.5) before assay for residual cellular LPL activity. In some experiments, as indicated, the basal or constitutive release of LPL into the perfusate during working heart perfusions preceded a subsequent Langendorff perfusion to measure HR-LPL activity.

LPL activity in preheparin and postheparin (HR-LPL) perfusate samples and the residual LPL activity remaining in postheparin heart homogenates were measured in vitro from the hydrolysis of a [3H]triolein emulsion, as described by Carroll et al. (10). The standard assay contained 0.6 mM glycerol-[9,10-3H]trioleate ([3H]triolein), 25 mM PIPES (pH 7.5), 0.05% essentially fatty acid-free BSA, 50 mM MgCl2, 2% chicken serum as a source of the apolipoprotein CII LPL activator, and appropriate quantities of perfusate (100 μl) or heart homogenate (5–25 μl) in a final volume of 400 μl. After a 30-min incubation at 37°C, the release of [3H]triolein was measured by liquid-liquid extraction. All assays were performed in duplicate. To validate the use of [3H-chylomicrons as a substrate for endothelium-bound LPL in perfused working mouse hearts, LPL assays were also conducted with [3H-chylomicrons in place of the usual [3H]triolein emulsion.

**FA metabolism in isolated working hearts.** A rate of 30.4 ± 2.0 μmol·h⁻¹·g dry wt⁻¹ for the oxidation of albumin-bound [3H]palmitate (0.4 mM) was observed in the present study (Fig. 1A), very similar to oxidation rates reported previously by Belke et al. (2). The incorporation of radioactivity into tissue lipids at the end of the 60-min perfusion was also measured (Fig. 1B); total lipid incorporation was 13.8 ± 2.0 μmol·h⁻¹·g dry wt⁻¹ (n = 3). Separation of lipid classes by thin-layer chromatography revealed that the vast majority of radioactivity was incorporated (esterified) into TG (80 ± 3% of total), with a much smaller fraction (12 ± 1%) incorporated into the total PL fraction. When the metabolic fates of [3H]palmitate after 60 min of perfusion were compared, the extent of oxidation (80 ± 3% of calculated total) was much greater than esterification (20 ± 3%). Hearts perfused with 0.4 mM [3H]palmitate had consistent contractile function over the perfusion period.

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FA metabolism in isolated working hearts was measured under conditions where radiolabeled FA was presented as an albumin complex or incorporated into the TG component of chylomicrons, requiring previous hydrolysis by endothelium-bound LPL.

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period, with values for cardiac power averaging 1.37 ± 0.36 mW. This value was similar to cardiac power measurements for hearts perfused with chylomicrons (see Chylomicron metabolism by perfused working mouse hearts), indicating a similar metabolic demand.

**LPL activity in mouse hearts.** Chylomicron metabolism is dependent on the hydrolytic activity of endothelium-bound LPL. To demonstrate that the mouse heart was a suitable model to study chylomicron metabolism, LPL activity was characterized under a number of experimental conditions.

Perfusion of mouse hearts with heparin resulted in the rapid release of LPL activity into the perfusate (Fig. 2A); HR-LPL activity is generated by displacing the enzyme from heparan sulfate proteoglycan binding sites on the surface of coronary endothelial cells (7). This heparin-releasable fraction was 19 ± 3% of total LPL (Fig. 2B); residual LPL remaining in the heart after heparin perfusion was the largest fraction (79 ± 3% of total). The basal or constitutive release of LPL during the first 5 min of Langendorff perfusion (without heparin) was very low, only 1.6 ± 0.3% of total (Fig. 2B).

LPL release into the perfusate was also examined with perfused working mouse hearts (Fig. 3). Basal or constitutive release of LPL during 60 min of working heart perfusions was much greater than the basal rate of release with nonworking Langendorff-perfused hearts (Fig. 3A). This difference in basal LPL release between working and Langendorff-perfused hearts was only partly due to greater coronary flow rates in working hearts (~3 ml/min) compared with the nonworking perfused hearts (~2 ml/min). The enhanced rate of LPL release from working hearts could be reduced to rates that were similar to basal rates from Langendorff-perfused hearts by including 1.2 mM palmitate in the working heart perfusate (Fig. 3A); coronary flow rates in the working heart were not changed by palmitate addition. Palmitate had no effect on the very low rate of LPL release from nonworking hearts. The reduction in basal LPL release from working hearts by palmitate was concentration dependent (Fig. 3B). This inhibitory effect of palmitate on basal (constitutive) LPL release was specific; addition of either 2.4 mM octanoate (short-chain FA) or 5 mM pyruvate had no effect (results not shown). Interestingly, 60 min of working heart perfusion (+ palmitate) had no effect on subsequent HR-LPL activity (compare Fig. 3C with Fig. 2A), suggesting that the content of endothelium-bound LPL was not altered over the period of perfusion.

**Chylomicron metabolism by perfused working mouse hearts.** The hydrolysis of rat intestinal 3H-chylomicrons was first examined in vitro with a mouse heart homogenate to establish that the chylomicron preparation (from rat) was a valid substrate for LPL (from mouse). Addition of 5% rat serum to the LPL assay increased hydrolysis of 3H-chylomicrons 7.2-fold (from 40 to 289 nmol·min⁻¹·mg protein⁻¹), confirming the expectation that lymph chylomicrons are apolipoprotein CII deficient (33) and that mouse heart LPL hydrolyzes 3H-chylomicrons. A polyclonal antibody to LPL (12) reduced serum-stimulated 3H-chylomicron hydrolysis to 20 nmol·min⁻¹·mg⁻¹. Thus, on the basis of serum stimulation and immunoinhibition, the rat 3H-chylomicron preparation is a suitable LPL substrate; hence, investigations into chylomicron metabolism by perfused working mouse hearts were initiated.

Working mouse hearts were perfused with 3H-chylomicrons (0.4 mM TG) and 3% rat serum as a source of apolipoprotein CII. The fate of LPL-derived FA from 3H-chylomicron hydrolysis was determined by measuring the accumulation of 3H₂O in the perfusate (FA oxidation) and the incorporation of radioactivity into tissue lipids at the end of the 90-min perfusion (esterification). The rate of FA oxidation was curvilinear (0–30 min, 111 ± 35; 30–60 min, 247 ± 35; 60–90 min, 400 ± 71 nmol·min⁻¹·mg⁻¹·g dry wt⁻¹; Fig. 4A), perhaps reflecting the time required for FA generation by the LPL reaction. Cardiac power was relatively stable from 10 to 90 min of perfusion, averaging 1.67 ± 0.29 mW (n = 6). The rate of LPL-derived FA oxidation showed...
no correlation with different intrinsic levels of cardiac power generated by individual perfused working hearts. A substantial amount of LPL-derived FA was also incorporated into total tissue lipids (Fig. 4B); after thin-layer chromatography, the majority of label was in TG (73.6 ± 5%) and PL (14.6 ± 2%) bands. Thus the distribution of label among lipid classes for LPL-derived FA was very similar to the pattern observed for hearts perfused with 0.4 mM \(^3\)H-palmitate complexed to albumin (compare Fig. 1B with Fig. 4B).

For \(^3\)H-chylomicrons after 90 min of perfusion, the extent of oxidation and esterification was equal (each 50 ± 3% of total; \(n = 6\)). Because LPL-derived FA had a different pattern of metabolism (equal amounts oxidized and esterified) from palmitate-albumin (oxidation much greater than esterification), the effect of adding unlabeled palmitate to the perfusate on \(^3\)H-chylomicron metabolism was investigated (Fig. 5). Surprisingly, the presence of either 0.4 or 1.2 mM palmitate had no significant effect on either LPL-derived FA oxidation (Fig. 5A) or esterification (Fig. 5B).

**DISCUSSION**

FA are the primary fuel for heart metabolism (31). Although the metabolism of albumin-bound FA has been studied extensively (18), comparatively little is
known about the fate of FA derived from the action of LPL on lipoprotein substrates. The objective of this study was to examine the action of endothelium-bound LPL on a native lipoprotein substrate (chylomicrons) by use of a working mouse heart preparation.

Functional endothelium-bound LPL in perfused mouse hearts was determined by measuring LPL catalytic activity displaced into the perfusate by heparin (HR-LPL). The distribution of HR-LPL (19% of total) and residual LPL in heart homogenates (79%) on the basis of activity measurements (Fig. 2) was nearly identical to previous results (6) with the use of immunocytochemistry to localize LPL to the capillary endothelium (18% of total) and cardiac myocytes (77%) in mouse ventricles.

The basal or constitutive release of LPL was enhanced in working hearts compared with nonworking Langendorff-perfused hearts (Fig. 3). The addition of 1.2 mM palmitate to the perfusate inhibited this enhanced release of LPL from working hearts, consistent with the observation of Saxena et al. (25) that FA can reduce the transport of LPL from the basal to apical (luminal) surface of endothelial cell monolayers. It is possible that the increased energy demand of working hearts reduces interstitial FA content and so transfer of LPL to the endothelial cell surface and release into the perfusate are enhanced. The physiological significance of this enhanced basal (constitutive) release of LPL from working mouse hearts is unclear, however, because perfusion for 60 min in working mode (± palmitate) had no effect on subsequent HR-LPL (Fig. 3C) activity compared with hearts not subjected to the working heart protocol (Fig. 2A). Therefore, functional heparan sulfate proteoglycan binding sites on the endothelium were not depleted when basal (constitutive) release was enhanced by workload. Furthermore, the metabolic fate of LPL-derived FA from chylomicron catabolism was not altered in working hearts exhibiting different intrinsic workloads (cardiac power) or by the addition of FA to the perfusate. Because this functional heparin-releasable fraction of LPL bound to the coronary endothelium remained remarkably constant, the perfused working mouse heart is a suitable model to study chylomicron catabolism.

It was not technically feasible to isolate sufficient mass quantities of radiolabeled mouse chylomicrons to give a final concentration of 0.4 mM TG in a total recirculating perfusate volume of 35 ml. Therefore, a heterologous experimental system had to be utilized, with rat intestinal chylomicrons as the LPL substrate. Similarities in chylomicron structure and composition for rats and mice minimize the limitation of not being able to use a strictly homologous experimental system. The validity of using rat [3H]chylomicrons as an LPL substrate was established from in vitro experiments using mouse heart homogenate as an LPL source. When supplemented with a source of apolipoprotein CII, mouse heart LPL readily hydrolyzed TG in 3H-chylomicrons to 3H-FA. Furthermore, specific inhibition of chylomicron hydrolysis could be achieved with the addition of an antibody directed against LPL. Together, these results established the feasibility of using the isolated working mouse heart preparation (2) as a model system to examine chylomicron metabolism by endothelium-bound LPL.

LPL-derived FA will have two principal metabolic fates: 1) oxidation, determined by measuring the formation of 3H2O in the perfusate, and 2) esterification, assessed from the incorporation of radioactivity into tissue lipids. Esterification of FA converts potentially noxious unesterified FA into a neutral lipid (TG) for storage. This intracellular TG in cardiac myocytes can be mobilized by lipolysis as an additional energy source (23) or be exported as a lipoprotein particle (19). The rate of chylomicron FA oxidation by working mouse hearts after 1 h of perfusion (10.7 ± 0.1 nmol ⋅ h−1 ⋅ g dry wt−1; Fig. 4A) was greater than the corresponding rate observed with working rat hearts (32, 34) of 4.4 ± 0.7 nmol ⋅ h−1 ⋅ g dry wt−1 (calculated assuming a wet-to-dry ratio of 5). This difference may be due largely to the use of apolipoprotein CII-deficient chylomicrons as an LPL substrate with working rat hearts (33). However, differences in metabolic demand in working hearts from rats and mice may also be a factor, because 1.2 mM albumin-bound palmitate was oxidized at a higher rate (94 ± 1.1 nmol ⋅ h−1 ⋅ g dry wt−1) by working mouse hearts (2) compared with an oxidation rate of 43 ± 0.5 nmol ⋅ h−1 ⋅ g dry wt−1 by working rat hearts (32).

Fig. 5. Chylomicron metabolism by working mouse hearts in the presence of palmitate. The oxidation (A) and esterification (B) of LPL-derived 3H-FA were measured after a 90-min perfusion with 3H-chylomicrons in the absence (n = 6) and in the presence of 0.4 (n = 4) or 1.2 mM unlabeled palmitate (n = 6) in the perfusate.
A further objective of this study was to determine the relative importance of chylomicron catabolism as a FA source for myocardial metabolism, specifically compared with the direct metabolism of albumin-bound FA. The concentration of 0.4 mM chylomicron TG was chosen as being representative of a postprandial concentration in vivo (34). For comparative purposes, the metabolic fate of a relatively low, unesterified FA concentration of 0.4 mM palmitate was examined, chosen to approximate the plasma FA concentration in a fed state, when rates of adipose tissue lipolysis are low. To keep the perfusion system as simple as possible, other plasma factors that would be present in a fed state, such as insulin, were not added to the heart perfusate. Because the total perfusate FA concentration that could be derived from chylomicron hydrolysis (1.2 mM) was not the same as the concentration of albumin-bound palmitate (0.4 mM), results measuring metabolic fates of FA from these two different sources must be interpreted cautiously. Given this qualification, it was nevertheless surprising that the intracellular metabolic fates of LPL-derived FA and palmitate-albumin were significantly different in relation to the proportion that was oxidized vs. esterified. For chylomicron metabolism, rates of oxidation and esterification were equal (50:50%) after 90 min of perfusion. In contrast, much more palmitate-albumin was oxidized (80%) than was esterified (20%) after 60 min of perfusion. This difference in metabolic fate must be interpreted carefully, because perfusion times were not identical. One factor that could influence chylomicron metabolism relative to palmitate complexed to albumin is the slower rate of chylomicron utilization because of the additional requirement for an enzymatic step, hydrolysis of core TG by LPL, to generate FA for uptake and metabolism. However, it is possible that LPL-derived FA and FA dissociated from FA-albumin complexes may not enter a common pool for intracellular metabolism by cardiac myocytes. This consideration was tested by determining whether the addition of unlabelled palmitate to the perfusate (0.4 and 1.2 mM) had any effect on the metabolism of 3H-chylomicrons. Although chylomicrons and FA-albumin have been studied individually for comparative metabolism by perfused hearts (16, 32–34), the combination of chylomicrons and FA-albumin in the perfusate has not been examined previously.

If LPL-derived FA and the FA dissociating from albumin enter a common metabolic pool (Fig. 6A), then the presence of unlabelled FA-albumin in the perfusate will reduce the apparent utilization of LPL-derived 3H-FA by dilution of radiolabel specific activity (isotope dilution). In addition, unlabelled FA added to the perfusate may reduce the actual hydrolysis of 3H-chylomicrons due to product inhibition of endothelium-bound LPL (3) and/or displacement of LPL from functional binding sites on the endothelium of perfused mouse hearts (24, 26). Unexpectedly, palmitate bound to albumin (0.4 or 1.2 mM) did not affect either the oxidation or the esterification of LPL-derived 3H-FA (Fig. 5). Therefore, LPL-derived FA and FA dissociating from an albumin complex may have separate sites or mechanisms for uptake, producing a distinct pattern of metabolic fates (Fig. 6B). Scow and Blanchette-Mackie (27) originally proposed that FA generated from the action of LPL on chylomicrons in the capillary lumen are transported into cardiac myocytes by lateral movement in a continuum of cell membranes, an uptake process that could prevent mixing with FA that have dissociated from FA-albumin complexes that are taken up by both passive diffusion and interaction with membrane transport proteins (31). Interestingly, the mode of FA delivery to endothelial cells also influenced their metabolic fate (29). Given the role of FA in mediating
some of the deleterious effects on cardiac function in disease states (18), analysis of any differences in FA uptake and metabolism depending on the FA source should be made.

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