Myocardial Triglyceride Turnover and Contribution to Energy Substrate Utilization in Isolated Working Rat Hearts*

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The objective of this study was to determine the contribution of myocardial triglycerides to overall ATP production in isolated working rat hearts. Endogenous lipid pools were initially prelabeled (pulsed) by perfusing hearts for 60 min with Krebs-Henseleit buffer containing 1.2 mM [1-14C]palmitate. During a subsequent 60-min period (chase), hearts were perfused with either no fat, low fat (0.4 mM [9,10-3H]palmitate), or high fat (1.2 mM [9,10-3H]palmitate). All buffers contained 11 mM glucose. During the "chase," 14CO2 production (a measure of endogenous fatty acid oxidation) and 3H2O production (a measure of exogenous fatty acid oxidation) were determined. Oxidative rates of endogenous fatty acids during the chase were 279 ± 50, 88 ± 14, and 88 ± 8 nmol of [14C]palmitate oxidized per g dry weight/min in the no fat, low fat, and high fat groups, respectively, compared to exogenous palmitate oxidation rates of 0, 361 ± 68, and 633 ± 60 nmol of [3H]palmitate/g dry weight-min, in the no fat, low fat, and high fat groups, respectively. Endogenous [14C]palmitate oxidation rates were matched by loss of [14C]palmitate from endogenous myocardial triglycerides. Overall triglyceride content decreased during the no fat and low fat chase perfusion but did not change during the high fat chase. Loss of triglyceride [14C]palmitate during the high fat chase was matched by incorporation of exogenous [3H]palmitate in triglycerides. In a second series of perfusions, three groups of hearts were perfused under similar conditions, except that unlabeled palmitate was used during the "pulse" and that 11 mM [2-H14U-14C] glucose and unlabeled palmitate was present during the chase. During the chase, both glycolysis (3H2O production) and glucose oxidation (14CO2 production) rates were measured. Rates of glucose oxidation were inversely related to the fatty acid concentration in the perfusate (1257 ± 158, 366 ± 40, and 124 ± 26 nmol of glucose oxidized per min·g dry weight in the no fat, low fat, and high fat groups, respectively), while rates of glycolysis were not significantly different between these groups. Calculation of overall ATP production from both oxidative and glycolytic sources determined that even in the presence of high concentrations of fatty acids, myocardial triglyceride turnover can provide over 11% of steady state ATP production in the aerobically perfused heart. In the absence of fatty acids, myocardial triglyceride fatty acids can become the major energy substrate of the heart. Using this experimental model in which triglyceride turnover can be directly measured, we conclude that endogenous triglycerides are an important source of fatty acids for oxidative metabolism in the heart and their contribution is inversely related to the concentration of fatty acids in the perfusate.

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Circulating fatty acids are an important myocardial energy source that provides 60–70% of the heart's energy requirements (1). Endogenous myocardial triglycerides, through lipolysis, are also a potential source of fatty acids for oxidative metabolism (2, 3). The concentration of exogenous fatty acids plays an important role in the regulation of triglyceride lipolysis (3); increasing the concentration of exogenous fatty acids inhibits endogenous triglyceride lipolysis in isolated perfused hearts and stimulates triglyceride synthesis, possibly through stimulating the enzyme phosphatidic acid phosphohydrolase (4). Because of the potential importance of triglycerides as an extended substrate source, several studies have addressed the topic of triglyceride lipolysis in myocardial tissue (5–10). The most part, however, measurements of triglyceride turnover have been indirect; i.e. by measuring glycerol release by the myocardium. To our knowledge, no studies have measured myocardial triglyceride turnover directly. In addition, most of the studies conducted on intact isolated heart preparations have used perfusates devoid of fatty acids.

The other major substrate of the aerobically perfused heart is glucose. It has been well established that high levels of fatty acids interfere with glucose utilization; both glycolytic flux and glucose oxidation are inhibited by fatty acids (1, 11, 12). This occurs primarily through inhibition of both phosphofructokinase, the rate-limiting enzyme in the glycolytic pathway (13), and pyruvate dehydrogenase (14). It has been suggested that glucose contributes from 15 to 55% of oxidative metabolism of human hearts under fasting conditions (15–20), that glucose extraction significantly increases during acute hyperglycemia, and that glucose becomes the major substrate for oxidative metabolism under conditions of hyperglycemia (21–23). However, Wisneski (24) recently demonstrated in humans that despite an increase in glucose uptake during hyperglycemia, only 32% of the glucose extracted was oxidized, suggesting that the primary fate of the extracted glucose was not oxidative metabolism. The relationship between both endogenous and exogenous fatty acid oxidation, and both glycolytic flux and glucose oxidation has not been characterized.
In this study, we developed a protocol to measure triglyceride turnover directly in isolated working rat hearts. This was achieved by prelabeling the triglyceride pool during a “pulse” perfusion with [1-14C]palmitate, and subsequently measuring the rate of endogenous triglyceride fatty acid oxidation during a chase perfusion. During the chase, exogenous fatty acid oxidative rates were also determined. By measuring glucose utilization (both glycolysis and glucose oxidation) in a parallel series of hearts perfused under similar conditions, we were able to determine the contribution of endogenous triglycerides to overall myocardial ATP production. This was performed in the presence and absence of both low and high concentrations of exogenous fatty acids.

**EXPERIMENTAL PROCEDURES**

**Materials**—d-[U,14C]Glucose, d-[2-3H]-glucose, [9,10-3H]-palmitic acid, and [1,14C]palmitic acid were obtained from Du Pont-New England Nuclear (Wilmington, DE). Bovine serum albumin (fraction V) was obtained from Boehringer Mannheim. Hyamine hydroxide (1 M, methanol solution) was obtained from England Nuclear Research Products (Boston, MA). Dowex 1X-2 anion exchange resin was obtained from Amersham Canada Ltd. (Oakville, Ontario). Baker Si250-PA (19C)-Silica Gel plates were obtained from Johns Scientific (Toronto, Ontario). Triglyceride assay kits were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All other chemicals were obtained from Sigma.

**Heart Perfusion**—Adult male Sprague-Dawley rats (200-250 g) were anesthetized with sodium pentobarbital (60 mg/kg). Hearts were then quickly excised, the aorta cannulated, and a retrograde perfusion using Krebs-Henseleit buffer initiated, as described previously (25). During this initial perfusion the hearts were trimmed of excess tissue, the pulmonary artery was cut, and the point at which the pulmonary veins enter the left atrium was cannulated. Following a 10-min equilibration period, hearts were switched to the working heart mode and perfused at a 11.5 mm Hg left atrial preload and a 80 mm Hg aortic afterload, as described previously (11, 25). Spontaneously beating hearts were used throughout the studies, with heart rate and peak systolic pressure being measured by a Gould P21 pressure transducer in the aortic outflow line. All hearts perfused with fatty acids contained Krebs-Henseleit buffer with 11 mM glucose, 3% albumin, and 2.5 mM free Ca2+. The albumin used in these perfusions was routinely obtained from Johns Scientific (Toronto, Ontario). Triglyceride assay kits were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). All other chemicals were obtained from Sigma.

**Procedure**—The protocol for the first series of heart perfusions is shown in Table I. Initially, all hearts in this series were perfused for 60 min with recirculated Krebs-Henseleit buffer containing 1.2 mM [1-14C]palmitate (specific activity = 65,801 dpm/μmol) to label the endogenous lipid pools (pulse). During this labeling period, exogenous steady state fatty acid oxidation was also measured by quantitative collection of myocardial 14CO2 production (as described below). Additional preliminary experiments were also performed in which the pulse period was extended in order to determine the optimal labeling periods. At the end of the pulse period, hearts were switched to a retrograde Langendorff drip-out perfusion with unlabeled Krebs-Henseleit buffer. During this 10-min period, buffer containing [14C]palmitate was removed from the system and replaced with buffer devoid of fat (no fat), 0.4 mM [9,10-3H]palmitate (low fat) (specific activity = 284,185 dpm/μmol), or 1.2 mM [9,10-3H]palmitate (high fat) (specific activity = 116,314 dpm/μmol). One group of hearts was frozen at the end of this Langendorff perfusion (with Wollenberger washout perfusion for a subsequent 60-min period with the new buffers described above. In order to determine how much [14C]palmitate was left in the system at the very beginning of chase, and whether there was any back diffusion of fatty acids released from triglyceride pools to the perfusate, perfusate samples were taken at the beginning and at the end of chase and their [14C]palmitate content determined.

In the second series of heart perfusions, the same perfusion protocol and perfusion substrates as described above were used, except that palmitate was not labeled during the pulse period (Table I). In addition, the chase perfusion contained 11 mM [2-3H/U-14C]glucose (specific activity = 21,381 dpm of H/μmol of glucose and 37,636 dpm of 14C/μmol of glucose) instead of labeled palmitate.

In all hearts, mechanical function was monitored throughout the entire perfusion. At the end of the Langendorff washout perfusion, the perfusion rate was increased by 10% above the pulse perfusion rate. Hearts were then quickly frozen with Wollenberger clamps cooled to the temperature of liquid N2. The frozen ventricular tissue was then weighed and powdered in a mortar and pestle cooled to the temperature of liquid N2. A portion of the powdered tissue was used to determine the dry to wet ratio of the ventricles. The atrial tissue remaining on the landmark was removed, dried in an oven for 24 h at 100°C, and weighed. The dried atrial weight, frozen ventricular weight, and ventricular dry to wet ratio was then used for determination of total dry weight of the heart.

**Measurement of Exogenous and Endogenous Fatty Acid Oxidation Rates**—During the chase, steady state exogenous palmitate oxidation was determined by quantitatively measuring 14CO2 production by hearts, as described in detail previously (11, 12, 25). Hearts were perfused in a closed system that allowed collection of both perfusate and gaseous 14CO2. The 14CO2 liberated into the gaseous phase was trapped in a 1 ml hyamine hydroxide solution in the air outlet line. Both perfusate and hyamine hydroxide samples were obtained at 20-min intervals throughout the initial 60-min pulse period. Perfusate samples were immediately injected below a 1-ml volume of mineral oil to prevent liberation of perfusate 14CO2. The 14CO2 from the perfusate was subsequently extracted by injecting 1 ml of perfusate containing 0.1 M H2SO4 contained in sealed metabolic vials, which also contained 400 μl of 1 M hyamine hydroxide in suspended center wells (25). Vials were gently shaken for 1 h and the center wells removed and counted in ACS scintillant using standard scintillation counting procedures.

During the chase, 14CO2 production was used as a measure of endogenous fatty acid oxidation, while H2O production was a measure of exogenous fatty acid oxidation rates. Perfusate and gaseous samples were collected at 10-min intervals during the chase, and 14CO2 production measured as described above. During this period exogenous steady state palmitate oxidation was determined by measuring H2O content in the perfusate samples. H2O was separated from [3H]palmitate by treating 0.5-ml buffer samples with 1.88 ml of [1-14C]palmitate (specific activity = 8163 dpm/μmol) and then adding 0.625 ml of chloroform and 0.625 ml of a 2 M KCl:HCl solution. The aqueous phase was then collected using a Pasteur pipette and subsequently treated with a mixture of chloroform, methanol, and KCl:HCl with a ratio of 1:1:0.1. Two 0.5-ml samples of the aqueous phase were then counted for each perfusate sample for total H2O determination, taking into account the dilution factor. This technique resulted in a

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Pulse period</th>
<th>Washout period</th>
<th>Chase period</th>
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<tbody>
<tr>
<td></td>
<td>60-min prelabeling with 11 mM glucose, 1.2 mM [1-14C]palmitate</td>
<td>10-min perfusion</td>
<td>60 min reperfusion with:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(a) No fat (11 mM glucose)</td>
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<td></td>
<td></td>
<td></td>
<td>(b) Low fat (11 mM glucose, 0.4 mM [3H]palmitate)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(c) High fat (11 mM glucose, 1.2 mM [3H]palmitate)</td>
</tr>
<tr>
<td>Procedure</td>
<td>measure 14CO2 production</td>
<td>change perfusate</td>
<td>measure 14CO2 and H2O production</td>
</tr>
</tbody>
</table>
greater than 99.7% extraction and separation of H₂O from the [H] palmitate. Exogenous palmitate oxidation rates during the chase were expressed as nanomole of [14C]palmitate oxidized per min x g dry weight, while endogenous oxidation rates were expressed as nanomole of [3H]palmitate oxidized per min x g dry weight.

Measurement of Glycolysis and Glucose Oxidation Rates-In the second series of hearts, in which the chase perfusate contained 11 mM [2-3H/14C] glucose, quantitative 3H₂O production was used to measure steady state glycolytic rates (H₂O is liberated at the phosphoglucomerase step of glycolysis), while quantitative 14CO₂ production was used to measure glucose oxidation rates (14CO₂ is liberated at the level of pyruvate dehydrogenase and in the tricarboxylic acid cycle). 14CO₂ production was determined using the same methods described above for palmitate oxidation. To measure glycolysis, 3H₂O was separated from [H]glucose and [14C]glucose as described by Kobayashi and Neely (27), using columns containing Dowex 1-X4 anion exchange resin (200-400 mesh) suspended in 0.2 M potassium tetraborate (the volume of resin was 0.5 x 0.5 cm). The Dowex in the columns were extensively washed with H₂O prior to use. A 0.2-ml volume of perfusate was then added to the column and eluted into scintillation vials with 0.8 ml of H₂O. Following addition of ACS scintillant, the samples were subjected to standard double isotope counting procedures, with the windows set at 0-300 nm (3H) and 400-670 nm (14C). The Dowex columns were found to retain 98-99.6% of the total [H]glucose and [14C]glucose present in the perfusate. The H₂O (which passes through the column) was corrected for the small amount of [H]glucose that passed through the column. This could be accomplished since an equal amount of [14C]glucose also passed through the column, and could be used as an internal standard for the degree of [H]glucose contamination in the 3H₂O sample. Correction was also made for the degree of spillover of 14C into the 3H counting window, by measuring this degree of spillover using standards containing only [14C]glucose.

Glucose utilization was expressed as nanomole of glucose metabolized per min x g dry weight.

Measurement of Metabolic Intermediates—Tissue lipids were extracted as previously described by Borer (11) and Spiegelman (26). Triglycerides were separated from other neutral lipids using Baker Si250-PA (19C)-Silica Gel plates and a solvent system that was extensively washed with HzO prior to use. A 0.2-ml volume of perfusate was then added to the column and eluted into scintillation vials with 0.8 ml of H₂O. Following addition of ACS scintillant, the samples were subjected to standard double isotope counting procedures, with the windows set at 0-300 nm (3H) and 400-670 nm (14C). The Dowex columns were found to retain 98-99.6% of the total [H]glucose and [14C]glucose present in the perfusate. The H₂O (which passes through the column) was corrected for the small amount of [H]glucose that passed through the column. This could be accomplished since an equal amount of [14C]glucose also passed through the column, and could be used as an internal standard for the degree of [H]glucose contamination in the 3H₂O sample. Correction was also made for the degree of spillover of 14C into the 3H counting window, by measuring this degree of spillover using standards containing only [14C]glucose.

Results

Measurements of Heart Function—Heart function in the spontaneously beating working hearts was monitored throughout the perfusion protocol. Table III shows the heart rate, peak systolic pressure development, and the heart rate pressure product obtained during the pulse perfusion and during the chase perfusion. No significant deterioration in heart function was seen throughout the 2-h perfusion period in hearts perfused with no fat. The presence or absence of either low or high fat in the perfusate was also without significant effect on heart function, although the peak systolic pressure in hearts chased with high fat was slightly but significantly lower. The triglyceride turnover and energy substrate utilization measurements made in this study, therefore, were not complicated by major changes in heart work. Labeling of Endogenous Myocardial Lipids during the Pulse Perfusion—Initial experiments were performed to determine the optimal conditions for labeling myocardial lipids with [14C]palmitate. A concentration of 1.2 mM [14C]palmitate was used in the perfusate since we have previously observed that perfusion of isolated working hearts with this concentration of fatty acid does not deplete myocardial triglyceride stores (26). Hearts were initially “pulsed” with 1.2 mM [14C]palmitate for either a 60- or 120-min period, followed by a 10-min dripout perfusion as Langendorff hearts in the absence of labeled fatty acids. [14C]Palmitate content and oxidation in hearts subjected to a 60-min pulse are shown in Table IV. As expected, during the pulse the majority of the [14C]palmitate taken up by the heart was oxidized to 14CO₂ (63%). The actual steady state oxidation rates during this pulse period were 659 ± 51 nmol of [14C]palmitate oxidized per min x g dry weight. The [14C]palmitate taken up by the heart which was not oxidized was primarily incorporated into triglyceride stores (56%), with a small amount being incorporated into phospholipids (9.6%). If hearts were pulsed for 120 min, a further increase in [14C]palmitate incorporation into triglycerides was not seen (22.55 ± 4.15 = (n = 5) versus 20.41 ± 3.22 μmol/g dry weight, in hearts pulsed for 120 or 60 min, respectively). A slight increase in [14C]palmitate incorporation into phospholipids was seen in hearts pulsed for the longer period (4.71 ± 0.8 versus 2.28 ± 0.47 μmol/g dry weight in hearts pulsed for 120 or 60 min, respectively). Table III shows the heart rate, peak systolic pressure development, and the heart rate pressure product obtained during the pulse perfusion and during the chase perfusion. No significant deterioration in heart function was seen throughout the 2-h perfusion period in hearts perfused with no fat. The presence or absence of either low or high fat in the perfusate was also without significant effect on heart function, although the peak systolic pressure in hearts chased with high fat was slightly but significantly lower. The triglyceride turnover and energy substrate utilization measurements made in this study, therefore, were not complicated by major changes in heart work. Labeling of Endogenous Myocardial Lipids during the Pulse Perfusion—Initial experiments were performed to determine the optimal conditions for labeling myocardial lipids with [14C]palmitate. A concentration of 1.2 mM [14C]palmitate was used in the perfusate since we have previously observed that perfusion of isolated working hearts with this concentration of fatty acid does not deplete myocardial triglyceride stores (26). Hearts were initially “pulsed” with 1.2 mM [14C]palmitate for either a 60- or 120-min period, followed by a 10-min dripout perfusion as Langendorff hearts in the absence of labeled fatty acids. [14C]Palmitate content and oxidation in hearts subjected to a 60-min pulse are shown in Table IV. As expected, during the pulse the majority of the [14C]palmitate taken up by the heart was oxidized to 14CO₂ (63%). The actual steady state oxidation rates during this pulse period were 659 ± 51 nmol of [14C]palmitate oxidized per min x g dry weight. The [14C]palmitate taken up by the heart which was not oxidized was primarily incorporated into triglyceride stores (56%), with a small amount being incorporated into phospholipids (9.6%). If hearts were pulsed for 120 min, a further increase in [14C]palmitate incorporation into triglycerides was not seen (22.55 ± 4.15 = (n = 5) versus 20.41 ± 3.22 μmol/g dry weight, in hearts pulsed for 120 or 60 min, respectively). A slight increase in [14C]palmitate incorporation into phospholipids was seen in hearts pulsed for the longer period (4.71 ± 0.8 versus 2.28 ± 0.47 μmol/g dry weight in hearts pulsed for 120 or 60 min, respectively).
in a decreased amount of [14C]palmitate incorporated into triglycerides (6.18 μmol of [14C]palmitate/g dry weight) compared to hearts perfused with 1.2 mM [14C]palmitate. Overall myocardial triglyceride content also decreased under these perfusion conditions to 12.87 μmol of free fatty acid equivalents/g dry weight (normal triglyceride levels in unperfused rat hearts are 50–60 μmol of free fatty acid equivalents/g dry weight). As expected, “pulsing” hearts with 1.2 mM [14C]palmitate did not deplete myocardial triglycerides (Table IV).

Perfusion of hearts for a 60-min pulse period resulted in labeling of 42% of the total myocardial triglyceride stores, without changing the size of the overall pool (Table IV). The 60-min pulse period with 1.2 mM [14C]palmitate was therefore used in all subsequent experiments involving triglyceride turnover.

**Endogenous Triglyceride Turnover and Oxidation during the Chase Perfusion**—During the 60-min chase perfusion, [14C]palmitate was not present in the perfusate. Instead, hearts were perfused with buffer containing 11 mM glucose in the presence of no fat, low fat, or high fat (Table I). To measure exogenous fatty acid oxidation during the chase period in the low fat and high fat groups, [9,10-3H]palmitate was present in the perfusate. The production of 3H2O during the chase is a measure of exogenous palmitate oxidation. The chase is quantified as described under “Experimental Procedures.”

Perfusion of hearts for a 60-min pulse period resulted in labeling of 42% of the total myocardial triglyceride stores, without changing the size of the overall pool (Table IV). The 60-min pulse period with 1.2 mM [14C]palmitate was therefore used in all subsequent experiments involving triglyceride turnover.

**TABLE IV**

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>[14C]Palmitate as</th>
<th>CO2 production</th>
<th>Triglyceride content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/g dry wt</td>
<td>µmol [14C]palmitate/g dry wt</td>
<td>µmol free fatty acid/g dry wt</td>
</tr>
<tr>
<td>At end of pulse</td>
<td>20.41 ± 3.22</td>
<td>39.54 ± 3.06</td>
<td>48.1 ± 6.0</td>
</tr>
<tr>
<td>Following the 60-min chase perfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1) No fat (n = 6)</td>
<td>6.64 ± 0.30&quot;</td>
<td>17.85 ± 3.16&quot;</td>
<td>17.8 ± 3.4&quot;</td>
</tr>
<tr>
<td>2) Low fat (n = 6)</td>
<td>12.58 ± 0.29&quot;</td>
<td>5.29 ± 0.82&quot;</td>
<td>21.7 ± 2.7&quot;</td>
</tr>
<tr>
<td>3) High fat (n = 7)</td>
<td>11.80 ± 1.30&quot;</td>
<td>5.26 ± 0.46&quot;</td>
<td>56.5 ± 5.9&quot;</td>
</tr>
</tbody>
</table>

* Significantly different from hearts frozen at the end of the pulse.
* Significantly different from no fat hearts frozen following the chase.
* Significantly different from low fat hearts frozen following the chase.

**FIG. 1** 3H2O production from exogenous [3H]palmitate oxidation during the chase perfusion in isolated working hearts perfused with low or high fat. Values are the mean ± S. E. of at least six hearts in each group. *H2O production by hearts during the chase was quantified as described under “Experimental Procedures.” Oxidative rates were expressed as micromole of [3H]palmitate oxidized per g dry weight.

**TABLE V**

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Steady state exogenous palmitate oxidation</th>
<th>Steady state endogenous palmitate oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/g dry wt - min</td>
<td></td>
</tr>
<tr>
<td>Chased with no fat</td>
<td>361 ± 68</td>
<td>88.2 ± 13.6&quot;</td>
</tr>
<tr>
<td>Chased with low fat</td>
<td>633 ± 69&quot;</td>
<td>87.6 ± 7.7&quot;</td>
</tr>
</tbody>
</table>

* Significantly different from hearts chased with no fat.
* Significantly different from hearts chased with low fat.
perfusion.

palmitate oxidation during the chase period in the three experimental groups. The loss of \([^{14}C]\)palmitate from myocardial triglyceride stores (Table IV) was derived from triglycerides (greater than 90%). The contribution of \([^{14}C]\)palmitate from phospholipids, and from neutral lipids other than triglyceride, to oxidative metabolism during the chase was negligible.

The most dramatic utilization of \([^{14}C]\)palmitate from endogenous triglycerides during the chase was seen in hearts from the no fat group. Steady state rates of \([^{14}C]\)palmitate oxidation were over 3 times those seen in the low fat and high fat groups (Table V). This was accompanied by a dramatic loss of \([^{14}C]\)palmitate content from myocardial triglyceride stores (Table IV). Since no exogenous fatty acids were present with the buffer used in the no fat group to replace these fatty acids, a net loss of triglycerides was seen (Table IV). Comparison of the absolute triglyceride loss (from 48.1 to 16.71 μmol of free fatty acid equivalents/g dry weight) to the loss of \([^{14}C]\)palmitate from triglycerides (from 20.41 to 6.64 μmol/g dry weight), indicates that 44% of the triglyceride fatty acid loss consisted of \([^{14}C]\)palmitate. Interestingly, this almost equals the portion of the triglyceride pool that was initially labeled with \([^{14}C]\)palmitate (42%). This suggests that the same pool of myocardial triglycerides that was initially labeled during the pulse was being lost during the no fat chase.

Steady state oxidation rates of endogenous \([^{14}C]\)palmitate during the chase were similar in both the low fat and high fat groups (Table V). In both of these groups, endogenous triglyceride \([^{14}C]\)palmitate made a significant contribution to overall fatty acid oxidation. In the low fat group, 20% of total fatty acid oxidation obtained from exogenous or endogenous labeled fatty acids was obtained from myocardial triglycerides. Even in the presence of high fat, 12% of labeled fatty acid oxidation was obtained from myocardial triglycerides. This demonstrates that myocardial triglycerides are an important source of fatty acids for oxidative metabolism.

The loss of \([^{14}C]\)palmitate from triglycerides during the chase in the low fat and high fat groups was similar (Table IV). This would be expected since steady state endogenous \([^{14}C]\)palmitate oxidation rates during the chase were the same in these two groups. As expected, overall triglyceride content of glucose through glycolysis compared to rates of glucose oxidation. In the no fat group, only 47% of the glucose that passed through the perfusate into triglycerides (Table VI). Surprisingly, however, a significant loss of overall triglyceride content did occur in the low fat group (Table IV). This loss (from 48.1 to 19.61 pmol of free fatty acid equivalents/g dry weight) cannot be fully explained by the significant decrease in \([^{3}H]\)palmitate incorporation into triglycerides during the chase. In addition, the loss of \([^{14}C]\)palmitate during the low fat chase (from 20.4 to 12.6 μmol/g dry weight) accounts for only 30% of the total fatty acids released from triglycerides, even though 42% of the pool was initially labeled with \([^{14}C]\)palmitate. This suggests that a greater portion of unlabeled rather than labeled fatty acids are being lost from triglycerides during the chase.

**Myocardial Glucose Utilization during the Chase Perfusion**—A second series of heart perfusions were performed in order to determine the contribution of glucose as an energy substrate during the chase perfusion. This was achieved by using perfusion protocols similar to that described for the first series, except that nonlabeled palmitate was used throughout the entire protocol (Table II). Steady state rates of glycolysis and glucose oxidation were measured simultaneously by perfusing hearts with 11 mM \([2-^{3}H/^{14}C]\)glucose during the 60-min chase period.

Fig. 3 shows the steady state rates of glucose oxidation and glycolysis obtained during the chase in the no fat, low fat, and high fat groups. An interesting observation is the greater rates of glycolysis compared to rates of glucose oxidation. In the no fat group, only 47% of the glucose that passed through the glycolytic pathway was subsequently oxidized. This greater flux of glucose through glycolysis compared to glucose oxidation in the no fat group parallels what has previously been reported by Kobayashi and Neely (27) in hearts perfused in the absence of fatty acids. In our study, if fatty acids were added to the perfusate a dramatic drop in glucose oxidation occurred, with only a slight decrease in glycolytic rates. This effect was most dramatic in the high fat group. In this group of hearts, only 7% of the glucose that passed through glycol-
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Table VI

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
<th>Free fatty acids</th>
<th>Neutral lipids other than triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hearts chased with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low fat</td>
<td>5.29 ± 0.70</td>
<td>0.88 ± 0.09</td>
<td>0.21 ± 0.05</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Hearts chased with</td>
<td>13.23 ± 1.59</td>
<td>1.2 ± 0.4</td>
<td>0.70 ± 0.13</td>
<td>1.29 ± 0.37</td>
</tr>
<tr>
<td>high fat</td>
<td></td>
<td></td>
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</tbody>
</table>
*Significantly different from hearts chased with low fat.

Fig. 3. Steady state of glycolysis and glucose oxidation in isolated working hearts chased with no fat, low fat, or high fat. Values are the mean ± S. E. of at least six hearts in each group. Hearts were perfused as described under "Experimental Procedures." Values are expressed as micromoles of glucose utilized per g dry weight min.

ysis was eventually oxidized. This observation suggests that in the intact heart, fatty acids are a much more potent inhibitor in flux through pyruvate dehydrogenase than through phosphofructokinase.

Overall Myocardial Utilization of Palmitate and Glucose— With the steady state rates of both exogenous and endogenous radiolabeled palmitate during the chase, as well as steady state rates of both glycolysis and glucose oxidation, it is possible to calculate overall steady state ATP production by the heart. Table VII shows the steady state rates obtained from radiolabeled substrates during the 60-min chase perfusion. Since overall triglyceride content of hearts chased with no fat and low fat decreased during the chase perfusion, we also calculated ATP production during the 60-min chase perfusion in which nonlabeled fatty acid oxidation was considered (Table VIII). Values in Table VIII are expressed as overall ATP production during the 60-min chase, as opposed to steady state ATP production, since it cannot be determined from this study whether unlabeled triglyceride contribution to oxidative metabolism was linear during the chase. Table VIII assumes that all unlabeled fatty acids released from triglycerides were oxidized by the heart. To confirm this assumption we measured [14C]palmitate-specific activity in the perfusates at the beginning and at the end of the chase perfusion. The activity was slightly higher at the beginning of chase (equivalent to 4.3 ± 1.2 µmol of [14C]palmitate versus 2.5 ± 0.48 at the end of the chase). This suggests that all of the palmitate released from triglycerides during the chase was oxidized. It also demonstrates that only 3% of our original chase buffers were initially contaminated with [14C]palmitate from the pulse perfusion.

An interesting observation seen in Tables VII and VIII is that if fatty acids were omitted from the perfusate, a major portion of myocardial ATP production is derived from endogenous triglycerides. If only steady state oxidative rates are considered, endogenous [14C]palmitate oxidation accounted for 42% of the total ATP production (Table VII). Overall, endogenous triglycerides contributed to 59% of total ATP production during the chase period (Table VIII).

As expected, if fatty acids were present in the perfusate, the contribution of glucose to ATP production significantly decreased. The contribution of glucose utilization to steady state ATP production from radiolabeled substrate decreased from 58% in the no fat group to 24% in the low fat group, and 8% in the high fat group (Table VII). If overall ATP production during the chase were measured, glucose utilization decreased from 41% in the no fat group, to 15% in the low fat group, to 8% in the high fat group.

Another observation from Tables VII and VIII is the contribution of endogenous triglycerides to ATP production in hearts perfused with low fat and high fat. As expected, exogenous fatty acids were the major source of both steady state and overall ATP production in both groups. In the low fat group, however, endogenous [14C]palmitate contributed to 47% of overall ATP production, while exogenous fatty acids contributed to only 39% of overall ATP production (Table VIII). Even in the presence of high fat, 11% of steady state ATP production was derived from triglycerides. A similar contribution to overall ATP production was seen in the high fat group (Table VIII), since myocardial triglyceride levels remained constant, due to similar rates of triglyceride lipolysis and synthesis (Tables V and VI).

A series of experiments were also conducted in order to determine the effects of pharmacological dose of insulin on triglyceride turnover in hearts perfused with high concentrations of fatty acids. Addition of 500 microunits/ml of insulin resulted in a significant increase in both glycolytic rates (from 1678 ± 290 to 3280 ± 280 nmol/min·g dry weight) and glucose oxidation rates (from 124 ± 26 to 345 ± 65 nmol/min·g dry weight). Insulin was without significant effect, however, on exogenous fatty acid oxidation (from 642 ± 54 to 696 ± 68 nmol/min·g dry weight) or endogenous fatty acid oxidation (from 90.4 ± 10.2 to 73.0 ± 14.5 nmol/min·g dry weight). As a result, in hearts perfused with high concentrations of fatty acids, the contribution of fatty acids to ATP production increased.
Triglyceride Turnover in the Heart

Steady state myocardial ATP production during the chase perfusion calculated from steady state rates of glycolysis, glucose oxidation, endogenous palmitate oxidation, and exogenous palmitate oxidation

Values are the mean ± S. E. of at least six hearts in each group. ATP production was calculated as described under "Experimental Procedures." Values in brackets are the percentage of total ATP production for each perfusion condition.

<table>
<thead>
<tr>
<th>ATP source</th>
<th>No fat</th>
<th>Low fat</th>
<th>High fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose oxidation</td>
<td>45.24 ± 6.68</td>
<td>12.85 ± 1.43</td>
<td>4.46 ± 0.95</td>
</tr>
<tr>
<td>Glycolysis</td>
<td>(52.23%)</td>
<td>(16.96%)</td>
<td>(4.42%)</td>
</tr>
<tr>
<td>Endogenous [14C]palmitate oxidation</td>
<td>5.39 ± 0.92</td>
<td>4.96 ± 0.68</td>
<td>3.36 ± 0.56</td>
</tr>
<tr>
<td>Exogenous [3H]palmitate oxidation</td>
<td>35.99 ± 6.45</td>
<td>11.98 ± 1.75</td>
<td>11.30 ± 0.99</td>
</tr>
<tr>
<td>Total ATP production</td>
<td>46.56 ± 8.81</td>
<td>8.66 ± 7.74</td>
<td>(61.47%)</td>
</tr>
<tr>
<td></td>
<td>(41.55%)</td>
<td>(11.21%)</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

In this study, we developed and characterized an experimental protocol in which endogenous myocardial triglyceride turnover can be directly measured. This protocol also permits the direct measurement of endogenous triglyceride fatty acid oxidation. During the initial pulse perfusion, 42% of myocardial triglyceride fatty acids were labeled with [14C]palmitate. In contrast, only a small amount of [14C]palmitate was incorporated into nontriglyceride neutral lipids and phospholipids. During the chase perfusion, the [14C]-labeled fatty acids released from the triglyceride pool were readily oxidized and were not released into the perfusate. In contrast, the contribution of phospholipids, cholesterol esters, monacyclic and diacyclic glycerol to myocardial oxidative metabolism was very low (5-10%), demonstrating that triglycerides are the only endogenous lipid pools which significantly contribute fatty acids for oxidative metabolism. Using this pulse-chase technique, the importance of triglycerides as a source of fatty acids for oxidative metabolism in the heart is clearly demonstrated. In the absence of added fatty acids, the isolated working heart readily uses endogenous triglyceride reserves of fatty acids, with more than 50% of its energy requirements being met by this source. As would be expected, as increasing concentrations of fatty acids were delivered to the heart, the contribution of these pools to myocardial oxidative metabolism decreased. This occurs mainly due to an inhibition of lipolysis, since incorporation of exogenous fatty acids into the triglyceride pool continues. The contribution of myocardial triglycerides to oxidative metabolism was clearly apparent in isolated hearts perfused in the presence of high fatty acid concentrations (1.2 mM palmitate). Under these conditions triglyceride fatty acids accounted for 11% of total myocardial ATP production, without any change in the size of the endogenous triglyceride pool. Even in the presence of a pharmacological dose of insulin (500 microunits/ml) and high fatty acid concentrations, triglyceride turnover accounted for 7.9% of total myocardial ATP production. This could be achieved because the rate of triglyceride lipolysis was matched by the rate of incorporation of exogenously derived fatty acids into triglycerides. These observations support the concept that myocardial triglycerides are a readily mobilizable extended substrate source.

It has been previously proposed that the contribution of myocardial triglycerides to energy substrate utilization may be an in vitro phenomenon that is seen only in isolated hearts perfused with buffers devoid of fat (15). This suggestion was based primarily on the observation that overall myocardial triglyceride content decreases if hearts are perfused in the absence of fatty acids (1). As expected, a loss of triglyceride content in hearts perfused without fatty acids was also observed in our study. However, under conditions in which triglyceride content was maintained (high fat), we found that substantial triglyceride turnover occurs. This suggests that endogenous myocardial triglyceride pools are never static, and that a continuous lipolysis and synthesis of myocardial triglyceride pools occurs. These pools may serve the homeostasis of fatty acids in the cytosol. Surprisingly, in our experiments, hearts perfused with physiologic concentrations of exogenous fatty acids (0.4 mM palmitate) did not maintain their endogenous triglyceride content. This loss of overall triglycerides parallels what has previously been reported in isolated rats.
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hearts perfused with a similar concentration of fatty acid (6, 29). The reason for this loss of triglycerides at physiological levels of circulating fatty acids is not readily apparent. One potential explanation is that in vivo, interstitial fatty acid concentrations may be higher than circulating fatty acid concentrations. In vivo, chylomicrons and very low density lipoproteins (VLDL) are potential sources of fatty acids for the heart, in addition to circulating free fatty acids. Triglycerides in VLDL and chylomicrons particles are subjected to lipolysis by lipoprotein lipase, with the liberated fatty acids then passing to the interstitial spaces prior to uptake by the myocytes. If chylomicron and VLDL triglycerides lipolysis was actively occurring then it is possible that interstitial fatty acid concentrations in vivo may exceed the free fatty acid concentration in the blood. In contrast, in isolated hearts perfused with physiological levels of fatty acid as the sole source of exogenous fatty acids (0.4 mM palmitate), interstitial fatty acid levels will quickly equilibrate with the concentration of fatty acid in the perfusate. A reduction in the concentration of fatty acids to which the myocyte itself is exposed may therefore result in the observed depletion of endogenous triglyceride stores. This possibility is supported by the observation that at higher concentrations of exogenous fatty acids (1.2 mM palmitate), endogenous triglyceride stores were not depleted. Further proof, however, would be provided by providing chylomicrons or VLDL to isolated perfused hearts. Previous studies have demonstrated that chylomicrons are a significant source of fatty acids for oxidative metabolism in the isolated perfused heart (30-32). To our knowledge, however, no study has been conducted which determines the relative contribution of chylomicrons, VLDL, or plasma free fatty acids to overall myocardial fat uptake and energy requirements.

An interesting observation from this study was the marked differences in the rates of glycolysis and glucose oxidation in the heart. Simultaneous measurement of glycolysis and glucose oxidation in isolated hearts demonstrated that glycolytic rates were twice as high as glucose oxidative rates in hearts perfused in the absence of fatty acids. This observation parallels what has previously been demonstrated by Kobayashi and Neely (27) in which hearts were also perfused in the absence of fatty acids. In this study, we extended this observation by demonstrating that fatty acids inhibit glucose oxidation to a much greater extent than glycolysis, such that in the presence of high concentrations of fatty acids, rates of glycolysis are more than 13 times the rate of glucose oxidation. The higher ratio of glycolysis to glucose oxidation in fatty acid-perfused hearts is not a result of the heart being artificially subjected to hypoxic or ischemic conditions, since oxidative metabolism is maintained and provides the primary source of ATP (see Tables VII and VIII). This higher ratio in fatty acid-perfused hearts supports the concept that fatty acid inhibition of glucose utilization occurs to a greater extent at the level of pyruvate dehydrogenase than at the level of phosphofructokinase (1, 13, 14).

It has been proposed that under conditions of hyperglycemia, glucose can become the primary, and even sole, energy substrate to the heart (15, 22). Our data would suggest that this cannot occur, however, since even under optimal conditions which cannot be achieved in vivo (high glucose, no fat), glucose utilization provided only 40–50% of overall ATP production (Table VIII). The differences between our results and the previous studies can be explained by the observation that only a portion of the glucose taken up by the heart was oxidized (Fig. 3). Previous studies which have suggested that

1 The abbreviation used is: VLDL, very low density lipoprotein.
of fatty acids for oxidative metabolism, and that their contribution is inversely related to the concentration of fatty acids delivered to the heart. Triglyceride fatty acid contribution to overall energy production can range from 11% of myocardial ATP requirements in hearts perfused with high fat, to more than 60%, when the heart is deprived of an exogenous fatty acid supply. This study also demonstrates that glucose oxidation and glycolytic flux cannot meet the total energy demands of the heart, even in the absence of added exogenous fatty acids.

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REFERENCES