Insulin-mediated Modifications of Myocardial Lipoprotein Lipase and Lipoprotein Metabolism*

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Recirculating organ perfusion in vitro was conducted with hearts from control rats, animals given a single dose of streptozotocin (65 mg/kg) 48 h earlier, and streptozotocin-treated rats administered insulin (5 units), 2 h prior to decapitation during 45-min perfusions, the lipolysis of very low density lipoprotein (VLDL) triglyceride was significantly less in hearts from diabetic than in controls (41.9 ± 7.3% of control). This was associated with significant reductions in heparin-releasable (functional) lipoprotein lipase and tissue lipoprotein lipase of perfused hearts. The decreases in VLDL triglyceride metabolism and the levels of myocardial lipoprotein lipase were completely reversed by treatment of diabetic rats with insulin 2 h prior to study. Similar improvement of VLDL triglyceride metabolism and increases in myocardial lipoprotein lipase activity were observed in hearts from diabetic rats by direct addition of 100 milliliters/ml of insulin to the recirculating perfusion media. Under these conditions, the increase in both fractions of lipoprotein lipase in response to insulin was completely inhibited, and utilization of VLDL triglyceride was partially inhibited by pre-perfusion with cycloheximide for 10 min. The data derived from either VLDL triglyceride lipolysis in organ perfusion or direct measurement of myocardial lipoprotein lipase demonstrated a direct effect of insulin on myocardial lipoprotein lipase activity, and suggest that the response to insulin may be due in part to effects on protein synthesis.

It is generally considered that a primary cause of the hypertriglyceridemia associated with insulin deficiency is defective clearance of chylomicrons and/or very low density lipoproteins from the circulation (1-4). The peripheral metabolism of the triglycerides of these circulating lipoproteins is dependent on the activity of the enzyme, lipoprotein lipase, which appears to be functional at the endothelial surface of capillary beds associated with most tissues (5-7).

The lipoproteins clearance defect associated with insulin deficiency has been demonstrated in intact animals (3, 8) and in perfusion of intact myocardial tissue (e.g. Ref. 9). However, the demonstration of a direct relationship of this defect with the activity of lipoprotein lipase have been more elusive. Preliminary reports of these studies have been presented earlier (17, 20).

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Experimental Procedures

Materials—Bovine serum albumin (Fraction V; fatty acid-poor), crystalline bovine insulin, and Celite were obtained from Sigma. Glyceryl trioleate and lipid standards for chromatography were from Supelco Corp. (Bellefonte, PA). Glyceryl tri[1-14C]oleate (60 mCi/mmol), and [2-n-3H]glyceryl trioleate (430 mCi/mmol) were purchased from Amersham-Searle Corp. (Arlington Heights, IL). Streptozotocin was either obtained from Dr. William Dulin, The Upjohn Co., or was purchased from Boehringer Mannheim. All other chemicals and reagents were of highest purity.

Lipoprotein Isolation and Labeling—Thoracic duct lymph was obtained from fed male Wistar rats (200-300 g; Microbiological Assoc., Bethesda, MD) by procedures described earlier (21). Chylomicrons were removed by ultracentrifugation at d 1.006 g/ml for 1 × 10^6 g, min, and VLDL were isolated by centrifugation at d 1.006 g/ml for 10^6 g, min. These were recentrifuged once through d 1.006 g/ml, and were characterized by apolipoprotein composition using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (23), and by analysis of lipid composition (24, 25). Of the total lipid fatty acids, 57-68% were as triglyceride, 13-19% as phospholipid, 9-14% as cholesterol ester, 8-16% as partial glyceride, and 2-10% as unesterified fatty acid.

Glyceryl tri[1-14C]oleate (25 μCi) and [2-n-3H]glyceryl trioleate (100 μCi) were incorporated into VLDL core lipids using Celite according to the method described in Ref. 28.
Insulin and Myocardial Triglyceride Utilization

TABLE I

Plasma glucose levels of animals prior to heart perfusion

| Group        | Plasma glucose (mg/dl) |
|--------------|------------------------|
| Control      | 97.6 ± 4.0             |
| Diabetic     | 320 ± 8.0              |
| Diabetic injected with 5 units insulin | 37.2 ± 4.0 |

Diabetes was produced by a single intravenous dose of 65 mg/kg of streptozotocin and plasma glucose was determined after 48 h. Where indicated, a single dose of insulin was given 2 h prior to analysis. Figures in parentheses are the number of analyses, and data are expressed as means ± S.E.

MOLECULAR SIZE MARKERS

- Apo B: 80,000
- Apo AIV: 75,000
- Apo E: 45,000
- Apo AT: 42,000
- Apo AI: 36,000
- Apo C: 25,000

FIG. 1. Apolipoprotein profile of thoracic duct lymph VLDL. Molecular size markers, shown on the left, include phospholipase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soy bean trypsin inhibitor, and lysozyme. The apoproteins were identified by apparent molecular size, and include: Apo-B (small molecular weight apo-B), 220,000; Apo-AIV, 46,000; Apo-E, 31,000-35,000; Apo-AI, 26,000; and Apo-C, 14,000.

after, 2 ml of the perfusate were removed and extracted in chloroform-methanol (2:1, v/v) according to Folch et al. (29). Additional 0.5-ml aliquots were used for assay of 14C (30). Following perfusion, hearts were cleared of perfusate with 5 ml of Krebs' buffer. These were blotted, weighed, and homogenized in 20 volumes of chloroform-methanol for subsequent lipid analysis.

Studies on the effects of insulin and cycloheximide added to the heart perfusate were modified as follows. Hearts from control, diabetic, and insulin-treated diabetic rats were perfused for 10 min with 10 ml of Krebs' buffer containing rat serum (10%) and 5 units of insulin/ml. The hearts were homogenized in 50 ml of 50 mM Tris-HCl, pH 8.0, containing 1 mM ethylene glycol (31).

Labeled unesterified fatty acids were partitioned into the methanol-water phase and aliquots were assayed for radioactivity by liquid scintillation spectrometry. Under these conditions, 75.6 ± 3.0% of the labeled unesterified fatty acid was recovered in the methanol-water phase, confirming earlier studies on this liquid partition system (31).

The limitations of the assay (31) were confirmed on both perfusate and tissue lipoprotein lipase prior to study. Under the conditions of the present studies, the assay was linear for 30 min at perfusate additions of 10-200 µl. The substrate concentration (6.68 µmol/ml) was not rate-limiting and optimal albumin (1%) and serum (7.5-15.0%) concentrations were as described earlier (31). During recirculating perfusion of heparin, there was a rapid release of lipoprotein lipase activity during the first 2 min and a slow release of lipoprotein lipase for at least 60 min thereafter. This latter slow release phase was consistent in all preparations, and therefore comparisons of lipoprotein lipase activities were derived from the initial 10 min rather than 2 min of perfusion.

Control assays were conducted on perfusate obtained in the absence of heparin; heart homogenates of unperfused hearts, heart homogenate. The reaction was stopped by addition of 3.25 ml of methanol:chloroform:heptane (141:125:100, v/v) and 1.05 ml of 100 mM potassium carbonate-borate buffer, pH 10.5.

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Control assays were conducted on perfusate obtained in the absence of heparin; heart homogenates of unperfused hearts, heart homogenates to which heparin was added. Corrections were made for the recovery of labeled fatty acid in the partitioning system, and for labeled fatty acid release in the absence of heparin. Enzyme activity is expressed as milliunits where 1 milliunit is 1 nmol of oleic acid released/min at 37°C.

Analytical Procedures—Lipids in the perfusate and heart homogenates were extracted (29) and fractionated into major lipid classes by thin layer silicic acid chromatography in a solvent system of hexane:ethyl ether:acetic acid (80:16:2, v/v). Areas corresponding to authentic standards of unesterified fatty acid, triglyceride, diglyceride + monoglyceride, and cholesterol ester were individually scraped and eluted from silicic acid with methanol/ether (25:75, v/v) as described earlier (29). The origin containing phospholipid was also scraped but was subsequently handled without solvent elution (24). These were subjected to transmethylation with boron trifluoride-methanol and
Insulin and Myocardial Triglyceride Utilization

extracted prior to analysis of derivatized fatty acids by quantitative gas-liquid chromatography. The conditions for extraction, derivitization and chromatography, and corrections for recovery have been described previously (24). Unesterified and esterified cholesterol were determined on the silicic acid extracts by gas-liquid chromatography (24). Radioactivity in all silicic acid fractions was determined by liquid scintillation spectrometry (Beckman LS-250). Where indicated, total protein of the lipoproteins and heart was analyzed according to Markwell et al. (32).

Data on individual lipids concentrations of the lipoproteins and heart tissue are expressed as micromoles and are derived from the total fatty acid concentrations of each lipid class. Since the absolute level of triglycerides in VLDL varied between preparations (6.4 ± 0.75 µmol), all data were normalized to 6.4 µmol of VLDL triglyceride/perfusion. The data are expressed as means/g of wet weight ± S.E., and differences between values were analyzed for significance by the Student's t test.

RESULTS

Correlation of VLDL Triglyceride Mass and Radioactivity during Perfusion—The use of VLDL labeled in vitro with [2-3H]glyceryl tri[1-14C]oleate for measurement of VLDL triglyceride clearance and metabolism of triglyceride fatty acids was assessed by comparison of the disappearance of triglyceride mass and radioactivity during heart perfusion. As shown in Fig. 2, the disappearance of triglyceride fatty acids during recirculating perfusion of control hearts with VLDL was essentially linear over the 45-min perfusion period. As shown in Table II, these triglycerides are composed largely (90%) of linoleic (18:2 n-6), oleic (18:1 n-9), palmitic (16:0), and stearic (18:0) acids. During the disappearance of approximately 2.6 µmol of total triglyceride fatty acids, the proportions of the individual major fatty acids remained unchanged (Table II), suggesting the lack of fatty acid specificity during lipolysis of the VLDL core triglycerides by myocardial lipoprotein lipase.

During this same period, the disappearance of VLDL glyceryl tri[1-14C]oleate was proportional to disappearance of triglyceride mass (Table III), as were the proportional losses of glyceryl tri[1-14C]oleate and [2-3H]glyceryl trioleate. Similar correlations were obtained during perfusions of hearts from diabetic rats.

Lipolysis of VLDL [2-3H]Glycerol Tri[1-14C]oleate by Perfused Hearts from Control and Diabetic Rats—The comparative disappearance of labeled triglyceride from VLDL during 45-min recirculating perfusions of hearts from control and diabetic rats is shown in Fig. 2. With hearts from control animals, disappearance of fatty acid radioactivity from circulating triglyceride was linear over the perfusion period, and represented a lipolysis rate of 57.8 ± 2.9 nmol of fatty acids released/min. During this period, there was no significant accumulation of labeled unesterified fatty acid or of partial glycerides in the perfusion media. There was, however, accumulation of non-lipid 3H in the medium derived from lipolysis of [2-3H]glyceryl trioleate. This was assumed to be [2-3H]glycerol, since utilization of free glycerol by myocardial tissue is negligible (33). During the 45-min perfusion, accumulation of this label was also linear and was calculated to represent 19.8 ± 2.0 nmol of triglyceride glycerol released/min. Thus, the ratio of triglyceride fatty acid release and triglyceride glycerol release under these conditions was 2.9, compared to the theoretical ratio of 3.0.

With hearts from diabetic rats under identical perfusion conditions, disappearance of triglyceride fatty acids was also linear but occurred at a rate of 24.2 ± 4.2 nmol/min, or only 42% of that by control hearts. Again, this was not associated with significant accumulations of labeled unesterified fatty acids or partial glycerides in the medium. Accumulation of [3H]glycerol from lipolysis of [2-3H]glyceryl trioleate was linear, representing a release of 6.0 ± 2.0 nmol/min. Although the ratio of fatty acid to glycerol release with diabetic hearts differed from controls, the data were obtained from only three hearts.

![Fig. 2. Changes in perfusate lipids and glycerol during recirculating perfusion of control and diabetic hearts with VLDL [2-3H]glyceryl tri[1-14C]oleate. Perfusion conditions and analytical methods are described under "Experimental Procedures."](http://www.jbc.org/)

TABLE II

| Fatty acids (chain length/unsaturation) | Triglyceride fatty acid | % Released | Fatty acids (chain length/unsaturation) | Triglyceride fatty acid | % Released |
|--------------------------------------|------------------------|-----------|--------------------------------------|------------------------|-----------|
| Linoleic (18:2 n-6)                  | 2.78 ± 0.10            | 1.63 ± 0.06| 41.4 ± 2.2                           | Linoleic (18:2 n-6)    | 2.78 ± 0.10| 1.63 ± 0.06| 41.4 ± 2.2 |
| Oleic (18:1 n-9)                     | 1.29 ± 0.04            | 0.87 ± 0.02| 41.1 ± 1.6                           | Oleic (18:1 n-9)       | 1.29 ± 0.04| 0.87 ± 0.02| 41.1 ± 1.6 |
| Palmitic (16:0)                      | 1.21 ± 0.05            | 0.72 ± 0.03| 40.5 ± 2.5                           | Palmitic (16:0)        | 1.21 ± 0.05| 0.72 ± 0.03| 40.5 ± 2.5 |
| Stearic (18:0)                       | 0.47 ± 0.03            | 0.28 ± 0.02| 40.4 ± 4.2                           | Stearic (18:0)         | 0.47 ± 0.03| 0.28 ± 0.02| 40.4 ± 4.2 |

TABLE III

| Measurement  | 0 | 15 | 30 | 45 |
|--------------|---|----|----|----|
| [14C]TG/TG mass | 1.0 | ±0.01 | 1.03 | ±0.02 | 1.01 | ±0.03 |
| [3H]TG/[3H]TG | 1.0 | ±0.01 | 1.00 | ±0.02 | 0.99 | ±0.03 |
was 4.0, lipolysis was sufficiently low and the variance sufficiently large to yield no statistical difference with the theoretical ratio of 3.0.

The metabolic fates of the released fatty acid and glycerol labels are summarized in Table IV. With control hearts, almost 25% of the labeled fatty acid released was recovered as \(^{14}\text{CO}_2\) by 45 min of perfusion. Another 52% was recovered as tissue non-lipid (or water-soluble) radioactivity suggesting catabolism of the fatty acid to water-soluble intermediates or metabolites. Only 6.3% was recovered as tissue lipid, and of this, 71.1 ± 2.3% was as tissue triglyceride. Thus, 93% of the fatty acid label was utilized by myocardial tissue and only 7% remained in the perfusate. Of the labeled glycerol released during lipolysis, 90% was recovered in the perfusate, and only 5.3% was utilized for lipid synthesis. Of this, 69.5 ± 3.7% was associated with tissue triglyceride.

With hearts from diabetic rats, a greater percentage (60.7 ± 11.2%) of the released fatty acid radioactivity was recovered in tissue lipids (0.66 ± 0.12 \(\mu\)mol compared to 0.16 ± 0.03 \(\mu\)mol in controls), and of this, 61.2 ± 6.5% was as tissue triglyceride. Although the complete oxidation of fatty acid to \(^{14}\text{CO}_2\) was statistically comparable to that in controls (0.42 ± 0.16 \(\mu\)mol versus 0.64 ± 0.13 \(\mu\)mol), there was no radioactivity in the non-lipid fraction of heart extracts. Of the labeled glycerol released, 76% remained in the perfusate, and a greater percentage was recovered as tissue lipid, of which 62.6 ± 2.2% was as triglyceride.

**Effect of Insulin on Myocardial Triglyceride Utilization**—Diabetic rats were given a single intraperitoneal dose of insulin (5 units) 2 h prior to removal of hearts for perfusion. As shown in Table IV, hearts from these animals were normalized with respect to lipolysis of VLDL [2-\(^{3}\text{H}\)]glycerol tri[(1-\(^{14}\text{C}\)]oleate. The release of either \(^{14}\text{C}\)-fatty acid or [\(^{3}\text{H}\)]glycerol was comparable to that in hearts from control rats and significantly greater than that observed with diabetic tissue. Despite the normalization of lipolysis of the triglyceride, the subsequent metabolism of the released fatty acid and glycerol was different from either control or diabetic tissue. Only 41% (0.96 \(\mu\)mol) of the released fatty acid was accounted for by complete or partial catabolism of the fatty acid, and a greater percentage (21.9% or 0.52 \(\mu\)mol) was recovered as tissue lipid. Thus, catabolism of fatty acid was improved over that by diabetic tissue but still only 50% of that by control tissue. Accumulation of radioactivity as tissue lipid (primarily triglyceride), in contrast, was 3.2 times that in controls, and similar to that in diabetic tissue (0.52 \(\mu\)mol and 0.66 \(\mu\)mol, respectively). This increased formation of esterified lipid was also observed by analysis of the metabolic fate of the released glycerol. In this case 19.4% (0.21 \(\mu\)mol) was incorporated into tissue lipid compared to 5.3% (0.05 \(\mu\)mol) by hearts from control rats.

The direct effect of insulin on myocardial lipolysis of VLDL triglyceride was demonstrable by including insulin (100 milliunits/ml) in the perfusates of diabetic hearts (Table IV). At this high level of insulin over the 45-min recirculating perfusion period, almost 59% of the available \(^{14}\text{C}\)-triglyceride oleic acid was released, representing 1.4 times that by hearts from control rats, and 1.5 times that by hearts from diabetic animals treated with insulin. In this case, however, total catabolism of fatty acid was comparable to that in controls, while accumulation of label in tissue lipid (27.5% or 1.0 \(\mu\)mol) was still elevated (6.2-fold over controls), and even greater than that in hearts from insulin-treated diabetic rats (by 1.9-fold). This increased incorporation of label into tissue lipid was also evident by analysis of the fate of the released glycerol.

**Myocardial Lipoprotein Lipase Activity**—Heparin-releasable (functional) lipoprotein lipase and tissue lipoprotein lipase were determined on the perfusates and hearts following a 10-min recirculating perfusion with 5 units of heparin/ml perfusate. It was determined in preliminary studies that during perfusion of VLDL, or perfusions in the absence of heparin, release of lipoprotein lipase activity into the perfusate was only 7.5 milliunits (0.75 milliunits/ml for 10 ml of perfusate). When hearts from controls rats were perfused with heparin, about 71% of the total releasable and non-releasable lipoprotein lipase was found in the perfusate (Table V).

Total lipoprotein lipase activity of diabetic hearts was 74 ± 3% of that in control hearts. This decrease was a reflection of both the heparin-releasable fraction (78.6 ± 2.7% of control) and the tissue residual activity (63.2 ± 4.0% of control). With hearts from diabetic rats treated with 5 units of insulin 2 h before study, total lipoprotein lipase activity (1220 ± 74 milliunits), and the percentage of the total lipoprotein lipase released by heparin perfusion (70.7 ± 4.4% of total lipoprotein lipase) was the same as in controls. As a control, normal rats were also given a single dose of 5 units of insulin, 2 h prior to study. In this group, levels of total lipoprotein lipase, and the proportions of heparin-releasable and tissue residual lipoprotein lipase activities were indistinguishable from those of controls or of diabetic animals treated with insulin.

The direct effect of insulin on myocardial lipoprotein lipase activity was demonstrated by perfusion of either control or

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**Table IV**

| Metabolism of VLDL [2-\(^{3}\text{H}\)]glyceryl tri[(1-\(^{14}\text{C}\)]oleate by perfused rat hearts |
|-------------------------------------------------------------|
| Rat hearts were perfused for 45 min with VLDL (6.4 \(\mu\)mol of total triglyceride fatty acids) containing [2-\(^{3}\text{H}\)]glyceryl tri[(1-\(^{14}\text{C}\)]oleate. Pre-treatment of animals, perfusion conditions, and analytical methods are described under “Experimental Procedures.” Figures in parentheses represent the number of studies, and data are expressed as means ± S.E. |

| Measurement | Control (4) | Diabetic (8) | Diabetic treated with insulin (7) | Diabetic perfused with insulin (7) |
|-------------|-------------|-------------|--------------------------------|----------------------------------|
| Lipolysis of [\(^{14}\text{C}\)]triglyceride, \(\mu\)mol fatty acid released/g tissue | 2.09 ± 0.13 | 1.09 ± 0.19 | 2.36 ± 0.76 | 3.65 ± 0.38 |
| Fate of released [\(^{14}\text{C}\)]oleic acid (%) | | | | |
| Oxidation to \(^{14}\text{CO}_2\) | 24.8 ± 5.0 | 39.2 ± 14.0 | 7.4 ± 0.9 | 25.1 ± 7.7 |
| Recovery as tissue water-soluble intermediates | 51.9 ± 4.5 | 0 ± 11.2 | 32.8 ± 3.2 | 30.2 ± 3.3 |
| Recovery as tissue lipid | 6.3 ± 1.1 | 60.7 ± 11.2 | 21.9 ± 5.9 | 27.5 ± 6.5 |
| Lipolysis of [\(^{3}\text{H}\)]triglyceride, \(\mu\)mol glycerol released/g tissue | 0.89 ± 0.09 | 0.27 ± 0.09 | 0.92 ± 0.18 | 0.87 ± 0.14 |
| Fate of released [2-\(^{3}\text{H}\)]glycerol (%) | | | | |
| Per fusate | 90.3 ± 15.1 | 75.5 ± 3.8 | 64.5 ± 22.8 | 61.4 ± 6.4 |
| Tissue water-soluble | 4.0 ± 1.1 | 10.7 ± 2.0 | 16.1 ± 4.8 | 18.8 ± 7.0 |
| Tissue lipid | 5.3 ± 0.2 | 13.8 ± 1.5 | 19.4 ± 5.6 | 19.7 ± 6.3 |
Insulin and Myocardial Triglyceride Utilization

TABLE V

Functional and residual lipoprotein lipase activities of perfused rat hearts

Hearts from each of the animal preparations were perfused with buffer or insulin (100 milliunits/ml) as described under "Experimental Procedures." Heparin (5 units/ml) was added during the final 10 min of perfusion, and lipoprotein lipase activity was determined (31) on the perfusates and the heart homogenates. Figures are means ± S.E. for five perfusions in each group. Figures with the same superscripts are significantly different (p < 0.01).

| Rat and heart preparation          | Heparin-releaseable (functional) | Residual | Total | Functional/total x100 |
|-----------------------------------|---------------------------------|----------|-------|----------------------|
|                                    | milliunits                      |          |       |                      |
| Control                            | 813 ± 75*                       | 321 ± 18*| 1135 ± 82*| 70.7 ± 7.6          |
| Diabetic                           | 639 ± 22ab                      | 203 ± 13ab| 842 ± 31ab| 75.9 ± 2.6          |
| Control injected with insulin      | 826 ± 110*                      | 332 ± 39*| 1159 ± 120*| 71.3 ± 9.5          |
| Diabetic injected with insulin     | 826 ± 54b                       | 357 ± 39*b| 1220 ± 74b| 70.7 ± 4.4          |
| Control perfused with insulin      | 1009 ± 120b                     | 332 ± 39*| 1366 ± 22ab| 73.9 ± 8.8          |
| Diabetic perfused with insulin     | 860 ± 95b                       | 308 ± 22*| 1168 ± 104b | 73.7 ± 8.1          |

Fig. 3. Effects of insulin and cycloheximide on VLDL triglyceride utilization by perfused hearts from diabetic rats.

Hearts were pre-perfused for 10 min with buffer, 100 milliunits/ml of insulin, or insulin and 20 μM cycloheximide, prior to addition of VLDL glyceryl tri-[1-C]oleate and determination of lipolysis. Data represent means ± S.E. for three perfusions in each group. ——O, buffer pre-perfusion; △—△, insulin pre-perfusion; ⋅⋅⋅⋅, insulin plus cycloheximide.

diabetic hearts with insulin (100 milliunits/ml) prior to analysis of heparin-releaseable and residual lipoprotein lipase activities. Perfusion of hearts from diabetic rats with insulin resulted in an increase (p < 0.01) in total lipoprotein lipase activities to levels which were comparable to those in controls (1165 ± 104 versus 1135 ± 82 milliunits, respectively). This was a result of a proportional increase in both heparin-releaseable and residual fraction of the heart (73.7 ± 8.1% of the total activity was released by heparin). Insulin had only a small effect on the lipoprotein lipase activity of hearts obtained from control rats (1366 ± 22 and 1135 ± 82 milliunits, respectively), and again, this was a result of proportional increases in the two tissue lipoprotein lipase fractions.

Effect of Cycloheximide on Myocardial Clearance of VLDL Triglyceride and Lipoprotein Lipase Activities—Since the in vivo effect of insulin on myocardial VLDL triglyceride utilization and lipoprotein lipase activities was largely duplicated by perfusion of hearts with insulin in vitro, perfusion studies were designed to assess the effect of cycloheximide on the insulin-dependent improvement of VLDL triglyceride clearance and on myocardial lipoprotein lipase activity.

For studies on VLDL triglyceride clearance, hearts from diabetic rats were perfused for 10 min with buffer, insulin, or insulin and 20 μM cycloheximide. This was followed by addition of the VLDL preparation labeled with glyceryl tri[1-14C]oleate and perfusions were continued for 45 min. With these modified perfusion conditions, the effect of insulin on the improvement of the lipolysis of VLDL triglyceride by diabetic hearts (Fig. 3) was even more pronounced than without the 10-min pre-perfusion with insulin (Table IV). This effect of insulin was partially, but not completely, prevented when cycloheximide was present during the preperfusion period (Fig. 3).

 Comparable studies were designed to determine the effect of cycloheximide on functional and residual lipoprotein lipase activities (See "Experimental Procedures"). As shown in Fig. 4, these perfusion conditions gave control lipoprotein lipase activity levels comparable to those in Table V (921 ± 53 units and 303 ± 23 units of functional and residual LPL activity, respectively). Perfusates from diabetic hearts contained only 47% of the lipoprotein lipase activity of that from control hearts (p < 0.05), and tissue lipoprotein lipase activity was also proportionately less (51% of control) than in controls. The effect of diabetes in this study was more dramatic than in the earlier study (Table V), in which perfusions were for a

![FIG. 3. Effects of insulin and cycloheximide on VLDL triglyceride utilization by perfused hearts from diabetic rats.](image-url)

![FIG. 4. Effects of insulin and cycloheximide (CHX) on functional and residual lipoprotein lipase (LPL) activities of perfused rat hearts.](image-url)
Insulin and Myocardial Triglyceride Utilization

total of only 10 min. Perfusion of hearts from diabetic rats with 20 \( \mu \)M cycloheximide (40 min) had no effect on the already reduced levels or distribution of functional and residual lipoprotein lipase activities. Perfusion of diabetic hearts with buffer for 10 min, and 100 milliunits of insulin/ml for 30 min, completely reversed the effect of diabetes and resulted in functional and residual lipoprotein lipase activities comparable to those in control hearts. However, when diabetic hearts were perfused with cycloheximide for 10 min prior to addition of insulin, and continued perfusion for 30 min, the increase in lipoprotein lipase activity during perfusion with insulin alone was completely blocked. Under these conditions, both functional and residual lipoprotein lipase activities remained low, with no suggestion of an accumulation of tissue-associated lipoprotein lipase activity.

Since the effects of perfusions with insulin and cycloheximide on myocardial lipoprotein lipase were observed when lipoprotein lipase levels were low, an additional study was conducted to determine the effects of these agents on lipoprotein lipase levels in control hearts. During these 40-min perfusions, insulin had no significant effect on the already elevated levels of functional lipoprotein lipase (906 ± 76 \( \mu \)g/ml), on perfused and control hearts, respectively. However, in the presence of cycloheximide added 10 min prior to addition of insulin, functional lipoprotein lipase levels were significantly less (662 ± 77 units; \( p < 0.05 \)) than after perfusions with insulin alone.

**DISCUSSION**

Diabetes with uncontrolled hyperglycemia is generally associated with elevated VLDL triglycerides (e.g. Ref. 34). Nikkila et al. (34) have suggested that these individuals have VLDL secretion rates comparable to non-diabetics, and that the associated hypertriglyceridemia is due to defective clearance rates. Chen et al. (35) have demonstrated that VLDL secretion rates are not elevated in streptozotocin-induced insulin deficiency in rats, and the clearance defect has been clearly demonstrated in a similar animal model by infusion of labeled VLDL triglycerides (3, 36).

The enzyme responsible for peripheral clearance of VLDL triglycerides is lipoprotein lipase. In intact organs such as adipose tissue and heart, lipoprotein lipase exists in both functional and nonfunctional forms (5, 14). The functional form appears to be largely associated with the endothelial surface of tissue capillaries and is readily released by heparin (5). This lipoprotein lipase activity is presumably responsible for the degradation of lipoprotein triglyceride during tissue assimilation of the triglyceride fatty acids. The nonfunctional form of lipoprotein lipase which is not readily releasable by heparin, appears to be associated with the tissue parenchymal cells (5). The activity has also been termed "residual" and may represent the ultimate source of the vascular endothelial activity (e.g. Refs. 37 and 38).

Data attempting to relate the defective clearance of lipoprotein triglycerides during human or experimental diabetes, and the activity of either functional or total tissue lipoprotein lipase have not been convincing. The original observations by Bieman et al. (39) suggested a decrease in postheparin lipolytic activity in ketosis-prone diabetic patients. These data, however, have been questioned (e.g. Ref. 35) in part, on the basis that postheparin lipolytic activity represents multiple enzyme activities derived from various tissues (34, 40, 41). More direct measurements, however, suggested a decrease in adipose tissue lipoprotein lipase in untreated diabetics (42, 43). Others, however, have been unable to demonstrate changes in either adipose (38) lipoprotein lipase, or in plasma postheparin lipolytic activity (11) in insulin-deficient rats. In fact, the original studies of Kessler (10) suggested that insulin deficiency in rats, induced by alloxan treatment, resulted in increased heart muscle lipoprotein lipase and decreased adipose lipoprotein lipase and that both changes were reversible by insulin. These data on heart lipoprotein lipase, however, are not compatible with the observation (9, 17) that perfused hearts from diabetic rats demonstrated defective clearance of chylomicron triglycerides. It seems likely, that differences in results on lipoprotein lipase activities under various experimental conditions are due in part to methods of tissue preparation and to the assay procedures. Lipoprotein lipase has been assayed on fresh tissue homogenates (e.g. Refs. 5 and 44) in the absence or presence of heparin (16), on acetone powders (e.g. Refs. 44 and 45) and on organ perfusate (45, 46). Activity measurements have been made on a variety of emulsified and natural triglyceride substrates. These multiple variations have not allowed consistent findings or effective comparison of results. The differences in measurable activity and residual lipoprotein lipase activity are also evident in the present study. Lipoprotein lipase activity, calculated from the extent of VLDL triglyceride lipolysis during controlled perfusion conditions, is likely to represent a more physiological assay. Under these conditions, calculated lipoprotein lipase activity was about 58 milliunits, compared to 800-900 milliunits (Table V, and Fig. 3) of functional lipoprotein lipase activity assayed by using the artificial substrate preparation (31). Nevertheless, as discussed below, the results were internally consistent, and comparable changes during each experimental condition were observed using either approach.

In the present study, the intact heart in recirculating perfusion has been employed as an organ model to assess the role of insulin on both the clearance of VLDL triglycerides, and on the activity of the endothelial, membrane-associated lipoprotein lipase. Studies on the recirculating perfusion of VLDL clearly demonstrated a defective triglyceride clearance associated with insulin deficiency within 48 h of a single dose of 65 mg/kg of streptozotocin. The defect was observed by measurement of either disappearance of triglyceride in isolated plasma VLDL, or of [2-3H]glycerol [1-14C]oleate, or by the accumulation of [2-3H]glycerol in the perfusion media. These data (58% reduction in clearance of 0.32 \( \mu \)mol triglyceride) are entirely consistent with those of Keisberg (9), who initially demonstrated a 70% reduction in chylomicron triglyceride (0.5-0.5 \( \mu \)mol) clearance by perfused hearts from rats rendered insulin-deficient by a single injection of 45 mg/kg of alloxan, 3-5 days earlier.

Although the present studies and those of Keisberg (9) employed lymph lipoprotein particles for assessment of tissue clearance, the observed data on defective clearance of circulating triglyceride by perfused hearts of diabetic rats were duplicated using isolated plasma VLDL. Under identical perfusion conditions and triglyceride concentrations, lipolysis of plasma VLDL triglyceride during perfusion of controls hearts was 4.7 \( \mu \)mol/45 min while with lymph VLDL, 2.6 \( \mu \)mol were cleared. Lipolysis of plasma VLDL by hearts from diabetic rats was 54% of control while with lymph VLDL, lipolysis was 42% of control. Thus, based on all of the measured criteria, it seems clear that a major defect in the hearts from diabetic rats is a reduced ability to degrade circulating lipoprotein triglycerides. There were also characteristic metabolic differences in diabetic tissue, with respect to the fate of the released fatty acids, and these were also compatible with previous observations. The accumulation of myocardial triglycerides during

\[P. O'Looney and G. V. Vahouny, unpublished observations.\]
diabetes is well recognized, and can occur within 48 h of streptozotocin administration to rats (47). We have determined that myocardial triglycerides are increased by 71.4 ± 9.8% within 48 h of administration of the drug to rats. In the present study, diabetes was associated with an increased percentage of the extracted fatty acid recovered as tissue lipid, and of this, the majority was as triglyceride. Conversely, as had been reported earlier (9), catabolism of the extracted fatty acid to CO₂ and water-soluble catabolites was markedly reduced in diabetic hearts. Thus, acute diabetes, as defined by marked hyperglycemia in these animals, was associated with decreased myocardial clearance of VLDL triglycerides, and with increased esterification and reduced catabolism of the extracted triglyceride fatty acids.

It seems possible that abnormal tissue metabolism of fatty acids may subsequently influence VLDL triglyceride lipolysis without modifying the activity of functional lipoprotein lipase. However, using direct measurements of heparin-releasable lipoprotein lipase, it was possible to show a significant reduction in functional lipoprotein lipase activity in hearts from diabetic rats, suggesting that the clearance defect was at least in part due to reduced lipolytic capacity of this tissue. The reduced clearance of VLDL triglycerides by perfused hearts from diabetic rats, and the measureable decrease in functional lipoprotein lipase from these tissues are difficult to reconcile with earlier reports of unchanged or even increased levels of myocardial lipoprotein lipase associated with insulin deficiency (8, 10, 11, 18).

Administration of a pharmacological dose of insulin (5 units) to diabetic rats resulted in severe hypoglycemia within 2 h. This treatment also resulted in a normalization of VLDL triglyceride lipolysis by perfused hearts, but did not completely reverse the metabolic defects observed with hearts from diabetic rats. Thus, a significant amount of the tissue fatty acid was retained as lipid (0.52 μmol compared to 0.66 μmol in diabetic tissue and 0.16 μmol in control hearts), and this was largely (75%) as triglyceride. Although complete oxidation of fatty acid was not improved by treatment of diabetic rats with insulin, overall catabolism (to water-soluble products and CO₂) was improved (0.95 μmol compared to 0.43 μmol in diabetic hearts).

These data suggested that the improvement in VLDL triglyceride clearance by hearts from insulin-treated diabetic rats is not simply a result of altered metabolic characteristics of the tissue per se, but might reflect a direct or indirect effect of insulin on lipolytic activity of the coronary vasculature. This was again substantiated by the observation that functional lipoprotein lipase activity of hearts from diabetic rats treated with insulin was significantly increased over that in animals not given insulin, and was comparable to that in control hearts.

Studies on the addition of insulin to the recirculating perfusion media were designed to assess the direct effects of insulin on myocardial VLDL triglyceride clearance. With the high levels of insulin (100 milliunits/ml) present during the entire perfusion period, the lipolysis of VLDL triglyceride by diabetic hearts was markedly improved (by 3.5-fold), and was even greater than that observed by perfusion of control hearts or hearts from diabetic rats treated with insulin. Under these conditions, there was also improvement of the catabolism of tissue fatty acids to levels comparable to that in control hearts. However, tissue levels of lipid (primarily as triglyceride) were still elevated compared to controls. These studies suggest a direct effect of insulin on the ability of perfused hearts to metabolize VLDL triglyceride, and this is further supported by the observation that, under these experimental conditions, the activities of both functional and tissue lipoprotein lipase are significantly increased by insulin, and to levels comparable to those in control hearts.

Evidence for the involvement of protein synthesis during insulin-induced increases in myocardial triglyceride utilization was derived from both assay systems. Thus, the increased lipoprotein lipase activities in response to insulin, assayed in vitro under optimized conditions, were completely blocked by pretreatment of hearts with cycloheximide. Using the more physiological measurement of VLDL triglyceride lipolysis during organ perfusion, however, cycloheximide only partially prevented the insulin-stimulated improvement in lipolysis. Nevertheless, these studies collectively imply that protein synthesis is at least in part involved in the effect of insulin on lipoprotein lipase activity.

It has been estimated that the turnover time of the functional enzyme at the capillary endothelium of rat heart is about 2 h (39). Morgan et al. (48) have clearly demonstrated that insulin stimulates and cycloheximide inhibits protein synthesis during short-term perfusion of rat hearts. From analyses of polysome and ribosomal subunit profiles, it has been suggested (48), that insulin accelerates steps involved in initiation of peptide chain synthesis in this tissue.

Furthermore, a reduction of total protein synthesis has been demonstrated in perfused hearts from rats following alloxan-induced diabetes (49). This reduction can be largely restored to normal levels by the perfusion of insulin for 1 h. Considering these results and the data in the present study, it seems likely that the reduced lipoprotein lipase activity during diabetes is due in part to lowered lipoprotein lipase synthesis or depressed synthesis of an activator or processing enzyme. Therefore, a general stimulation of protein synthesis by insulin could restore lipoprotein lipase activity to normal levels in either a specific or nonspecific manner.

The present studies on myocardial tissue are entirely consistent with those employing adipose tissue. Insulin can, under specific experimental conditions, stimulate adipose lipoprotein lipase synthesis (6, 7) but may not be directly involved in post-translational modifications (50). Activation, presumably by glucose concentration, has also been considered important in regulation of functional lipoprotein lipase activity. Enzyme release from the adipocyte, however, appears to be independent of protein synthesis (52).

Thus, despite reported differences in the Km for the enzymes from adipose tissue and heart (6), and the possible differences in effects of circulating metabolite levels, such as VLDL (53), it appears that the enzyme from both tissues may be directly regulated by insulin. The mechanism(s) and site of this regulation in myocardial tissue require additional attention.

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Insulin-mediated modifications of myocardial lipoprotein lipase and lipoprotein metabolism.

P O'Looney, M Vander Maten and G V Vahouny

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