Uptake and Tissue Content of Fatty Acids in Dog
Myocardium under Normoxic and Ischemic Conditions

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SUMMARY. The effect of ischemia on the myocardial content of nonesterified fatty acids (NEFA), triacylglycerol, cholesteryl esters, and phospholipids assayed with gas-liquid chromatography was studied in an open-chest dog preparation. Ischemia was induced by partial occlusion of the left interventricular coronary artery during 120 minutes \( n=20 \). Tissue content of the lipid classes was assessed in biopsies taken from ischemic and normoxic areas of the left ventricular free wall. Local venous blood from the concomitant vein of the left interventricular coronary artery was collected to determine myocardial extraction of lipids. In eight other dogs, no ischemia was induced (control group). Under normoxic conditions, NEFA appeared to be present in trace amounts: about 25 nmol/g wet weight of tissue, representing less than 0.1% of total myocardial fatty acids. During ischemia, NEFA increased in the affected area. This accumulation was most pronounced in the least perfused layer: the subendocardium (up to 172 nmol/g). Blood flow, estimated with radioactively labeled microspheres fell from 0.55 to 0.06 ml/min per g in this particular layer. The uptake of NEFA by the ischemic myocardium was decreased, indicating that enhanced lipolysis of endogenous lipids or reduced combustion may be held responsible for the accumulation of NEFA in ischemic tissue. Since arachidonic and linoleic acids showed the highest relative increase, lipolysis of endogenous phospholipids, rich in these fatty acids, seems to be reasonable. Ischemia had no significant effect on the content of triacylglycerol and cholesteryl esters. Phospholipids tended to decrease in the affected subendocardial layers. 

FATTY ACIDS may be harmful to the heart during shortage of oxygen. Raised plasma levels of NEFA have been suggested to induce or facilitate arrhythmias in patients with myocardial infarction (Oliver et al., 1968), or to extend the infarct area (Opie et al., 1981). Despite the indications that increased concentrations of NEFA can inhibit enzyme activities and mitochondrial energy production (Katz and Messineo, 1981). Ischemia had no significant effect on the content of triacylglycerol and cholesteryl esters. Phospholipids as well as the arterio-local venous differences of the aforementioned lipid classes were determined. To investigate the behavior of saturated and unsaturated fatty acids under ischemic circumstances, the individual long-chain fatty acids were determined with gas-liquid chromatography.

Methods

Animal Preparation

The experiments were performed on 28 mongrel dogs of either sex and unknown age, ranging in weight from 18 to 40 kg. The animals were premedicated intramuscularly with 10 mg of fluanisone and 200 µg of fentanyl citrate per kilogram of body weight. Anesthesia was induced intravenously with sodium pentobarbital (10 mg/kg of body weight) and, after endotracheal intubation, was maintained with nitrous oxide in oxygen (60:40, vol/vol) in combination with a continuous infusion of sodium pentobarbital (2 mg/kg per hr). Pulmonary ventilation was kept constant during the experiments with a positive-pressure respirator. Rectal temperature was measured with a thermistor probe. Body temperature was kept constant at 37.5°C.

The chest was incised through the left 5th intercostal space and the pericardium was opened over the anterolateral aspect of the heart. Ascending aortic pressure was measured via the femoral artery with a polyethylene catheter connected to a pressure transducer (Ailtech). The pressure in the left interventricular coronary artery distal to the site of stenosis (see below) was measured through a small side branch of this artery (Van der Meer and Reneman, 1972) with a polyethylene catheter (PE-50 Clay Adams) connected to a pressure transducer (Ailtech). The

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catheters used for pressure measurements and sampling of blood were kept patent with a continuous infusion of saline (6 ml/hr) using a Harvard infusion pump or a Sorensen infusion system (Sorensen Research Company). The use of heparin was avoided to prevent both in vivo and in vitro lipolysis of esterified fatty acids (Van der Vusse et al., 1980a; Giacomini et al., 1980). Left intraventricular pressure was measured with a catheter-tip micromanometer (Millar) inserted through the left brachial artery, and its maximal first derivative (dP/dtmax) was determined with an analogue differentiator (Schaper et al., 1965). The ECG was derived from limb leads. The hemodynamic variables were recorded continuously on a multichannel Schwarzer recorder.

**Experimental Set-up**

The animals were allocated to 2 groups. In group I (ischemia group, n=20), an inflatable cuff was placed on the left interventricular coronary artery just distal to the diagonal branch. The cuff was connected through silicon tubing to a micrometer. The whole system was filled with distilled water so that the cuff could be inflated carefully until the desired degree of stenosis—mean coronary artery pressure distal to the stenosis of approximately 3.0 kPa—was reached. This degree of stenosis was maintained throughout the experimental period of 120 minutes with a servo-motor pump with an autoregulating feedback system controlled by the mean coronary artery pressure (Jageneau et al., 1975). In group II (control group, n=8), the cuff remained deflated during the experimental period.

Arterial blood samples were collected through the catheter used for aortic blood pressure measurements. Local venous blood samples were obtained through a polyethylene catheter (PE-60 Clay Adams) inserted into the concomitant vein of the left interventricular coronary artery with a Seldinger technique. Six-tenths milliliter of blood was used for determination of blood gases and blood pH, and 4.0 ml of blood were immediately centrifuged. The supernatant of these samples was quickly frozen and stored at —80°C for 7 days. Routinely, two adjacent biopsies were obtained from the area perfused by the left interventricular coronary artery (ischemic area in group I, normoxic area in group II) and two adjacent biopsies from tissue perfused by the hemodynamic variables, which were recorded continuously, were also calculated at these moments.

Transmural biopsies from the free wall of the left ventricle were taken with an electrically driven drill bore at time 120 minutes. Routinely, two adjacent biopsies were obtained from the area perfused by the left interventricular coronary artery (ischemic area in group I, normoxic area in group II) and two adjacent biopsies from tissue perfused by the circumflex branch of the left coronary artery (normoxic in both groups). The biopsies were taken in eight dogs of group I and in five dogs of group II. The wet weight of the transmural biopsies ranged from 450 to 900 mg. Adhering blood was removed with an ice-cold physiological saline solution and the tissue was dried between gauze pads. The transmural biopsies were divided into subepicardial, subepicardial layers. Subsequently, the pieces of tissue were harvested from the tongue of an aluminum clamp into 500-750 mg of tissue; 500-750 mg of tissue after the injection of the microspheres and was continued during at least 1 min. After the experiment, the heart was excised, rinsed and stored in formaldehyde 5%. The lipid content in the first layer of the myocardial biopsies was compared with the blood flow in these layers. Since the subdivided biopsies were too small for reliable flow determination, we have measured the flow in the area closely surrounding the tissue used for lipid analysis. Pieces of tissue for the determination of myocardial blood flow weighed about 1 gram. After weighing the tissue and blood samples, they were counted in a gamma counter (Packard Multichannel Analyzer). From these data, regional myocardial blood flow was calculated with the MTC II program (Schosser et al., 1979).

**Biochemical Analysis**

Glucose, lactate, inorganic phosphate and potassium were measured with a Technicon Auto-analyzer. Blood gasses were analyzed with an IL 413. Hemoglobin and oxygen saturation were determined with a Radiometer OSM-2. Oxygen content was calculated from the latter variables.

Tissue biopsies were homogenized and lipids were extracted as described before (Van der Vusse et al, 1980b). Aliquots of deeply frozen tissue (150-300 mg of wet weight) were pulverized in an aluminum mortar with a stainless steel pestle, previously cooled in liquid nitrogen. The tissue powder was transferred to test tubes cooled with liquid nitrogen. The test tubes were placed at —21°C and the tissue powder was weighed with 1 ml of methanol at —21°C. The content of the test tubes was allowed to warm up to room temperature and was subsequently weighed. Chloroform was added until a mixture of chloroform and methanol of 2:1 vol/vol was obtained. The anti-oxidant butylated hydroxytoluene (0.1%) was present in the methanol and chloroform. Subsequently, a mixture of heptadecanoic acid, cholesteryl heptadecanoate, and triheptadecanoin was added to the extraction mixture to correct for losses during the assay procedure. Non-esterified fatty acids, triacylglycerol, cholesteryl esters, and total phospholipids were isolated from the extracts by thin-layer chromatography using TLC plates coated with Silica gel F 254 (Merck, FRG). The lipid spots were predeveloped with chloroform:methanol:H2O:acetic acid (10:10:1:1 vol/vol) until the liquid front had reached a level 1 cm above the site of application of these spots. Hexane:diethyl ether:acetic acid (24:5:0.3, vol/vol) was used as developing solvent. The lipid spots were made visible with Rhodamine G, scraped from the plate and transferred into test tubes, containing 0.5 ml BF3-methanol solution (7%BF3). The fatty acid moiety of the various lipid classes was methylated at 20°C for 15 minutes (non-esterified fatty acids), at 100°C for 30 minutes (triacylglycerol), and at 100°C for 45 minutes (cholesterol esters and phospholipids). The methyl esters were extracted from the methylating mixture with pentane. After evaporation of the pentane under a stream of N2 at 37°C, the methyl esters originating from the non-esterified fatty acids, triacylglycerol, and phospholipids were dissolved in trimethyl pentane, containing appropriate amounts of methyl pentadecanoate as internal standard. The methyl esters of the cholesterol esters were rechromatographed on silica gel plates to remove the anti-oxidant butylated hydroxytoluene.
The lipid spots were extracted from the silica gel powder with diethyl ether:methanol (50:1, vol/vol). The methyl ester mixtures were analyzed by gas-liquid chromatography using glass columns packed with 5% DEGS on chromosorb W-AW-DMCS (length 6 feet; inner diameter 2 mm). Starting temperature of the column was routinely 150°C; the temperature gradually increased up to 190°C, with an increase rate of 4°C/min. Carrier flow of N₂ was 45 ml/min. One-tenth milliliter of serum was extracted with 2.0 ml of methanol:chloroform (1:2, vol/vol) at room temperature. Serum extracts were subsequently treated in the same manner as were the extracts of myocardial biopsies (see above).

Statistical Analysis

Statistical analysis of differences between tissue content of lipids in normoxic and in ischemic myocardium was performed using the two-tailed Wilcoxon rank-sum test (aligned ranks). Differences between the hemodynamic values and arterio-local venous differences of substrates and oxygen before and after induction of ischemia were evaluated for statistical significance by applying Wilcoxon’s matched-paired signed-rank test (two-tailed probability). Statistical analysis of uptake or release of individual fatty acids, triacylglycerol, cholesteryl esters and phospholipids was performed, using a two-tailed sign test. P<0.05 was considered to be a significant difference.

Results

Lipid Content in Various Layers of Normoxic Dog Myocardium

The lipid content in the various layers of dog myocardium was investigated in five animals of the control group. Biopsies were taken from the regions supplied with blood by the left interventricular coronary artery and by the circumflex branch of the left coronary artery. In the various layers of these regions, the median content of NEFA varied between 24 and 31 nmol/g, that of cholesteryl esters between 205 and 235 nmol/g, and that of phospholipids between 30 and 36 μmol/g. All values are expressed as fatty acid moieties per gram of wet tissue. No significant differences in the content of these fatty acids could be detected, either between the various layers or between these layers in both regions. On the contrary, in both areas, the content of triacylglycerol was significantly higher in the subepicardial layer as compared with the meso- and subendocardial layers. The median triacylglycerol content in the subendocardial, meso-, and subepicardial layers in both regions ranged from 4.0 to 5.9, from 4.9 to 5.5, and from 10.8 to 14.2 μmol triacylglycerol fatty acids per gram tissue. Since, in most animals, fat can be observed macroscopically in the vicinity of the superficial epicardial coronary arteries, it is likely that the gradient of triacylglycerol is caused by fat cells at the epicardium and does not necessarily reflect differences in the content of esterified fatty acids in the myocytes in various layers of the left ventricular myocardium. In Table 1, the results obtained in the mesolayer of the area perfused by the left interventricular coronary artery are given in more detail. With respect to the total content of the various lipid classes in dog myocardium, the data in Table 1 show that less than 0.1% of the fatty acids present in myocardial biopsies were in the nonesterified form. About 0.5% was bound to cholesterol, whereas 13.7% was incorporated in triacylglycerol and 85.7% in phospholipids.

The four lipid classes in dog myocardial biopsies differed markedly in their relative fatty acid composition. However, the composition within one class was

| Lipid class and Relative Fatty Acid Composition of Each Class in Normoxic Dog Myocardium |
|---------------------------------|-----------------|----------------|----------------|
| Lipid class | NEFA | Triacylglycerol | Cholesteryl esters | Phospholipids |
| Amount (nmol/g wet wt of tissue) | 25 | 5530 | 203 | 34,480 |
| Myristic acid | 0.4 | 2.2 | 1.0 | 0.5 |
| Palmitic acid | 0.3-3.6 | 1.9-3.6 | 0.5-2.0 | 0.2-1.6 |
| Palmitoleic acid | 26.3-35.6 | 20.7-24.5 | 7.6-13.6 | 7.0-10.8 |
| Stearic acid | 6.7-12.3 | 4.0-10.0 | 2.9-4.9 | 0.5-1.9 |
| Oleic acid | 28.2 | 9.6 | 3.2 | 16.4 |
| Linoleic acid | 16.6-39.8 | 7.3-11.4 | 1.2-5.8 | 14.9-17.8 |
| Arachidonic acid | 14.2 | 41.2 | 21.2 | 18.0 |
| Linoleic acid | 11.8-22.5 | 36.0-45.5 | 15.6-25.5 | 15.4-23.7 |
| Arachidonic acid | 8.8 | 16.6 | 41.3 | 25.2 |
| Linoleic acid | 2.2-17.3 | 12.2-21.1 | 35.4-57.0 | 22.0-27.1 |
| Arachidonic acid | 5.0 | 0.7 | 14.4 | 28.3 |
| 0-8.9 | 0.3-1.8 | 11.8-20.2 | 26.7-34.4 |

Median values and 95% confidence limits of 10 biopsies from the mesolayer of the area perfused by the left interventricular coronary artery of five dogs of group II (control group) are presented.
similar in the various layers of the two areas investigated. For that reason, only the data of the mesolayer of the region perfused by the left interventricular coronary artery are given (Table 1). Palmitic and stearic acid were the main constituents of the nonesterified fatty acids. Oleic acid was present in abundance in triacylglycerol. Linoleic acid accounted for about 40% of the fatty acids in the cholesteryl ester group. More than half of the fatty acid moieties of total phospholipids was linoleic and arachidonic acid.

**Extraction of Lipids by Normoxic Dog Myocardium**

The concentration of NEFA, triacylglycerol, cholesteryl esters, and phospholipids and the relative fatty acid composition of each lipid class in arterial blood obtained during the control period in both group I and II are summarized in Table 2. Less than 3% of the total fatty acids was in the unesterified form. About 8% was esterified as triacylglycerol, whereas 34% and 55% were present in the esterified form as cholesteryl esters and phospholipids, respectively. Oleic acid was the predominant fatty acid in NEFA and triacylglycerol. Linoleic acid accounted for about 50% of the fatty acids bound to cholesterol. Stearic and arachidonic acid were the main constituents of the fatty acid moiety of the phospholipid class. Approximately half of the fatty acids in the four lipid classes together was linoleic and arachidonic acid.

The arterial concentration of NEFA did not change significantly during the experimental period and varied between 0.28 (0.08-0.35) and 0.24 (0.11-0.38) mm (median values and 95% confidence limits). The arterio-local venous differences were rather constant during the whole experimental period. There was no significant difference between the concentrations of these lipids in arterial and local venous blood at any sample time (data not shown).

**Regional Myocardial Ischemia—Effect on Some Hemodynamic and Biochemical Parameters**

In the experimental model used in this study, the area perfused by the left interventricular coronary artery was made ischemic by partial occlusion (stenosis) of this artery. Heart rate remained unaffected, whereas aortic pressure and the maximal value of the first positive derivative of left ventricular pressure fell during the ischemic period (Table 4). The arterio-local venous differences of glucose, lactate, inorganic phosphate, potassium, and oxygen reflect the changes in

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**Table 2**

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>NEFA (mmol fatty acid/liter)</th>
<th>Triacylglycerol</th>
<th>Cholesteryl esters</th>
<th>Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>0.18</td>
<td>0.76</td>
<td>2.37</td>
<td>3.82</td>
</tr>
<tr>
<td>Relative composition (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myristic acid</td>
<td>1.1</td>
<td>0.9</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>24.5</td>
<td>17.3</td>
<td>8.5</td>
<td>17.4</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>4.9</td>
<td>5.2</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Searic acid</td>
<td>13.4</td>
<td>7.2</td>
<td>1.2</td>
<td>27.5</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>11.5-15.6</td>
<td>6.6-7.8</td>
<td>0.9-2.1</td>
<td>25.4-29.5</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>40.9</td>
<td>42.1</td>
<td>19.0</td>
<td>10.9</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>14.9</td>
<td>19.9</td>
<td>48.1</td>
<td>14.1</td>
</tr>
</tbody>
</table>

Median values and 95% confidence limits are presented. n (NEFA) = 27, n (triacylglycerol) = 18, n (cholesteryl esters, phospholipids) = 10. Sera were obtained during the control period from dogs belonging to groups I and II.
myocardial metabolism induced by shortage of oxygen (Table 5). The ischemic tissue released lactate, inorganic phosphate and potassium, whereas the arterio-local venous difference of glucose increased to a value about seven times higher than the control value. The arterio-local venous difference of oxygen increased by about 25%. In the control experiments, the measured hemodynamic and biochemical parameters did not change significantly during the experimental period (data not shown).

The Lipid Content in Various Layers of Regional Ischemic Dog Myocardium

In eight dogs of group I, two biopsies were taken from the normoxic area supplied with blood by the circumflex branch of the left coronary artery and two biopsies were taken from the ischemic part of the heart, following 120 minutes of ischemia. Just before the biopsies were performed, regional myocardial blood flow was assessed by injection of radioactive microspheres. Blood flow in the subepi-, meso-, and subendocardial layers of the normoxic area was found to be about 0.55 ml/min per g (Fig. 1). The decline of blood flow in the ischemic area was most pronounced in the subendocardial layers (0.06 ml/min per g), whereas flow in the subepicardial layers fell to 0.22 ml/min per g).

The content of NEFA in the subepicardial layers increased from 32 to 51 nmol/g, but these changes were not significant (Fig. 1). NEFA significantly increased in the ischemic meso (up to 110 nmol/g) and subendocardial layers (up to 172 nmol/g). The NEFA content and the relative fatty acid composition in the normoxic area of the regional ischemic myocardium were similar to those of the normoxic dog hearts of group II.

The individual NEFA content in normoxic and ischemic heart tissue is summarized in Figure 2. Since accumulation of this lipid class was most pronounced in the subendocardial layers, only the data in this particular layer are presented. Palmitic, stearic, oleic, linoleic, and arachidonic acid content increased significantly during ischemia. The ratio of ischemic and normoxic tissue content of myristic, palmitic, palmitoleic, stearic, oleic, linoleic, and arachidonic acid was 3.0, 4.0, 1.7, 4.0, 6.0, 7.3, and 11.0, respectively. This indicates that the longer chain, unsaturated fatty acids, such as linoleic acid (by about 600%) and arachidonic acid (by about 1000%), show the highest relative increase.

Ischemia had no significant effect on the content of cholesteryl esters (data not shown). The content of fatty acid moieties in the total phospholipid class tended to decrease in the ischemic subendocardial layers (P = 0.10). The amount of cholesteryl esters and phospholipids in the normoxic region of regionally ischemic myocardium was not different from amounts measured in the corresponding area in the control dogs. The content of triacylglycerol in the meso- and subendocardial layers of the ischemic area was significantly higher than the values of the normoxic part of the same heart, but comparable with the content of triacylglycerol in normoxic hearts of group II, indicating a decrease of triacylglycerol in the normoxic area of the partially ischemic heart.

### Extraction of Lipids by Ischemic Dog Myocardium

Arterial NEFA levels were not influenced by regional myocardial ischemia (data not shown). The arterio-local venous differences of total NEFA tended to increase during the period of ischemia from 0.05 (0.02-0.11) mm at time −15 minutes to 0.10 (0.06-0.15) mm at time 120 minutes (median values and 95% confidence limits, n = 15, P = 0.05). The data in Table
TABLE 5
Arterio-Local Venous Differences of Glucose, Lactate, Inorganic Phosphate, Potassium, and Oxygen before and during Left Interventricular Coronary Artery Stenosis

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>-15</th>
<th>20</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mm)</td>
<td>0.15</td>
<td>0.70*</td>
<td>0.90*</td>
<td>1.0*</td>
</tr>
<tr>
<td>Lactate (mm)</td>
<td>0.19</td>
<td>-0.51*</td>
<td>-0.40*</td>
<td>-0.42*</td>
</tr>
<tr>
<td>Inorganic phosphate (mm)</td>
<td>0.04-0.55</td>
<td>(-1.26)-(-0.06)</td>
<td>(-1.76)-(-0.10)</td>
<td>(-0.89)-0.24</td>
</tr>
<tr>
<td>Potassium (mm)</td>
<td>(-0.01)</td>
<td>-0.36*</td>
<td>-0.10</td>
<td>-0.05</td>
</tr>
<tr>
<td>Oxygen (mm)</td>
<td>-0.10</td>
<td>-0.40*</td>
<td>-0.22</td>
<td>-0.20</td>
</tr>
<tr>
<td>Median values and 95% confidence limits are presented (n = 10).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
* Significantly different from pre-ischemic values at P < 0.05.

3 show that the overall uptake of NEFA by ischemic myocardial tissue was greatly reduced as compared with the uptake of the normoxic myocardium of the dogs in group II. However, the preponderance of oleic acid as NEFA substrate for the heart remained. The relative contribution of palmitic, palmitoleic, stearic, and linoleic acid to NEFA extracted under ischemic conditions was also comparable with that under normoxic circumstances. An uptake of arachidonic acid such as that during normoxia could not be detected.

Regional myocardial ischemia had no effect on the arterial concentration and relative fatty acid composition of triacylglycerol, cholesteryl esters and phospholipids. No arterio-local venous differences of these three lipid classes across the ischemic region could be detected as in the normoxic situation (data not shown).

Discussion
Myocardial Content of Fatty Acids under Normoxic Conditions

The content of NEFA in normoxic dog myocardium found in the present study is considerably lower than the values published by other investigators (Haider et al., 1977; Weishaar et al., 1977; Andrieu et al., 1979). Besides differences in the animal preparation, such as feeding conditions and type of anesthesia used during the experimental procedure, differences in methodology and specificity of the assay method of NEFA are likely to be responsible for the overestimation of

![Figure 1](image1.png)
**Figure 1.** Regional myocardial blood flow and NEFA content in subepi, meso-, and subendocardial layers of normoxic and ischemic regions of dog left ventricular myocardium. Values shown in this figure are median values and 95% confidence limits of 16 biopsies from the area perfused by the partially occluded left interventricular coronary artery (ischemic region, dark bars) and of 16 biopsies from the area perfused by the left circumflex coronary artery (normoxic region, light bars) in eight dog hearts from group I. * Significantly different from the values in the corresponding layers of the normoxic area of the heart (P < 0.05).

![Figure 2](image2.png)
**Figure 2.** Content of individual NEFA in the subendocardial layer of the normoxic and ischemic area of dog left ventricular myocardium. Median values and 95% confidence limits of 16 biopsies from the subendocardial layer of the normoxic region (light bars) and 16 biopsies of the ischemic region (dark bars) in eight dog hearts from group I are shown. Biopsies were taken after 120 minutes of regional ischemia. Data are expressed as individual fatty acid per gram of wet tissue. 14:0 refer to myristic acid, 16:0 to palmitic acid, 16:1 to palmitoleic acid, 18:0 to stearic acid, 18:1 to oleic acid, 18:2 to linoleic acid, and 20:4 to arachidonic acid. * Significantly different from the values in the corresponding layer in the normoxic region of the left ventricle at P < 0.05.
the NEFA content in myocardial tissue (Kramer and Hulan, 1978; Van der Vusse et al., 1980b).

Information about the content of individual NEFA in normoxic dog myocardium is limited. Besides, the results published on this subject matter are conflicting. In their 1977 paper, Weishaar and co-investigators found palmitic acid to be present far in excess of myristic, stearic, oleic, and linoleic acid. In a more recent communication of these investigators (Weishaar et al., 1979), the relative content of the individual fatty acids is in good agreement with the data in the present study, despite the considerably higher total amounts of NEFA found in heart tissue by Weishaar and co-workers (1979).

Investigations of Rose and Goresky (1977a) might provide support for the correctness of the low content of NEFA in normoxic myocardial tissue. From experiments performed with the multiple indicator dilution technique, they calculated that in dog hearts under steady state conditions, the concentration of NEFA in plasma, interstitial space, and intracellular space should relate as 1:0.25:0.06. Just before biopsies were taken in our study, the median arterial and local venous NEFA concentrations were found to be 0.22 and 0.10 mm, respectively, resulting in a mean capillary NEFA concentration of 0.16 mm. From the aforementioned relation, the median interstitial and intracellular concentrations can be calculated to be 40 and 10 mm, respectively. Calculations based on these fluid concentrations and on a ratio of interstitial to intracellular fluid volume of 0.24 (Rose et al., 1977b) as well as on the assumption that 10% of myocardial tissue consisted of blood, lead to a median content of NEFA of 23 mm or about 2.3 nmol/g in normoxic dog myocardium. This value is in the same order of magnitude as the directly determined amount of NEFA in myocardial biopsies in the present study, being 25 nmol/g.

Under steady state conditions and in the presence of oxygen, NEFA will be extracted from extracellular sources and combusted to CO₂ and H₂O. The first step in the oxidative breakdown of fatty acids is activation by fatty acyl thiokinase. The question arises as to whether the calculated intracellular NEFA concentration is compatible with the kinetic properties of this enzyme system. The Kₘ value of fatty acid thiokinase for palmitic acid has been reported to be 8 mm (Groot et al., 1976). From the aforementioned relationship between the NEFA content in plasma, interstitial space, and intracellular space published by Rose and co-workers (1977a, 1977b), we calculated from our experimental data an intracellular NEFA content of about 10 nmol/g or 10 μmol/liter. This value refers to both free and bound NEFA. Since specific binding proteins for NEFA have been described in myocardial tissue (Mishkin et al., 1972; Grostetter and Harris, 1977), the concentration of NEFA available for the fatty acid-activating enzyme system will be considerably lower than the Kₘ value. Aas (1971) reported a maximal activation rate for NEFA of 2 μmol/min per g heart tissue. This value was found to be far in excess of the actual amount of NEFA extracted and combusted by the heart under steady state conditions. An uptake rate of 33 nmol NEFA/min per g can be calculated from a median arterio-local venous difference of NEFA of 0.1 nmol/liter, a myocardial blood flow of about 0.55 ml/min per g, and a hematocrit of 40%. The in vivo conversion rate of NEFA is compatible with the above-described hypothesis that the concentration of NEFA available for the activating enzyme system is lower than the affinity constant of the enzyme.

Extraction of Lipids by Normoxic Dog Myocardium

The arterial serum concentration of NEFA is at least eight times higher than the NEFA content measured in biopsies. The real gradient from serum to intracellular fluid is even higher because the biopsies consist, in part, of interstitial fluid and serum, trapped inside the tissue. This observation favors the hypothesis that the extraction of NEFA by myocardial tissue is based on diffusion (Spector, 1968). The finding that oleic acid accounted for about 50% of total NEFA extracted by the heart is in good agreement with the data reported by Rothlin and Bing (1961). The tissue content of triacylglycerol exceeds the concentration in arterial serum. The abundance of these esterified fatty acids in myocardial tissue might emphasize the role of triacylglycerol in the potential storage of substrates, required for energy production by aerobic breakdown of the fatty acid moieties. Considering an uptake of 33 nmol NEFA/min per g in normoxic myocardial tissue, the calculated intracellular concentration of 10 nmol/g indicates that endogenous NEFA, in contrast with triacylglycerol, represents no substantial store of substrates.

The absence of a positive arterio-local venous difference for triacylglycerol and phospholipids across the myocardium might indicate the virtual inertness of these circulating substances for the heart, although uptake of small quantities can readily be overlooked due to the rather high concentration of these substances in serum.

The Effect of Regional Ischemia on Myocardial Extraction and Tissue Content of Lipids

The finding that the content of NEFA is significantly increased in ischemic myocardial tissue is in agreement with the data of Weishaar and co-workers (1977, 1979) but in contrast with the results of Haider and colleagues (1977), who could not find any difference in NEFA content between ischemic and nonischemic myocardial tissue. A possible explanation for this discrepancy might be the difference in the method of assaying NEFA. The NEFA content estimated in normoxic tissue by the latter authors is about 300 times higher than in our study, which might mask the changes that take place during ischemia.

Analysis of tissue samples from various layers of the myocardium reveals that the increase in NEFA content is most pronounced in the layer with the lowest blood flow, i.e., the subendocardium. The
origin of the increased amount of NEFA remains to be clarified. A potential source for the NEFA accumulated in ischemic myocardial tissue are nonesterified fatty acids circulating in blood. Although the uptake of NEFA is greatly reduced during ischemia, these fatty acids are still extracted under these circumstances. Studies in which accumulation of fatty acid derivatives as acyl-CoA and acylcarnitine has been reported (Shug et al., 1978), demonstrate that fatty acid oxidation is impaired under ischemic conditions. As a consequence, part of the NEFA extracted from the blood, with oleic acid as predominant fatty acid, might be responsible for the accumulated NEFA in the affected myocardium. However, because of the observed inhomogeneities in ischemic myocardial blood flow, the uptake of substrates across the ischemic tissue might also vary from layer to layer. Therefore, we cannot definitely conclude whether the observed extraction and tissue accumulation of NEFA occur in the same layer or cells of the affected myocardium.

The finding that the uptake of arachidonic acid by the ischemic tissue is absent and that arachidonic acid shows the highest relative increase in the ischemic area, might indicate an endogenous source of the accumulated NEFA. The most likely candidates are myocardial phospholipids, rich in arachidonic and linoleic acid. This is supported by the observation of Vasdev and co-workers (1980) that the individual phospholipid classes in sarcolemmal and mitochondrial fractions isolated from ischemic dog myocardium are significantly decreased. Release of fatty acids from phospholipids may be induced by enhanced phospholipase A2 activity. After hydrolysis of the fatty acid moiety at the β-position in the phospholipid molecule, lysophosphoglycerides will be produced. In this respect, the observation of Sobel and co-workers (1978) that the content of lysophosphatidylethanolamine and lysophosphatidylcholine was found to be increased in ischemic rabbit hearts is worthy of mentioning. The elevated release of linoleic and arachidonic acid and the accompanying increase of lysophosphoglycerides in an isolated blood perfused dog heart preparation, subjected to global ischemia for 30 minutes, strongly support the hypothesis of activated phospholipase activity under ischemic circumstances (Weglicki et al., 1973).

Another possible source for some of the accumulated NEFA in ischemic myocardial tissue may be endogenous triacylglycerol, rich in oleic acid. Although oleic acid accumulates in the ischemic myocardium, we have no indication that this fatty acid is derived from intracellular triacylglycerol, since the content of this lipid class in the ischemic area is similar to that in the corresponding area of the control dogs. The lower triacylglycerol content in the meso- and subendocardial layers of the normoxic part of regional ischemic hearts might indicate an enhanced hydrolysis of endogenous triacylglycerol, reflecting an increased need for more combustible substrates for energy production in the normoxic area in order to compensate for the loss of work by the ischemic region. The finding of Crass and co-workers (1971) that isolated rat hearts, forced to deliver work, consumed more rapidly their endogenous triacylglycerol stores, supports this hypothesis.

The impact of the accumulation of long-chain unsaturated fatty acids, especially in the inner layers of ischemic myocardial tissue, is incompletely understood. According to the hypothesis of Oliver et al. (1968) and Opie and co-investigators (1977), the nonesterified fatty acids themselves will be toxic for the jeopardized myocardial tissue. Besides, accumulated lysophosphoglycerides, the residues of partly hydrolyzed endogenous phospholipids, might exert, alone or in combination with increased NEFA levels, a noxious effect on myocardial function (Sobel et al., 1978; Corr et al., 1979). On the other hand, increased cellular concentrations of linoleic and arachidonic acid may result in an increased biosynthesis of prostaglandins in ischemic myocardial tissue. Enhanced prostaglandin F2α synthesis and increased release of this substance from regional ischemic dog hearts have been reported (Berger et al., 1976). Protective effects of this prostaglandin have been described in animals with occluded coronary arteries (Goldfarb and Glenn, 1974).

The conclusion as to whether the increased amount of NEFA in the ischemic area is noxious for the myocardium or merely reflects a disturbance in myocardial lipid metabolism under ischemic circumstances, is subject to further investigations.

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