Experimental Study on the Lipid Metabolism in the Heart Muscle*


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Metabolic activity of the heart muscle is derived from the source of energy of the carbohydrate, fat, and aminoacid metabolism in the organ. Although it had been observed for a long time that the myocardial R. Q. became quite low under certain experimental conditions, it was not known that uptake of fat by the myocardium was directly implicated until 1938 (2) (3). The important participation of fatty acids in the myocardial metabolism has been demonstrated by Bing et al. (1), using the method of coronary sinus catheterization and reporting that fatty acids were contributed 67 per cent of total myocardial oxygen usage.

A previous report from this laboratory (5) showed that neither cholesterol nor phospholipid were utilized, but nonesterified fatty acid (NEFA) were consumed in some extent, and that a relatively large amount of oleic acid in the plasma total fatty acid was extracted in the heart muscle. The present investigation is aimed to clarify the mode of utilization of esterified fatty acids, NEFA, and the effect of epinephrine or some components of electron transport system on the myocardial uptake of fatty acids in normal dogs.

Experimental Method

The experiments were performed on mongrel dogs weighed 14–16kg. Sodium Pental anesthesia was used. Under the inhalation of 100% Oxygen, thoracotomy was performed, and the coronary vein was wedged with polyethylene catheter of 4–6mm in diameter. The other end of the catheter was inserted to one of the jugular veins. (Fig. 1) After the chest was closed and then the respiration' the blood pressure and the pulse became physiologically stabilized, the blood samples were obtained in order to measure the coronary blood flow, glucose, lactate, pyruvate, and fatty acids concentration in the blood. The methods adopted were as follows:

Coronary blood flow was measured with the drop method. Blood gas analysis was chacked by the method of Van Slyke and Neill. Blood sugar (Somogyi-Nelson's method), lactate (Barker-Summerson's method), pyruvate (Friedmann-Haugen's method), phospholipid (Allen's method), cholesterol (Zak-Henly's method), NEFA (Dole's method) were analyzed on simultaneously drawn blood samples from a systemic artery and coronary sinus. Fatty

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acids were prepared for gas-liquid chromatographic analysis by the following method: The plasma lipid was extracted with Folch reagent, and divided into two parts. One was used for analyzing the total fatty acid composition, and the other was prepared for silicic acid column chromatography from which triglyceride, cholesterol, cholesterol ester, NEFA, phospholipid were eluted. Their fatty acids were methylated with diazomethane. Individual fatty acids were identified on the basis of retention times of known fatty acids. Fatty acids of neutral fat, NEFA, and total fatty acids were measured by internal normalization technique.

RESULTS

28 dogs, as the control group, were used for the determination of the difference of lipids between their arterial and coronary venous bloods. The results were shown in the Table I, and Fig. 2. The A–V difference was hardly recognized in cholesterol and phospholipid. Therefore, they were considered to be unutilized in the heart muscle.

As for the total fatty acids and NEFA, the A–V differences were recognized and their values were 3.1 ± 2.4 mg/dl (0.0112 mEq/dl) and 3.4 ± 0.7 μEq/L respectively.

**Table I** Mean values of 28 dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF</td>
<td>68.5</td>
<td>cc/min.</td>
</tr>
<tr>
<td>BP</td>
<td>120.3</td>
<td>mmHg</td>
</tr>
<tr>
<td>CVR</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>A-O₂</td>
<td>16.68</td>
<td>Vol%</td>
</tr>
<tr>
<td>A–V O₂</td>
<td>11.68</td>
<td></td>
</tr>
<tr>
<td>R Q</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>A-glucose</td>
<td>96.4</td>
<td>mg/dl</td>
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<tr>
<td>A–V glucose</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>A–V lactate</td>
<td>5.83</td>
<td></td>
</tr>
<tr>
<td>A–V pyruvate</td>
<td>1.05</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2.** Mean Values of Lipids

**Fig. 3.** Myocardial Extraction of Individual Fatty Acids (16 Dogs)

**Fig. 4.** Arterial NEFA Composition (16 Dogs)

**Fig. 5.** Arterial NEFA Concentration and Myocardial Extraction of NEFA

**Fig. 6.** Effect of Drugs

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Fig. 3 illustrated the myocardial extraction of individual fatty acids in the total, esterified, and nonesterified fatty acids, measuring their composition by gas-liquid chromatography. In this figure only the five major fatty acids were listed. And the figure showed that palmitic, palmitoleic, stearic, oleic, and linoleic acid were extracted in the heart muscle, and that the amount of uptake was the largest in oleic acid followed by palmitic acid.

Fig. 4 revealed the mean distribution of NEFA in the arterial samples of 16 dogs. Palmitic, stearic, oleic and linoleic acids were the main components.

The correlation between arterial NEFA concentration and myocardial uptake in normal dogs is shown in Fig. 5. Each dot on this scattergram represents each experimental animal. This illustrated that the myocardial uptake became higher, accompanying with the raise of the arterial NEFA concentration.

Oleic, palmitic, and linoleic acid were main components of neutral fat, which was extracted with ether by silicic acid column chromatography, and their total level was 30.2 ± 10.5 mg/dl (0.112 mEq/dl) with A-V difference 1.6 ± 1.0 mg/dl (0.0058 mEq/dl).

Fig. 6 represented the mean differences for the several experimental conditions. Blood pressure and myocardial uptake of glucose were elevated by epinephrine (10007 I.V.) administration. The increased uptake was associated with an increased coronary blood flow. Although arterial NEFA level was raised, the increase in A-V difference was not significant.

Administration of cytochrome C (20 mg I.V.) or coenzyme Q (10 mg I.V.) in normotensive animals revealed a slight increase in coronary blood flow in spite of lowered blood pressure, and increased the myocardial uptake of NEFA and glucose. Such an effect of electron transport system was almost similarly exhibited even when these preparations used together. In the myocardial uptake of NEFA, palmitic, palmitoleic, stearic, oleic, and linoleic acid were extracted in proportion to their arterial levels.

No significant change of individual NEFA extraction was observed as the effect of a given preparation. (Fig. 7)

**DISCUSSION**

In the fat metabolism of the heart muscle, neither cholesterol nor phospholipid were extracted as already been studied by Fredrickson and Gordon[3]. A concise review of the metabolic activity of the intact heart had been presented by Bing[7]. In this present study, as for the fatty acids, the total extraction was 3.1 ± 2.4 mg/dl and the myocardial oxygen extraction rate was 56 per cent. Therefore, 56 per cent of extracted oxygen by myocardium was suggested to be used for the oxidation of fatty acids (Table II).

In accordance with previous reports, the present investigation showed a consistent coronary artery-sinus differences in total fatty acid and NEFA. Myocardial extraction of NEFA was 3.4 μEq/dl and its contribution to the total fatty acid was calculated as 29.1 per cent. And hence the myocardial uptake of esterified fatty acid was theoretically supposed to be considerably high. According to the results of the present study, fatty acids extracted from

**Table II Relative contribution of metabolites to total myocardial oxygen usage**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>% Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>45 % (18 %)</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.6 % (0.5 %)</td>
</tr>
<tr>
<td>Lactate</td>
<td>37 % (17 %)</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>56 % (67 %)</td>
</tr>
</tbody>
</table>

(R.Q. Bing)

**Table III Relative contribution of fatty acids to total myocardial oxygen usage of EFA**

<table>
<thead>
<tr>
<th>Component</th>
<th>% Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA</td>
<td>29.1 %</td>
</tr>
<tr>
<td>EFA</td>
<td>56.0 %</td>
</tr>
<tr>
<td>Error</td>
<td>14.9 %</td>
</tr>
</tbody>
</table>
the plasma neutral fat were relatively high and their myocardial oxygen extraction was contributed 56 per cent to total fatty acids as was shown in Table III.

Lipoprotein lipase was observed to be abundant in the heart muscle by Rothlin et al. and therefore hydrolysis of plasma neutral fat should have been occurred in the heart muscle and in turn their fatty acids should have been extracted.

The metabolic importance of the extracted NEFA had been emphasized by several workers. All five major fatty acids, palmitic, palmitoleic, stearic, oleic, and linoleic acid, that were present in plasma NEFA were extracted altogether. It could be observed from Fig. 4 that the amount of oleic acid was the largest in these extracted fatty acids. Comparing these NEFA distribution with similar analysis by Miller and Spitzer, percentage value for palmitic acid was higher, and linoleic acid was lower in the latter than the present study. Although a part of the discrepancy was undoubtedly due to the nutritional conditions of experimental animals, differences in extraction and separation procedures seemed to be more important.

Bing et al., Carlsten et al., and Spitzer et al., called attention to the positive correlation between arterial NEFA level and coronary A-V differences. Present study was completely agreement with these previous reports (Fig. 5). P value of the slope was between 0.05 and 0.01.

In the net removal of NEFA by myocardium, the uptake of oleic acid was the largest and that of palmitic acid was the next. Myocardial extraction of the individual fatty acids were almost similar in proportion to their arterial concentrations.

The metabolism of the lipid and carbohydrate in the heart muscle influenced by the administration of epinephrine, cytochrome C, and co-enzyme Q, was shown in Table IV. Administration of epinephrine induced an increase of myocardial uptake of glucose more than that of NEFA, and the administration of cytochrome C or co-enzyme Q elevated myocardial uptake of both glucose and NEFA. In accordance with the reports of Spitzer et al., normal dogs reacted to epinephrine by a moderate increase of arterial NEFA concentration, coronary blood flow, and myocardial uptake of NEFA. Epinephrine has been acknowledged as an important regulator of fat metabolism. It increases the release of NEFA from adipose tissues, and thus raises blood NEFA level. Therefore it was expected that epinephrine would increase myocardial uptake of NEFA from the correlation between arterial NEFA level and its myocardial uptake. But in this study we could not reveal the elevated myocardial uptake of NEFA. This discrepancy might be partly due to the marked increase of coronary blood flow and consequent reduction of A-V difference of NEFA to the level which undetectable by the present method.

The concept of the mitochondrion as a complete system in which citric acid cycle and the electron transport system functioning in conjunction with the production of ATP was become largely accepted by Green et al. More than 30 per cent of the weight of the mitochondrion is composed of lipid. This lipid plays a key role in the process of electron transport and oxidative phosphorylation, being associated with DPNH flavoprotein, succinic flavoprotein, cytochrome C, and co-enzyme Q. From this point of view, we administered cytochrome C and co-enzyme Q to this experiment, and confirmed the increase of myocardial uptake of both glucose and NEFA.

**Summary**

Myocardial arteriovenous difference in the lipids and coronary blood flow was determined in dogs with coronary sinus catheterization. Neither cholesterol nor phospholipid were utilized. Total fatty acids were markedly participated in the myocardial metabolism, and NEFA was extracted in some extent by the metabolism.
myocardium. Esterified fatty acids, especially those in neutral fat fraction were mainly consumed. A significant correlation was found between arterial NEFA concentration and myocardial uptake in normal dogs. In the composition of NEEA in arterial blood, the percentage value of oleic acid was the largest followed by palmitic and linoleic acids. The myocardium removed mainly oleic, palmitic, and linoleic acids, and stearic, palmitoleic acids in lesser amount.

Epinephrine elevated myocardial uptake of glucose more than that of NEFA. Cytochrome C and co-enzyme Q increased the myocardial uptake of both glucose and NEFA.

Acknowledgement:

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REFERENCES