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Effect of Fasting on Myocardial Glucose Uptake in Rabbits

MONA A. NESSIM, M.D.; MAGED A. HAROUN, M.D.; HANAA Z. YASSIN, M.D. and WAFAA A. MATTAR, M.D.

The Physiology Department, Faculty of Medicine, Cairo University.

Abstract

In the present study, the effect of fasting on myocardial glucose uptake was studied. Two different techniques were used, namely, cardiac muscle slices (0.5 mm thick) and the isolated heart perfusion using Langendorff's coronary technique. The effect of fasting for 1, 2, 3, and 4 days on glucose uptake was studied in both groups of experiments and the results were compared with control fed animals. The present results showed that in both groups of experiments (slices and isolated heart perfusion) fasting caused a significant increase in glucose uptake, an effect which was potentiated by increasing the duration of fasting. These results differed from previous studies which reported decreased glucose uptake by starvation. This difference could be explained by the difference in the substrate used, since previous studies used glucose and free fatty acids in the used solution or injected radioactive glucose in vivo then measured radioactivity after killing the animals while in the present study glucose only was used as a substrate. The increase in glucose uptake could, also, be explained by its use for glycogen synthesis in cardiac muscle.

Introduction

THE heart is a constantly working organ and therefore its energy use is high. Important differences exist between the metabolism of cardiac muscle and that of the skeletal muscle. More mitochondria are present in the heart muscle, thus it can utilize more substrates than skeletal muscle which relies predominantly on glucose. Energy production in the cardiac muscle is entirely aerobic under normal conditions, while skeletal muscle can readily use the anaerobic pathway [1].

Starvation is a stressful state characterized by adaptive mechanisms which ensure an adequate glucose supply to the tissues which have an obligatory requirement for it, e.g. brain tissue [2]. The transition from the fed to the starved state is therefore characterized by and associated with depletion of stored carbohydrate, together with a fundamental switch in fuel selection

in certain oxidative tissues [3]. It has been known that fasting increased glycogen concentration in hearts of male rats [4]. The cardiac response to starvation is characterized by inactivation of the pyruvate dehydrogenase complex, doubling of citrate content, changes in metabolite concentrations consistent with inhibition of phosphofructokinase and increased glycogen [5].

The aim of the present work was to study the effect of fasting on myocardial glucose uptake in adult male rabbits.

Material and Methods

Adult male rabbits weighing about 1-1.5 kg were used in the present study (75 rabbits). Animals were kept on carbohydrate rich diet, bread, before the experiments. Fasting animals were allowed free access to drinking water. All animals were killed by cutting the throat.

Two groups of experiments were performed using two different techniques.

Group I : in which the effect of fasting on glucose uptake by cardiac muscle slices (0.5 mm thick) was studied.

After being prepared, the slices were immediately dropped into wide short tubes containing 3 ml of purely oxygenated freshly prepared Krebs Ringer Phosphate Buffer (K. R. P. B.) containing 100 mg% glucose. The tubes were then shaken in a metabolic shaker at 37°C at a rate of 60 oscillations/minute for half an hour, then the fluid was changed and shaking resumed for one hour [6].

Five subgroups were included in this group, each consisted of 10 rabbits.

Subgroup (1) (Control); glucose uptake by cardiac slices of fed rabbits was estimated.

Subgroup (2), (3), (4) and (5): glucose uptake by cardiac slices of fasting for 1. 2, 3 and 4 days, respectively, was estimated.

The uptake of glucose by the cardiac muscle slices was first calculated as the difference between the control and incubating media, then as mg/gm wet weight of heart slices/hour according to the following equation, taking into consideration that the volume of the incubating fluid was 3 ml :

$$\frac{\text{The difference in glucose content between each sample \& control sample} \times 3 \times 1000}{100 \times \text{Wet weight of heart muscle slices in mg}}$$

Group II: isolated heart perfusion using Langendorff's coronary perfusion set of the isolated rabbits heart (Langendorff, 1895). The coronary flow was measured by collecting the fluid dropped from the heart in a graduated cylinder. Glucose uptake by cardiac muscle was calculated as mg/ coronary flow in ml/hour.

Estimation of glucose content in the medium was done by the enzymatic glucose oxidative method of Stanbio [7]. Statistical analysis was done using Student's test [8].

Group I: The effect of fasting on glucose uptake in cardiac muscle slices.

As shown in table (1) The mean value of glucose uptake of the cardiac muscle slices in fed rabbits (control subgroup) was 5.44 ± 1.2 mg/gm wet weight cardiac tissue/hour. Fasting for one day resulted in a significant increase in glucose uptake by cardiac muscle ($p < 0.001$) with a mean value of 14.8±3.6 mg/gm wet weight cardiac tissue/hour. The significant increase in glucose uptake by cardiac muscle slices was potentiated by prolonging the time of fasting. Thus, the mean values of glucose uptake after 2,3 and 4 days of fasting were 18.25 ± 5.4, 24.6 ± 4 and 27.3±2.3 mg/gm weight cardiac tissue/hour, respectively.

Group I: The effect of fasting on glucose uptake using Langendorff's coronary perfusion set.

As shown in table (2) the mean value of glucose uptake by cardiac muscle was 17.8 ± 1.1 mg/coronary flow in ml/hour. After one day of fasting a significant increase in glucose uptake by cardiac muscle ($p < 0.001$) was noticed. Increasing the duration of fasting potentiated the increase in glucose uptake by cardiac muscle. Thus, the mean values of glucose uptake after 1, 2, 3 and 4 days of fasting were 25 ± 1.2, 28.8 ± 1.2, 31.4 ± 1 and 37.4 ± 1 2.1 mg/coronary flow in ml/hour, respectively. All results were highly significant ($p < 0.001$).

Tabl (1) : Glucose Uptake by Cardiac Muscle Slices (0.5 mm thickness) Control Subgroup, Fasting 1 Day, 2 Days, 3 Days and 4 Days. Calculated as mg/gm Wet Weight Cardiac Tissue/Hour.

Exp . No	Control	1 day fasting	2 days fasting	3 days fasting	4 days fatsing
1	5.3	15.3	22.5	18.9	29.6
2	4.5	11.4	15.1	26.5	29.5
3	4.8	10	21.3	19.5	29.2
4	6.4	15	21.9	28.8	26.5
5	6.9	13.3	20.1	28	31
6	4.3	11.2	24.5	28.5	28.9
7	4.8	20	23	27.2	24
8	6	15.9	10.5	26.5	25.5
9	3.9	15.3	11.5	22.3	27
10	7.5	21	12.1	20	25
Mean	5.44	14.8**	18.25**	24.6**	27.3**
S.D.	± 1.2	± 3.6	± 5.4	± 4	± 2.3
S.E.	± 0.4	± 1.1	± 1.7	± 1.3	± 0.7

** Very highly significant

Table (2): Glucose Uptake by Cardiac Muscle Using Langendorff' Scoronary Perfusion Set. Control Subgroup' Fasting 1 Day, 2 Days, 3 Days and 4 Days Calculated as mg/ Coronary in ml/ Hour

Exp . No.	Control subgroup	1 day fasting	2 day fasting	3 days fasting	4 days fatsing
1	19.8	25	30	32.5	38.8
2	17.5	23.8	27.6	31	35
3	17.3	25.2	28.8	32	34
4	17	24.3	27.6	30	38.8
5	17.3	27	29.8	31.7	38.3
Mean	17.8	25*	28.8*	31.4*	37*
S.D.	±1.1	±1.2	±1.2	±1	±2.1
S.E.	±0.4	±0.4	±0.4	±0.3	±0.7

*** Very highly significant

Discussion

Fasting is associated with changes in carbohydrate and lipid metabolism of the whole organism, including a fall in blood glucose and a rise in plasma lipids and free fatty acids. Because the myocardial uptake of glucose and free fatty acids is intimately related to their circulating levels, Opie et al. [9] reported that it is desirable to separate the effects of fasting on the level of circulating substrates from any direct effect on myocardial metabolism. Therefore, the present study was carried out on isolated myocardium (slices and isolated perfused heart) where the glucose available to the myocardium was kept unchanged.

The results of the present study demonstrated a significant increase in glucose uptake by myocardium derived from fasted rabbits compared to fed animals. This increase was potentiated by prolonging the duration of fasting. These results are not in accord with previous reports. Thus, studies using radioactive glucose demonstrated that fasting decreases myocardial glucose uptake in rats. The decrease in glucose uptake by myocardium was potentiated after 4 days of fasting and was approximately proportional to the decrease in blood sugar [9]. In a more recent study, these results were confirmed through measuring glucose-6-phosphate content of cardiac muscle, which

was decreased by fasting for 48 hours [10].

Isaad and his cowrkers [11] measured glucose utilization index in heart ventricle of rats by using radioactive glucose. Glucose utilization was found to be decreased by fasting. The authors attributed this decrease to lower plasma insulin concentration found in skeletal muscles of fasted rodents as a consequence of increased insulin binding and higher efficiency of insulin on postbinding steps [12]. Still, a decrease in cardiac muscle glucose utilization index by starvation for 6, 24 and 48 hours was reported [13]. This was explained by the preferential oxidation of fatty acids and ketone bodies through the operation of glucose-fatty acid cycle [13].

The difference between the present results and previous reports could be attributed to the media used. Opie et al [9] used Krebs-Henseleit bicarbonate buffer as a perfusion medium for isolated heart which contained 5 mM radioactive glucose and 0.75 mM labelled free fatty acid. In the present study the solutions used were Krebs Ringer phosphate buffer and Ringer Locke solution, no free fatty acids were added. Randle et al. [14] found that perfusing rat heart with fatty acids or ketone bodies or acetoacetate inhibits glucose uptake, glycolysis and pyruvate oxidation. The role of the addition of another substrate, e.g. free fatty acids, in decreasing glucose uptake by cardiac muscle is clarified by previous reports[15] which proved that there is a negative correlation between myocardial extraction of glucose and arterial plasma free fatty acids concentration in healthy resting men. The additional finding that infusion of an antilipolytic or plasma fatty acid lowering agent, nicotinic acid, leads to increased myocardial glucose extraction suggested that plasma fatty acids are determinants of myocardial carbohydrate metabolism.

Another difference is that in the present study, isolated muscle was used, while other rinvestigators injected radioactive glucose in vivo, then measured radioactivity in the heart after killing the animal [13]. Thus, the myocardium was exposed to both high concentrations of free fatty acids resulting from fasting, as well as radioactive glucose, which was not the case in the present work. Moreover, since those previous studies were performed in vivo, the role of the endogenous regulators of glucose metabolism cannot be ignored. Such regulators were not present in the present study. This was proved by Arnall et al. [16] who noticed no change in myocardial glycogen content in female rats compared with an increase in male rats after fasting of both groups for 48 hours. They added that differences in gonadal hormones, growth hormone or glucagon might be responsible for the different responses to fasting, but the mechanism remains to be determined.

Glycogen content of the hearts of fed rats was reported to be 2-3 times lower

than that in the hearts of fasted rats [10 , 17]. This finding was attributed to the glucose fatty acid cycle which is operative in the hearts of the starved rats. These findings could provide another explanation for the difference between the present results and those of previous studies. The higher fasting glycogen content of cardiac muscle is due to the sparing action of free fatty acids on the use of the endogenous store of energy, namely, glycogen. However, in the present study, the extra glucose uptake during fasting is used both for building glycogen molecules as well as it is consumed for energy purposes. This suggestion is confirmed by previous reports [18], which demonstrated the presence of cardiac autophagic vacuoles that participate in the turnover of cardiac glycogen. When the myocardium was placed in vitro and deprived of exogenous substrate but supplied with oxygen, an increase in these autophagic vacuoles was observed suggesting an increase in the glycogen turnover [18]. The increase in glucose uptake in the present study using cardiac muscle from fasted rabbits suggests that the extra glucose uptake is used to replenish the glycogen stores.

It can thus be concluded that the increase in myocardial glucose uptake in the present work results from absence of another substrate beside glucose, e.g. free fatty acids. As free fatty acids were shown to exhibit an inhibitory effect on glucose uptake by myocardial cell according to facilitated diffusion from high concentration outside to low concentration inside [19].

Second, the extra glucose uptake observed in the present study is explained by its use for glycogen synthesis.

Thirdly, the absence of hormonal control in increasing glucose uptake by fasted myocardium.

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