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DIABETES-LIKE ACTION OF INTERMITTENT FASTING ON SARCOPLASMIC RETICULUM Ca^{2+}-PUMP ATPase AND MYOSIN ISOENZYMES CAN BE PREVENTED BY SUCROSE

Heinz Rupp, Vijayan Elimban and Naranjan S. Dhalla

Division of Cardiovascular Sciences,
St. Boniface General Hospital Research Centre,
and Department of Physiology, University of Manitoba,
Winnipeg, Canada R2H 2A6

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Summary: Experimental diabetes results in a reduction of the sarcoplasmic reticulum (SR) Ca^{2+}-stimulated ATPase activity and a redirection of myosin isoenzymes from V_1 to V_3. Similar, but less pronounced, changes were induced by subjecting rats to intermittent fasting for 6 weeks. Low amounts of sucrose (0.8%) in the drinking water prevented the subcellular changes in fasted rats; however, sucrose neither affected the levels of plasma thyroid hormones nor normalized the reduced body weight. Plasma glucose was lowered without any changes in plasma insulin in the fasted rats receiving sucrose; this suggested an enhanced peripheral glucose utilization. Thus, the signals in the diabetic heart leading to changes in SR and myosin can be mimicked by intermittent fasting and seem to be linked to a shift in fuel utilization by the myocytes.

In a number of studies on experimental diabetes, it has been shown that subcellular structures including SR (1-3) and myosin (4) are altered in the myocytes. The nature of the underlying signals is, however, unclear and the role of subcellular alterations in the pathophysiology of heart dysfunction in chronic diabetes remains to be elucidated (5). The characterization of the signals is complicated by the fact that experimental diabetes is associated with low levels of plasma insulin and thyroxine (6). In this regard it should be noted that a reduction in plasma thyroid hormones is known to induce changes in SR (7) and myosin (8,9) which resemble those seen in the diabetic heart. Because metabolic derangements in the diabetic myocardium are considered to play a crucial role in heart dysfunction (5), it is possible that alterations in subcellular organelles can be simulated by changing the fuel utilization under conditions such as fasting. In this study, rats were

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fasted intermittently (one day fasting, one day feeding) for 6 weeks before monitoring changes in the heart. Since sucrose or fructose feeding has been shown to prevent subcellular changes in the myocardium under different pathological situations (10-13), the effect of sucrose was tested in the intermittently fasted animals.

Methods

Male Sprague-Dawley rats (6 weeks old) received a regular chow either ad libitum or every other day with 1 day fasting in between (intermittent fasting). Tap water was ad libitum; in one group of intermittently fasted rats tap water contained sucrose (0.8%, w/v). After 6 weeks, rats in the intermittent fasting group were killed either on the day of fasting or the day of feeding. Diabetes was induced in rats fed ad libitum by an intrafemoral injection of 65 mg streptozotocin/kg body weight (2). Fragmented SR was isolated from the left ventricle as described previously (14) and Ca^{2+}-stimulated ATPase was assayed (2) in a medium containing 100 mM KCl, 5 mM NaN_3, 5 mM $MgCl_2$, 0.01 mM free Ca^{2+}, 5 mM ATP and 20 mM Tris-Cl, pH 6.8; the basal ATPase activity in the absence of Ca^{2+} was subtracted. The proportion of myosin isoenzymes was derived from polyacrylamide gels in the presence of pyrophosphate (15). The plasma concentrations of thyroxine (T_4), triiodothyronine (T_3) and insulin were determined by using radioimmunoassays and plasma glucose was measured by the glucose oxidase method (2). Statistical comparisons were performed by the analysis of variance or in the presence of unequal variances by the test of Kruskal and Wallis. The differences were considered significant when the P value was < 0.05.

Results

Following 6 weeks of intermittent fasting, the body weight and the left ventricular weight were reduced; these were not significantly different from those of the diabetic rats (Table 1). Sucrose feeding did not affect

Table 1. Comparative effect of diabetes and intermittent fasting with or without sucrose feeding on the rat growth characteristics and ventricular myosin isoenzyme population

	Control	Diabetic	Fasting	Fasting Sucrose-fed
Body weight (g)	446 ± 13	360 ± 12**	372 ± 25**	365 ± 15
Left ventricular weight (mg)	857 ± 57	690 ± 78**	707 ± 52**	700 ± 55
V_1 (%)	53.3 ± 12.5	24.7 ± 8.3**	38.1 ± 7.2*	48.5 ± 6.3**
V_2 (%)	27.7 ± 5.1	29.8 ± 2.3	31.2 ± 1.6	29.4 ± 3.4
V_3 (%)	19.0 ± 8.1	45.5 ± 9.2**	30.7 ± 7.4*	22.1 ± 3.4*

Each value is a mean ± S.D. of 12 animals. The intermittently fasted rats fed sucrose were compared with intermittently fasted rats. No significant differences were observed between rats of a given group killed either on the day of feeding or on the day of fasting. * $P < 0.05$, ** $P < 0.01$ vs control.

Table 2. Comparative effect of diabetes and intermittent fasting with or without sucrose feeding on rat ventricular SR protein yield and SR Ca^{2+}- stimulated ATPase activity

	Control	Diabetic	Fasting	Fasting Sucrose-fed
SR yield (mg/g)	1.44 ± 0.34	1.91 ± 0.36*	1.71 ± 0.51	1.52 ± 0.17
SR ATPase activity (nmol P_i/mg/min)	135 ± 29	75 ± 12**	95 ± 14**	123 ± 30*

Each value is a means ± S.D. of 12 animals. The intermittently fasted rats fed sucrose were compared with intermittently fasted rats. No significant differences were observed between rats of a given group killed either on the day of feeding or on the day of fasting. * $P < 0.05$, ** $P < 0.01$ vs control.

significantly the growth characteristics of the fasted rats, most probably because the additional calorie intake was only approximately 1% of total calories consumed by the animal. The intermittently fasted rats consumed 0.5 ± 0.2 g sucrose on the day of ad libitum feeding and 0.3 ± 0.1 g sucrose on the day of fasting.

In the intermittently fasted rat hearts, the proportion of myosin V_1 was markedly reduced and the proportion of myosin V_3 increased (Table 1). A similar, but more pronounced shift was observed in the diabetic heart. When the intermittently fasted rats received sucrose in the drinking water, then no significant reduction in the proportion of myosin V_1 was observed. The intermittent fasting also provided a trigger for reducing the activity of Ca^{2+}-stimulated ATPase in the SR; similar but greater changes were seen in the diabetic heart SR (Table 2). Sucrose feeding again counteracted the effect of intermittent fasting on cardiac SR Ca^{2+}- stimulated ATPase.

In order to elucidate the mechanisms operating during intermittent fasting or sucrose feeding, plasma levels of T_4, T_3, insulin and glucose were determined. In contrast to diabetic rats where both T_4 and T_3 were reduced, only T_3 was reduced in the intermittently fasted rats (Tables 3 and 4). Both thyroid hormones were not significantly different when determined either on the day of feeding or the day of fasting at the end of the experiment. Because the plasma T_3 was reduced to a similar extent in the intermittently fasted and diabetic rats, one would expect a comparable effect on myosin and SR if the thyroid hormone was the only signal for subcellular

Table 3. Plasma glucose, insulin, T_4 and T_3 levels of intermittently fasted rats killed on the day of feeding

	Control	Fasting	Fasting Sucrose-fed
Glucose (mg/dl)	151 ± 10	169 ± 12	133 ± 7*
Insulin (uU/ml)	16.5 ± 3.0	19.1 ± 3.8	16.9 ± 4.2
T_4 (ug/dl)	6.8 ± 0.9	6.3 ± 1.0	6.6 ± 0.6
T_3 (ng/dl)	119 ± 16	86 ± 10*	91 ± 15

Each value is a mean ± S.D. of 12 animals. * $P < 0.05$ vs control.

alterations in the heart. Because diabetes had a significantly greater effect on myosin and SR in comparison to the fasted rats, it is likely that other signals may also be operating in this condition. This view is supported by the finding that the sucrose feeding which prevented the changes in myosin and SR in fasted rats did not result in an increased plasma T_3 level. On the other hand, it should be noted that at the day of fasting the plasma insulin level was reduced to 58% of that observed in ad libitum fed rats (Tables 3 and 4). Because fasting was carried out intermittently, reduced insulin levels can be seen to occur every other day. The fasted rats, however, did not show changes in the level of insulin comparable to that in the diabetic rats (Table 4). Sucrose feeding reduced the plasma glucose level at the day of feeding and this appears to represent an increased utilization of glucose.

Discussion

The data show that intermittent fasting for 6 weeks was sufficient for inducing changes in the pattern of myosin isoenzymes and in the activity of SR Ca^{2+}-pump ATPase which are similar to those seen in the diabetic heart. The

Table 4. Plasma glucose, insulin, T_4 and T_3 levels of diabetic rats or intermittently fasted rats killed on the day of fasting

	Diabetic	Fasting	Fasting Sucrose-fed
Glucose (mg/dl)	799 ± 207	130 ± 14**	117 ± 1
Insulin (uU/ml)	3.8 ± 0.6	9.5 ± 2.4**	10.9 ± 5.6
T_4 (ug/dl)	4.4 ± 0.8**	6.2 ± 0.8	5.4 ± 0.5
T_3 (ng/dl)	91 ± 14*	101 ± 15	81 ± 20

Each value is a mean ± S.D. of 12 animals. * $P < 0.05$, ** $P < 0.01$ vs respective values in Table 3.

intermittent fasting can be seen to mimick to a certain extent the effect of diabetes, most probably because it reduces the availability of plasma insulin for action on cardiac metabolism. It is possible that changes in cardiac metabolism in the fasted rats may occur in a manner similar to that suggested for the diabetic hearts (16) because glucose uptake by the myocardium is reduced upon fasting (17). In the diabetic heart, where glucose utilization is reduced to a greater extent, the effect on the heart should be expected to be more dramatic in comparison to the fasted animals and in fact this is what was observed in the present study. In favour of such a metablic signal for the alteration of subcellular structures is the finding that sucrose feeding prevented the observed changes in cardiac myosin and SR due to fasting. It should be pointed out that in the present study a small amount of sucrose was given in the drinking water which resulted in a steady sucrose intake modulating gastro-intestinal glucoregulatory signals (18). In previous studies focussing on myosin isoenzymes, glucose or fructose were given in high doses either through a nasogastric tube (74% of total calories in the form of sucrose) (10) or in the rat chow containing 60% sugar (11). When a diet of 60% fructose was given to diabetic rats, the proportion of myosin V_1 increased but this was also associated with hyperglycemia which resulted in further deterioration of the metabolic state of the animal (19).

Since sucrose feeding prevented the effect of intermittent fasting without influencing the plasma T_4 and T_3 levels, circulating thyroid hormones do not seem to be involved in the observed changes in the myocardium. Physiological doses of thyroid hormones also failed to reverse the diabetes-induced changes in the cardiac SR (2). Only when a severe fasting is imposed on the animal, the levels of circulating thyroid hormones are markedly depressed. Thus, when food intake was limited to 50% of ad libitum intake, the proportion of V_1 was reduced by 49% (compared to 15% in the present study) and the plasma T_4 and T_3 levels were reduced by 45 - 50%(20). Although the present evidence favours the signals linked to fuel utilization by the heart in the fasted animal, it is not our intention to rule out the

possibility of other factors such as adrenergic mechanisms. Since catecholamines favour the expression of myosin V_1 (21-23) and because sympathetic activity is reduced during fasting (24,25) whereas beta-adrenergic receptors are down-regulated in the diabetic heart (26,27), a reduced sympathetic influence could be seen to contribute towards the reduced expression of myosin V_1. If this mechanism would operate during fasting then one would have to assume that sucrose feeding results in a higher adrenergic activity. In fact sucrose (8%) has been reported to activate the sympathetic system in rats (28).

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