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METABOLIC EFFECTS OF ACARBOSE ADMINISTRATION IN NORMAL AND DIABETIC RATS*

NIELS HAUGAARD,† MARILYN E. HESS, CATHERINE L. LOCKE, ALIZA TORBATI and GARY WILDEY

Department of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.

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Abstract—The effect of acarbose on cardiac and hepatic metabolism was investigated in normal and diabetic rats. Groups of rats were fed one of the three following diets for 7 days: (1) ground Purina chow, (2) ground Purina chow fortified with raw corn starch and sucrose, and (3) the above high carbohydrate diet, with added acarbose (40 mg/100 g food). At the end of the dietary period the rats were decapitated, and a sample of liver tissue was removed and frozen in liquid nitrogen. The heart was extirpated for subsequent perfusion by the Langendorff technique. Increases in liver and heart glycogen produced by the high carbohydrate diet in the normal rats were prevented completely when acarbose was incorporated into the food. In diabetic animals, liver glycogen was uniformly lower than normal, irrespective of the diet or the presence of acarbose. With animals fed the control diet, cardiac glycogen was higher in diabetic than in normal rats. The high carbohydrate diet caused a lowering of heart glycogen in diabetic rats and this reduction in glycogen content was reversed by including acarbose in the diet. Effects of isoproterenol on myocardial phosphorylase *a* activity were determined in hearts from normal and diabetic rats given one of the three diets. The high carbohydrate diet decreased the enzymatic response to the catecholamine in hearts from both normal and diabetic animals, and this phenomenon was prevented by the presence of acarbose in the diet. In diabetic rats fed any of the three diets, the activation of cardiac phosphorylase by isoproterenol was greatly accentuated. Measurements of heart uridine kinase showed that the activity of this enzyme was lower than normal in hearts from diabetic rats given either the control or the high carbohydrate diet. The presence of acarbose in the latter diet resulted in a significant decrease in cardiac uridine kinase activity in hearts from normal rats. The results of this study demonstrate the effectiveness of acarbose in modulating tissue metabolism in normal and diabetic animals.

Substances that inhibit the enzymatic breakdown of sucrose and starch in the gastrointestinal tract have potential value as therapeutic agents in the treatment of diabetes mellitus and obesity [1, 2]. One compound which has been investigated extensively is acarbose, a glucosidase inhibitor with equal inhibitory action on the hydrolysis of sucrose and starch [1].

Many of the previous studies with acarbose have been concerned with measurements of plasma levels of glucose, triglycerides and insulin in animals [1, 3, 4] or human subjects [5–7] following ingestion of diets with a high content of starch and sucrose. In these investigations, the effectiveness of acarbose in reducing postprandial increases in blood glucose and insulin usually seen following intake of carbohydrate was clearly demonstrated. Although there is considerable information concerning the clinical use of acarbose, comparatively little data are available pertaining to the action of acarbose on tissue metabolism. The studies presented here describe effects of acarbose on heart and liver metabolism in normal and diabetic rats.

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† Send correspondence to: Dr. Niels Naugaard, University of Pennsylvania, Department of Pharmacology, School of Medicine G-3, Philadelphia, PA 19104.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats weighing 180–200 g were used throughout the investigation. The animals were housed in plastic cages with free access to water and fed *ad lib.* with Purina Lab Chow Checkers (Canine Diet 5006).

Before experimental measurements were made, groups of normal and diabetic rats were placed on the following diets for 7 days: (1) Purina Lab Chow Checkers finely ground; (2) ground chow with sucrose and raw starch (high carbohydrate diet) [20 g corn starch (Argo pure corn starch by Best Foods), and 10 g of sucrose were thoroughly mixed with 70 g ground chow]; and (3) ground chow with sucrose and starch to which was added 40 mg acarbose 100 g (high carbohydrate diet with acarbose).

Experimental diabetes was produced by injecting rats intraperitoneally with 100 mg/kg streptozotocin (100 mg/ml in 0.1 M citrate buffer, pH 4.5) following an overnight fast. The rats were given solid lab chow checkers for 5 days, and their blood glucose was determined after fasting overnight. Those with val-

ues greater than 200 mg/dl were included in the study. The values for fasting blood glucose concentrations ranged from 207 to 553 mg/dl.

On the day following 7 days of dietary regimen, the animals were decapitated, hearts were extirpated for perfusion, and a portion of each liver was removed and frozen immediately with Wollenberger tongs cooled in liquid nitrogen. The hepatic tissue was stored in liquid nitrogen for subsequent analytical measurements.

Heart perfusions

The hearts were perfused by the Langendorff technique as modified by Shanfeld *et al.* [8]. A miniature Palmer heart clip was attached to the apex of the heart and connected to a Grass force-displacement transducer. Heart rate and force of contraction were recorded on a Sanborn oscillograph. A heart was allowed to stabilize during a preliminary 10-min perfusion period, after which 0.2 ml of either 0.15 M NaCl or 2.4×10^{-7} M isoproterenol in 0.15 M NaCl was injected at the tip of the cannula.

At approximately 20 sec after the injection, the point of maximal inotropic response to isoproterenol, the heart was frozen with Wollenberger tongs precooled in liquid nitrogen. All tissues were stored in liquid nitrogen for subsequent determination of glycogen, phosphorylase and uridine kinase activity.

Analytical methods

Measurement of glycogen. Glycogen was determined by an adaptation of the method of Sølling and Esmann [9]. Tissue samples were digested in hot 10% KOH and ethanol was added to give a concentration of 66%. After standing overnight in the cold room, the tubes were centrifuged, the supernatant fraction was discarded, and the glycogen was dissolved in warm water. Aliquots of 15–50 μl of the glycogen solutions were spotted on 1.5 × 1.5 cm filter papers (Whatman 4). The papers were washed three times with 66% alcohol and once with acetone. The glycogen which remained bound to the filter paper was hydrolyzed to glucose by incubation with the enzyme 1,4-α-D-glucan glucohydrolase (Boehringer-Mannheim). The glucose released was assayed by incubation with hexokinase and glucose-6-phosphate dehydrogenase in the presence of NADP. The NADPH formed was measured spectrophotometrically at 340 nm.

Phosphorylase activity. Phosphorylase *a* and total phosphorylase activities were determined by the method of Cori and Illingworth [10], as modified by Aronson and Hess [11]. Under liquid nitrogen, a 150–200 mg sample of ventricular tissue was chipped from the frozen heart and homogenized in 4 ml of 0.02 M NaF–0.001 M EDTA–0.05 M Tris solution (pH 6.8). The homogenate was brought to a 1:30 dilution (100 mg/3 ml) and centrifuged (3500 *g*). After centrifugation, the supernatant fluid was decanted and diluted 1:5 (equivalent to 1:150 final dilution) with NaF–EDTA–Tris solution. Aliquots (0.2 ml) of diluted extract were incubated with 0.2 ml substrate (0.032 M glucose-1-phosphate, 2% glycogen with and without 0.002 M AMP) at 37.5° for 5 min. Inorganic phosphate liberated during incubation was measured by the method of Fiske and SubbaRow [12]. Phosphorylase *a* activity was expressed as the percentage of total phosphorylase.

Uridine kinase. The assay is based on the procedure described by Sköld [13]. Samples of heart tissue were homogenized with a Polytron homogenizer in 10 vol. of 0.1 M Tris buffer–0.01 M EDTA (pH 7.5). The homogenates were centrifuged at 100,000 *g* for 1 hr. Aliquots of 100 μl were incubated for 30 min at 37° in a total volume of 150 μl containing 15 mM ATP, 16.6 mM $MgCl_2$ and 0.33 mM [^{3}H]-uridine (5000 cpm/nmole). At the end of the incubation, the reaction was terminated by the addition of 200 μl of 0.01 M non-radioactive uridine and heating in a bath with boiling water for 2 min. The amount of uridine phosphorylated was determined by absorbing uracil nucleotides on Whatman DE-81 disks and counting the radioactivity in a liquid scintillation counter, as previously described [14]. Activity is expressed as nmoles/per mg protein/per min.

Protein determination. Protein of tissue extracts was measured by the procedure of Lowry *et al.* [15].

Drugs

Streptozotocin was obtained from the Sigma Chemical Co., St. Louis, MO; D,L-isoproterenol HCl was from Winthrop Laboratories, New York, NY. Acarbose was a gift from Miles Institute for Preclinical Pharmacology, New Haven, CT.

Statistics

For statistical evaluations, the Newman–Keuls multiple range test [16] and Student's *t*-test were employed [17]. A P value of < 0.05 was considered significant.

RESULTS

Effect of diet on body weight

Rats fed a high carbohydrate diet, with or without acarbose, did not appear different from rats fed the control diet, indicating that the animals had no obvious ill effects from the ingestion of the high carbohydrate or the acarbose.

Body weight changes of normal and diabetic rats on the different dietary regimens are presented in Table 1.

It can be seen that the weight gain in normal rats on a high carbohydrate diet was significantly smaller than that observed in rats fed the control diet. This unexpected result may be related to the palatability of the two diets. When acarbose was included in the high carbohydrate diet, the weight gain was even smaller, probably due to the delayed absorption of carbohydrate.

Diabetic rats on the control diet lost weight, while those on a high carbohydrate diet, with or without acarbose, remained stable.

Measurements of heart function

Recordings of ventricular contractions showed that neither diabetes nor variation in diet significantly affected heart rate or contractile force. The administration of isoproterenol caused approximately a 100% increase in contractility in all hearts. These results demonstrate that the mechanical functions of

Table 1. Changes in body weights of normal and diabetic rats during a 7-day dietary period

Diet	Changes in body wt (g)	
	Normal	Diabetic
Control	+46.4 ± 3.36 (17)*	−22.9 ± 5.17 (12)
High carbohydrate	+31.7 ± 3.28† (11)	−7.0 ± 6.50 (11)
High carbohydrate plus acarbose	+18.1 ± 2.37‡ (15)	+0.4 ± 4.53 (12)

* Number of animals.
† Significantly different from normal animals on control diet, $P < 0.005$.
‡ Significanty different from normal animals on high carbohydrate diet, $P < 0.005$.

the heart were minimally influenced by diet or the diabetic state.

Changes in liver glycogen

Normal rats fed a diet high in carbohydrate showed, as expected, a marked elevation in hepatic glycogen (Table 2).

When acarbose was present in the high carbohydrate diet, glycogen content of the liver returned to that measured in normal rats given the control diet. These findings demonstrate the effectiveness of acarbose in decreasing the rate of breakdown and delaying the absorption of carbohydrate in the intestinal tract under the conditions of our experiments. In diabetic animals, the liver glycogen was uniformly lower than that measured in the correspondingly treated normal rats.

Alterations in cardiac phosphorylase activity

The results of determinations of cardiac phosphorylase are given in Table 3.

It is apparent that dietary changes did not alter basal levels of cardiac phosphorylase in either normal or diabetic rats, and that only in rats given the control diet did diabetes increase phosphorylase *a* activity. On the other hand, when hearts were stimulated by isoproterenol, the enzyme was activated to a much greater extent in hearts from diabetic rats than in hearts from normal animals. In hearts from rats fed a high carbohydrate diet, the enzymatic response to isoproterenol tended to be smaller than in hearts from animals given the control diet. The decrease in isoproterenol-induced activation of cardiac phosphorylase observed in rats fed a high carbohydrate diet was highly significant in the diabetic animal. In both normal and diabetic rats, inclusion of acarbose in the diet containing excess carbohydrate restored the activation of myocardial phosphorylase to that observed in rats given the control diet.

Changes in cardiac glycogen

Cardiac glycogen was increased significantly in normal rats on the high carbohydrate diet (Table 4). Addition of acarbose to the high carbohydrate diet fed to nondiabetic animals prevented the rise in heart glycogen produced by excess carbohydrate, similar to the observation with liver glycogen.

The usual elevation in heart glycogen associated with the diabetic state [18, 19] is clearly evident in the rats given the control diet. Diabetic rats fed a high carbohydrate diet had a lower cardiac glycogen content than diabetic animals given the control diet. This was an unexpected finding for which we can offer no reasonable explanation. When acarbose was present in the diet with extra carbohydrate, cardiac glycogen exceeded the high value observed in the control diabetic group.

Table 2. Effects of diet, acarbose and diabetes on liver glycogen

Diet	Glycogen (μmoles glucose/g wet wt)	
	Normal	Diabetic
Control	261 ± 13 (17)*	77 ± 11 (12)
High carbohydrate	479 ± 29† (11)	115 ± 15 (11)
High carbohydrate plus acarbose	238 ± 15 (15)	93 ± 9 (12)

* Number of animals.
† Significantly different from normal animals on control diet and from animals on high carbohydrate diet with acarbose, $P < 0.01$.

Table 3. Effects of diet and acarbose on activation of cardiac phosphorylase by isoproterenol in normal and diabetic rats

	Cardiac phosphorylase *a* activity (% of total)					
	Normal			Diabetic		
	Basal	Isoproterenol	Δ	Basal	Isoproterenol	Δ
Control	5.8 ± 1.72 (8)*	21.9 ± 5.02 (9)	+16.1 ± 5.31	11.4 ± 1.80† (6)	68.4 ± 5.56 (6)	+57.0 ± 5.84‡
High carbohydrate	4.6 ± 1.42 (8)	13.0 ± 2.43 (9)	+8.4 ± 2.81	7.3 ± 2.10 (5)	36.8 ± 7.08 (6)	+29.5 ± 7.38‡
High carbohydrate plus acarbose	7.3 ± 1.78 (7)	33.4 ± 3.61§ (8)	+26.1 ± 4.02	8.2 ± 1.71 (5)	59.1 ± 4.70‖ (7)	+50.9 ± 5.00‡

* Number of animals.
† Significantly different from normal animals on control diet, $P < 0.05$.
‡ Significantly different from corresponding Δ in normal rats, $P < 0.02$.
§ Significantly different from normal animals on high carbohydrate diet, $P < 0.001$.
‖ Significantly different from diabetic animals on high carbohydrate diet, $P < 0.05$.

Measurements of cardiac uridine kinase activity

Changes in myocardial uridine kinase in the different groups of animals are presented in Table 5.

The high carbohydrate diet had no significant effect on the level of uridine kinase activity in the myocardium of normal rats. However, when acarbose was present, there was a highly significant lowering of enzyme activity. Since acarbose delays the digestion of polysaccharides present in the control diet, as well as in the high carbohydrate diet, the data reflect the consequence of lowered blood glucose and insulin concentrations in animals given acarbose. It is of importance in this connection that, in the investigation of Gertz and Haugaard [14], it was shown that fasting of rats was as effective as diabetes in lowering heart uridine kinase activity. As is well established in both fasting and diabetes, there is a lowering of plasma insulin and, in muscle, a shift from carbohydrate metabolism to utilization of fat.

In the groups of rats given the control diet or the high carbohydrate diet, diabetes was associated with a marked decrease in myocardial uridine kinase activity, and among the diabetic animals, there were no significant differences in enzyme activity when the different dietary groups were compared. With acarbose present in the diet, heart uridine kinase activity was low in both normal and diabetic animals and there was no significant difference between the values obtained.

Results from experiments in which isoproterenol was given to the perfused heart showed that uridine kinase was activated in two groups of rats, i.e. normal rats on control diet and normal rats on a high carbohydrate diet containing acarbose. Although these results are of interest for future investigation, they are not included in Table 5.

Table 4. Effects of diet, acarbose and diabetes on heart glycogen

	Glycogen (μmoles glucose/g wet wt)	
Diet	Normal	Diabetic
Control	10.2 ± 0.70 (17)*	19.3 ± 2.41† (12)
High carbohydrate	15.3 ± 1.54‡ (11)	12.3 ± 1.73§ (11)
High carbohydrate plus acarbose	10.8 ± 1.06 (15)	31.3 ± 2.48§ (12)

* Number of animals.
† Significantly different from normal rats on control diet, $P < 0.005$.
‡ Significantly different from normal rats on control diet, $P < 0.01$.
§ Significantly different from diabetic rats on control diet, $P < 0.01$.

DISCUSSION

The actions of acarbose in normal rats in reversing the change in both liver and cardiac glycogen produced by a diet high in carbohydrate are very evident. Acarbose had little effect on the depressed hepatic glycogen content in diabetic animals.

The increase in myocardial glycogen in diabetic animals seen in our experiments is in agreement with previous observations [18, 19] and has been attributed to a decrease in glycogenolysis associated with increased oxidation of fatty acids [20]. The lowering of the glycogen content of the heart in diabetic rats fed the high carbohydrate diet is not readily explained. A reasonable possibility, however, is that the excess carbohydrate had promoted an increase in the utilization of glucose, despite the diabetic state. When acarbose was present in the high carbohydrate diet, glycogen in the heart was even higher than that in diabetic rats fed the control diet. This overcompensation may have been due to the fact that acarbose prolonged the absorptive period of the added polysaccharides, as well as of those present in the ground chow, the basic ingredient of all the diets.

The results of the investigations of cardiac phosphorylase bring out several important points. In the normal rat, ingestion of a high carbohydrate diet had no effect on basal phosphorylase *a* activity but markedly decreased the isoproterenol-induced activation of the enzyme. The decrease in response to isoproterenol was most likely a consequence of an increase in glucose utilization by the heart when

Table 5. Effects of diet, acarbose and diabetes on heart uridine kinase activity

Diet	Uridine kinase activity [nmoles·(mg protein)$^{-1}$·min^{-1}]	
	Normal	Diabetic
Control	0.345 ± 0.013 (8)*	0.287 ± 0.012† (6)
High carbohydrate	0.367 ± 0.020 (5)	0.283 ± 0.012† (6)
High carbohydrate plus acarbose	0.266 ± 0.013‡ (7)	0.304 ± 0.014 (5)

* Number of animals.
† Significantly different from corresponding normal animals, $P < 0.005$.
‡ Significantly different from normal animals on control and high carbohydrate diet, $P < 0.005$.

excess carbohydrate was provided. When rats were placed on the diet containing acarbose, isoproterenol produced an increase in phosphorylase *a* activity, which was not significantly different from that measured in hearts from rats on the control diet. These findings illustrate the influence that altered cardiac metabolism exerts on the action of catecholamines on phosphorylase activation in the myocardium.

Enhanced activation of cardiac phorphorylase by the catecholamine in diabetic rats is similar to the results of Miller *et al.* [21], using epinephrine, and Vadlamudi and McNeill [22], using isoproterenol. It is evident from the observations of these investigators and from our results that the diabetic heart is supersensitive to the metabolic effects of catecholamines. The most intriguing aspect of this phenomenon of hypersensitivity is that the accentuated enzymatic response is not due to a greater number of cardiac β-receptors nor to an increase in responsiveness of adenylate cyclase to catecholamines [21–23]. Actually, the number of ventricular β-receptors has been reported recently to be decreased by streptozotocin-induced diabetes [24], and it has been demonstrated that the production of myocardial cyclic AMP in response to epinephrine is the same in normal and diabetic rats [21].

The reason for the supersensitivity of the diabetic heart to the metabolic effects of catecholamines is not clear at present, but Miller *et al.* [21] and Vadlamudi and McNeill [22] have speculated that the diabetic state may alter the availability of intracellular calcium or augment the sensitivity of phosphorylase *b* kinase to activation by calcium ions.

The effectiveness of acarbose in altering tissue metabolism in normal animals is again illustrated by the result of the experiments in which cardiac uridine kinase was determined. In nondiabetic rats, the activity of this enzyme was depressed significantly when acarbose was present in the diet.

Acarbose and other agents that inhibit glucosidases in the gastrointestinal tract have potential therapeutic value in the treatment of obesity and as adjuncts in the treatment of diabetes mellitus [25, 26]. Therefore, it is essential for the rational use of these agents in therapy to understand the manner in which they influence tissue metabolism in both normal and diabetic animals.

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