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The effect of insulin on the uptake and metabolic fate of glucose in isolated perfused hearts of dyslipemic rats

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Male Wistar rats chronically (15 weeks) fed a sucrose-rich diet (SRD; 63% w/w) developed hypertriglyceridemia and impaired glucose homeostasis. Hearts from these animals were isolated and perfused using the Langendorff recirculating method. Glucose at levels similar to those found in the animal in vivo was used as the only exogenous substrate. The hearts were perfused for 30 minutes in the presence or absence of insulin (30 mU/mL) in the perfusion medium. In the absence of the hormone, glucose uptake was impaired and the glucose utilization was reduced, with a significant increase of lactate release. Glucose oxidation, which was estimated from the activation state of the enzyme pyruvate dehydrogenase complex (PDHc), was depressed mainly due to both an increase of PDH kinase and a decrease of PDHa (active form of PDHc) activities. Although the addition of insulin in the perfusion medium improved the above parameters, it was unable to normalize them. The present results suggest that at least two different mechanisms might contribute to insulin resistance and to the impaired glucose metabolism in the perfused hearts of the dyslipemic SRD-fed animals: (1) reduced basal and insulin-stimulated glucose uptake and its utilization or (2) increased availability and oxidation of lipids (low PDHa and high PDH kinase activities), which in turn decrease glucose uptake and utilization. Thus, this nutritional experimental model may be useful to study how impaired glucose homeostasis, increases plasma free fatty acid levels and hypertriglyceridemia could contribute to heart tissue malfunction. (J. Nutr. Biochem. 11: 30–37, 2000) © *Elsevier Science Inc. 2000. All rights reserved.*

Keywords: sucrose-rich diet; isolated rat heart; hypertriglyceridemia; insulin resistance; glucose intolerance

Introduction

High carbohydrate intake (fructose or sucrose) has long been recognized to induce hypertriglyceridemia and contribute to the development of hypertension in humans as well as in experimental animals.[1–6] We have demonstrated that the impaired glucose homeostasis and insulin insensitivity that accompany the hypertriglyceridemia that develops in normal rats fed with a sucrose-rich diet (SRD) depend on both the amount of carbohydrate and the length of time the diet is administered.[7,8] Plasma glucose and insulin levels evolve from normoglycemia and hyperinsulinemia after short-term (3–5 weeks) feeding of a SRD to hyperglycemia with normoinsulinemia during a long-term (15–30 weeks) feeding of a SRD. Hypertriglyceridemia has been considered an associated or indirect cause of coronary heart disease (CHD), operating through one or more of other well-known risk variables. Moreover, Fontbonne and Eschwege[9] showed that hypertriglyceridemia is an important predictor of CHD mortality in subjects with impaired glucose tolerance or noninsulin-dependent diabetes mellitus (NIDDM).

Myocardial glucose metabolism is regulated by competing substrates and hormonal influence. Both glucose uptake and oxidation are decreased in the heart muscle of rats with experimental diabetes or starvation,[10] whereas insulin in vitro enhances glucose uptake and lactate release from isolated perfused hearts of both diabetic and fasted rats.[11,12] Mitochondrial pyruvate dehydrogenase complex activity

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(PDHc) is a key enzyme in the conversion of pyruvate to acetyl-coenzyme A (acetyl-CoA), and hence in the control of glucose oxidation in animal cells. It is under metabolic and hormonal control in several tissues including heart muscle (for a review see Randle,[13]). Recently, Wu et al.[14] showed an increase in the activity of the pyruvate dehydrogenase kinase (PDH kinase), which is the enzyme responsible for the inactivation of the PDHc, in hearts of both starved and diabetic rats. In these animals plasma insulin levels were low whereas plasma free fatty acid concentrations were highly increased. However, either re-feeding or insulin treatment in vivo restored the enzyme activity to control levels. On the other hand, experimental studies demonstrated that an increase in plasma free fatty acid levels together with their enhanced uptake by the heart results in both an elevation and accumulation of fatty acids intermediates in the myocardium. A high concentration of these metabolites in cardiac muscle could potentiate tissue ischemic injury.[15]

Hypertriglyceridemia with altered glucose homeostasis could have profound effects on myocardial substrate oxidation. However, these aspects have been only partially investigated in the nutritional model of long-lasting hypertriglyceridemia, increased plasma free fatty acids, glucose intolerance and insulin insensitivity induced by long-term SRD feeding. In this regard, Chicco et al.,[16] using nonperfused hearts from rats fed a SRD for 15 weeks, showed a significant reduction of PDHc activity. This was accompanied by an increased ratio of acetyl-CoA/reduced CoA (CoASH), as well as higher concentrations of glucose-6-phosphate, citrate, and glycogen. These findings are symptomatic of impaired glucose utilization. To test this hypothesis the present work was designed to study, under controlled conditions in the ex vivo perfused heart preparation, the following issues: (1) uptake and metabolic fate of glucose and the activities of PDHc and PDH kinase when glucose at levels similar to those found in the animal in vivo were used as the only exogenous substrate, and (2) whether insulin added to the perfusion medium could reverse the biochemical abnormalities observed in vivo in the heart of rats fed a SRD for an extended period.

Methods and materials

Animals and diets

Male Wistar rats, weighing 180 to 200 g and purchased from the National Institute of Pharmacology, Buenos Aires, were used in the present study. The animals were housed in an animal room under controlled temperature (22 ± 1°C), humidity, and air flow conditions, with a fixed 12-hour light/dark cycle (light 7:00 AM–7:00 PM). They were fed a standard laboratory chow (Ralston Purina, St. Louis, MO USA). After 1 week of acclimatization, rats were randomly divided into two groups: control and experimental. The experimental group received a semisynthetic SRD containing by weight (g/100 g): 63% sucrose, 17% of casein free of vitamins, 5% corn oil, 10% cellulose, 3.5% salt mixture (AIN-93M-MX), 1% vitamin mixture (AIN-93M-VX), 0.2% choline clorohidrate, and 0.3% methionine. Composition of both the vitamin and salt mixtures added to the SRD comply with the recommendations made by the Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet.[17] The control group received the same semisynthetic diet, except that sucrose was replaced with starch (high starch: CD). The animals had free access to food and water and were maintained on their respective diets for 15 weeks. Both diets provided approximately 15.28 kJ/g of food. The weight of each animal was recorded twice a week during the experimental period. In a separate experiment, the individual caloric intake and weight gain of eight animals in each group were assessed twice a week. On the day of the experiment, food was removed at 7:00 AM and the experiments were performed between 8:00 AM and noon. The experimental protocol was approved by the Human and Animal Research Committee of the School of Biochemistry, University of Litoral, Argentina.

Perfusion technique

The perfusion system for the heart described by Langendorff and previously used by the authors[4] was extended to a recirculating mode. Briefly, in rats anesthetized with pentobarbital (60 mg/kg body weight), a transverse abdominal incision was made and the thoracic cavity opened. The heart was rapidly removed and placed in a modified ice-cold Krebs Henseleit saline buffer (mmol/L: NaCl 118, KCl 4.70, $CaCl_2$ 2.54, KPO_4H_2 1.17, $MgSO_4$ 1.64, EDTA-Na_2 0.03, and $NaHCO_3$ 24.88, pH 7.4). After cessation of contraction, the heart was cannulated via the aorta. Perfusion was begun as a retrograde perfusion at a constant pressure of 60 mmHg and a single pass (nonrecirculating) for 5 minutes (washout period). The perfusion medium, Krebs Henseleit bicarbonate buffer containing either 5.5 or 8.5 mmol/L of glucose (pH 7.4), was gassed with a 95% oxygen (O_2), 5% carbon dioxide (CO_2) mixture and was filtered through a Millipore (Bedford, MA USA) SSWP type filter before use. Then the heart was switched to a recirculation perfusion mode with 90 mL of the above buffer in the presence or absence of 30 mU/mL of insulin. The buffer was kept at 37°C, continuously gassed with 95% O_2, 5% CO_2, and the pH maintained at 7.4. The flow rate (10–11 mL/min) was controlled by adjusting the speed of the perfusion pump (rotary pump Model Gilson Minipuls II) to maintain a perfusion pressure of 60 mmHg. The frequency of the heart contraction (220–260 beat/min) remained constant during the perfusion period.

Perfusate (1 mL) was removed at 0, 5 and 30 minutes of the recirculating perfusion for the analysis of glucose and lactate. This allowed the determination of the overall glucose metabolism by glucose disappearance from the medium. The glucose disappearance and the lactate production were linear at least for 30 minutes. At the end of the perfusion period, the heart was quickly frozen with a Wollenberger clamp that had been previously cooled in liquid nitrogen and kept at this temperature until the time of assay. Tissue wet weight/dry weight ratios were recorded in all experiments. Details of the methodology used have been given elsewhere.[4] Values of metabolites measured either in the perfusate or in the heart tissue are expressed per gram of dry weight, thus correcting any differences in the tissue water content.

Extraction and assay of PDH and PDH kinase activities

Frozen tissues were placed in 20 vol (w/v) of ice-cold homogenizing buffer [50 mmol/L 2-hydroxyethyl-1-piperazinyl ethanol sulfonic acid (HEPES) buffer (pH 7.4) containing 3% Triton X-100, 2 mmol/L EDTA, 5 mmol/L dithiothreitol, 0.5 mmol/L thiamine pyrophosphate, 2 mmol/L dichloroacetate (PDH kinase inhibitor), 50 mmol/L potassium fluoride (phosphoprotein phosphatase inhibitor), 2% bovine albumin, 0.1 mmol/L N-tosyl-L-phenylamine-chloromethyl ketone, 0.1 mg/mL trypsin inhibitor, and 0.02 mg/mL leupeptin] and homogenized with a Polytron homogenizer three times during 30 seconds. After centrifugation, the supernatant was precipitated once with 9% polyethylene

glycol.[18] The pellet was resuspended in the homogenizing buffer and divided to measure the active form of PDHc (PDHa) and total PDHc (PDHt). One portion was immediately used to the measure PDHa. For the measurement of PDHt, the other portion was incubated with broad specificity phosphoprotein phosphatase[19] in the presence of 10 mmol/L $MgCl_2$ at 30°C for 20 minutes. The activity of PDHc was spectrophotometrically determined at 30°C by measuring the reduction of NAD^+.[20] The complete assay mixture contained 30 mmol/L phosphate buffer (pH 7.4), 2.5 mmol/L NAD^+, 0.5 mmol/L CoA, 0.5 mmol/L thiamin pyrophosphate, 0.5 mmol/L dithiothreitol, 5 mmol/L $MgCl_2$, 10 units pig heart dihydrolipoyl dehydrogenase, 0.5 mmol/L pyruvate, and appropriate amounts of PDHc (0.5–1.0 mg protein) in a final volume of 2 mL. The reaction was started by enzyme addition, and all assays were performed at 30°C. The PDH activity was expressed as nmoles of nicotinamide adenine dinucleotide (NADH) formed per minute, per gram of dry tissue, or per milligram of soluble protein.

Isolation and assay of the PDH kinase were done as previously described by Popov et al.[21] Briefly, the complete reaction mixture containing 30 mmol/L HEPES, 1.5 mmol/L $MgCl_2$, 5 mmol/L dithithreitol, 0.1 mmol/L EDTA, 0.05% (w/v) Triton X-100, 0.1 umol/L leupeptin, 10 μg/mL trypsin inhibitor, 0.5 mmol/L adenosine triphosphate (ATP; pH 7.35), and 0.1 mg PDH complex, in a final volume of 0.2 mL, was incubated at 30°C for 10 minutes. At various intervals (0–5 min), aliquots (20 μL) were removed and transferred to the PDH assay mixture and the residual dehydrogenase activity was spectrophometrically measured, as previously described.[20] The apparent first-order rate constant for the ATP-dependent inactivation of the enzyme was calculated from a least-squares linear regression analysis of ln (inactivation by ATP) against time of incubation.

Analytical methods

Rats were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/kg of body weight). Blood samples were obtained from the jugular vein and immediately centrifuged at 4°C. The plasma samples obtained were either immediately assayed or stored at −20°C and examined within the following 3 days. Plasma triglyceride[22] and free fatty acid[23] were determined by spectrophotometric methods. The glucose levels were determined by fluorometric methods.[24] Immunoreactive insulin (IRI) was measured by Herbert et al.'s method.[25] The IRI assay was calibrated against the rat insulin standard (Novo, Nordisk, Copenhagen, Denmark). The frozen heart was removed from either the whole animal or the perfused chamber and pulverized in a mortar precooled in liquid nitrogen. Extracts of aliquots of frozen heart powder were used for the determination of ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), phosphocreatine (PCr), creatine (Cr), and glucose-6-phosphate by standard enzymatic methods as described by Bergmeyer.[24] Energy charge was estimated as

$$\frac{1}{2}\{[ADP + 2(ATP)]/(AMP + ADP + ATP)\}$$

according to Atkinson.[26] Other aliquots of frozen tissue were used for determinations of glycogen,[27] triglyceride,[22] and proteins.[28] The perfusion medium was analyzed for lactate and glucose.[24]

Statistical analysis

Results were expressed as mean ± SEM. The statistical significance between groups was determined by Student's *t*-test, or, when appropriate, data were subjected to a two-way analysis of variance[29] with diet and insulin as the main effects. When significance was found, Scheffe post hoc comparisons were made. Differences having *P*-values less than 0.05 were considered statistically significant.

Reagents

Highly purified bovine insulin was kindly provided by Mary Root of the Lilly Co. (Indianapolis, IN USA). Enzymes for the assays, substrate, and coenzymes were purchased from Sigma Chemical Co. (St. Louis., MO USA) or from Boehringer Mannheim Biochemical (Indianapolis, IN USA). All other chemicals were of reagent grade.

Results

Similar weight gain and food intake were recorded in animals fed SRD or CD during the 15 weeks of observation. Values are mean ± SEM of eight animals in each group. Caloric intake (kJ/day) was 289 ± 18 in CD rats and 293 ± 23 in SRD rats. The weight gain (g/day) was also similar: 2.00 ± 0.08 in CD rats and 2.03 ± 0.05 in SRD rats. At the end of the experimental period the weight of hearts (g) were also similar in both groups: 1.12 ± 0.02 in CD rats and 1.14 ± 0.03 in SRD rats.

Plasma triglyceride, free fatty acids, and glucose were all significantly higher in SRD-fed rats at the end of the experimental period. Values (mean ± SEM, $n = 8$) for triglyceride (mmol/L) were 0.49 ± 0.04 in CD rats and 1.63 ± 0.14 in SRD rats ($P < 0.01$). Free fatty acids were (μmol/L): 291 ± 19 in CD rats and 597 ± 20 in SRD rats ($P < 0.01$). Glucose (mmol/L) were 5.98 ± 0.08 in CD rats and 8.30 ± 0.10 in SRD rats ($P < 0.01$). On the other hand, comparable plasma IRI levels were recorded in both groups of animals (μU/mL): 50.3 ± 4.9 in CD rats and 53.1 ± 5.1 in SRD rats.

According to previous reports from our laboratory[16] and confirmed by the present findings, nonperfused hearts from SRD-fed rats showed significant increases ($P < 0.01$) of triglycerides, glucose-6-phosphate, and glycogen concentrations. Values (mean ± SEM, $n = 6$, μmol/g dry weight) for triglycerides were 24.8 ± 1.9 in CD rats and 46.3 ± 2.4 in SRD rats; glucose-6-phosphate values were 1.12 ± 0.06 in CD rats and 2.29 ± 0.19 in SRD rats; and glycogen values were 80.5 ± 3.9 in CD rats and 112.3 ± 5.1 in SRD rats. The present results show that these changes were accompanied by both a significant decrease of PDHa and increase of PDH kinase activities when compared with values obtained from age-matched rats fed a CD. Values were as follows (mean ± SEM, $n = 6$): PDHa (% of PDHt) 68.6 ± 2.9 in CD rats and 21.0 ± 3.1 in SRD rats ($P < 0.01$); PDH kinase activity ($K.min^{-1}$): 0.97 ± 0.03 in CD rats and 3.10 ± 0.15 in SRD rats ($P < 0.01$). Moreover, PDHt, expressed on a dry weight basis or relative to milligrams of a soluble protein, remained unchanged (data not shown).

The results shown in *Table 1* (upper panel) indicate that at the end of the perfusion period, in the absence of insulin, the cumulative glucose uptake of heart from SRD-fed rats was only half that recorded in age-matched rats fed a CD. A significant increase ($P < 0.05$) of cumulative lactate production was also observed. The tissue glycogen content decreased in hearts from rats fed both SRD and CD during the time of the nonrecirculating Langendorff perfusion and continued to decrease, reaching similar values in both

Table 1 Rate of glucose uptake and lactate release and tissue levels of glucose-6-phosphate (Glucose-6-P) and glycogen on perfused hearts of rats fed a sucrose-rich diet (SRD) or control diet (CD)

	Additions to the perfusion medium		Glucose uptake[†]	Lactate release[†]	Glucose-6-P	Glycogen[‡]
Diet	Glucose* (mmol/L)	Insulin (mU/mL)	(μmol/g dry weight 30 min)		(μmol/g dry weight)	
CD	5.5	−	240.8 ± 22.6[§a]	74.7 ± 5.4	0.66 ± 0.08	61.2 ± 2.5
CD	5.5	+	400.6 ± 27.2[b]	118.0 ± 10.4	1.40 ± 0.08	102.5 ± 3.3
SRD	8.5	−	127.7 ± 11.7[c]	111.4 ± 7.2	1.18 ± 0.11	61.6 ± 3.9
SRD	8.5	+	203.4 ± 7.9[a]	151.4 ± 10.3	1.65 ± 0.13	98.2 ± 4.9
SRD	5.5	−	141.2 ± 16.9	106.3 ± 9.8	1.09 ± 0.12	53.0 ± 3.2
SRD	5.5	+	242.0 ± 19.0	142.1 ± 13.6	1.59 ± 0.10	101.9 ± 11.0
(Upper panel) Two-way ANOVA(2 × 2)[‖]						
Diet			S	S	S	NS
Insulin			S	S	NS	S
Diet × insulin			S	NS	NS	NS
Residual mean square			1836	438	0.054	86.81

Hearts were perfused with a Krebs Henseleit bicarbonate buffer (pH 7.4) containing glucose as shown. After 5 min of nonrecirculating Langendorff perfusion, the hearts were switched to a recirculating type and perfused for 30 min in the absence or presence of insulin. Insulin (30 mU/mL) was present in the perfusate throughout the 30 min experimental period. Perfusion was carried out as described in Materials and methods. At the end of the perfusion, heart tissues were instantly frozen in liquid nitrogen, powdered and extracted for assays.
*Initial concentration of glucose.
[†]Cumulative glucose uptake (as glucose disappearance from the perfusion medium) and lactate release were calculated as the difference between the level of each metabolite in the perfusate at the beginning of the recirculating perfusion and at 30 min.
[‡]Glycogen is expressed as μmol glucose-glycogen. Values in nonperfused hearts were as follows (mean ± SEM, $n = 6$; μmol/g dry weight): 80.5 ± 3.9 in CD vs. 112.3 ± 5.1 in SRD. Values of glycogen at the beginning of the recirculating perfusion were as follows (mean ± SEM; μmol/g dry weight): 71.9 ± 5.4 in CD vs. 82.9 ± 6.4 in SRD.
[§]Values are expressed as mean ± SEM. Six animals were used in each experimental group.
[‖]Analysis of variance (ANOVA): Effects significant (S; $P < 0.05$) or not significant (NS).
Values in each column that do not share the same superscript were significantly different ($P < 0.05$) when one mean at a time was compared by Scheffe's test. No significant differences were obtained by the two-way ANOVA when compared hearts of SRD-fed rats perfused either with glucose 8.5 mmol/L (upper panel) or glucose 5.5 mmol/L (lower panel) in the presence or absence of insulin (not shown).

groups of rats at the end of the recirculating perfusion period. These data confirm a net glycogen degradation in hearts perfused with glucose as the sole substrate. Note that the glycogen concentrations present in the nonperfused hearts of SRD-fed rats (112.3 ± 5.1 μmol/g dry weight) was significantly higher than those recorded in rats fed with CD (80.5 ± 3.9 μmol/g dry weight). This indicates a more pronounced drop of glycogen stores in the hearts of the former animals. On the other hand, at the end of the perfusion period, the glucose-6-phosphate concentration was still significantly higher in the hearts of SRD-fed rats compared with CD-fed rats.

As expected, the addition of insulin (30 mU/mL) to the perfusate resulted in an approximately 70% increase in the cumulative glucose uptake and a 60% increase in lactate release in hearts of rats fed CD, using glucose as the only exogenous substrate. Tissue glucose-6-phosphate and glycogen concentration were also significantly higher at the end of the perfusion period. Note that under the action of the hormone the net glycogen content reached a value that was 30% higher than those recorded in nonperfused hearts (102.5 ± 3.3 versus 80.5 ± 3.9 μmol/g dry weight).

Hearts from SRD-fed rats were also sensitive to insulin. The percent increase in glucose uptake reached similar values in both dietary cohorts in the presence of the hormone. However, in absolute terms, glucose uptake was still lower in the hearts of SRD-fed rats than in those of CD-fed rats. The increase of lactate release was identical in absolute terms in both dietary groups under the action of insulin. Meanwhile, insulin tended to increase the absolute amount of glucose-6-phosphate more in the heart of rats fed a CD than in those fed a SRD (0.74 and 0.47 μmol/g dry weight, respectively). Insulin increases the glycogen content to similar levels in both dietary groups. Note, however, that SRD-fed animals did not reach the value recorded in nonperfused hearts (98.2 ± 4.9 and 112.3 ± 5.1 μmol/g dry weight, respectively).

In perfused hearts the maximal insulin effect on glucose uptake occurs at physiologic concentrations of glucose (5.5 mmol/L) when this is used as the only exogenous substrate. Then the glucose concentration in the perfusate may constitute a confounding factor that could influence treatment effects on the variable of interest. Thus, hearts from SRD-fed rats were perfused with glucose at physiologic levels (5.5 mmol/L) and insulin levels (30 mU/mL) (*Table 1*, lower panel) and compared with those perfused with 8.5 mmol/L of glucose and the same amount of insulin. The results show that the behavior of all the metabolic variables measured in the presence or absence of the hormone were similar when the hearts were perfused at either 5.5 (physiologic level) or 8.5 mmol/L of glucose (*Table 1*), the latter concentration being similar to those found in the animal in vivo.

We found no significant changes in ATP, ADP, and AMP myocardial levels of SRD rats and consequently, the energy charges (EC) remained within the control range. EC

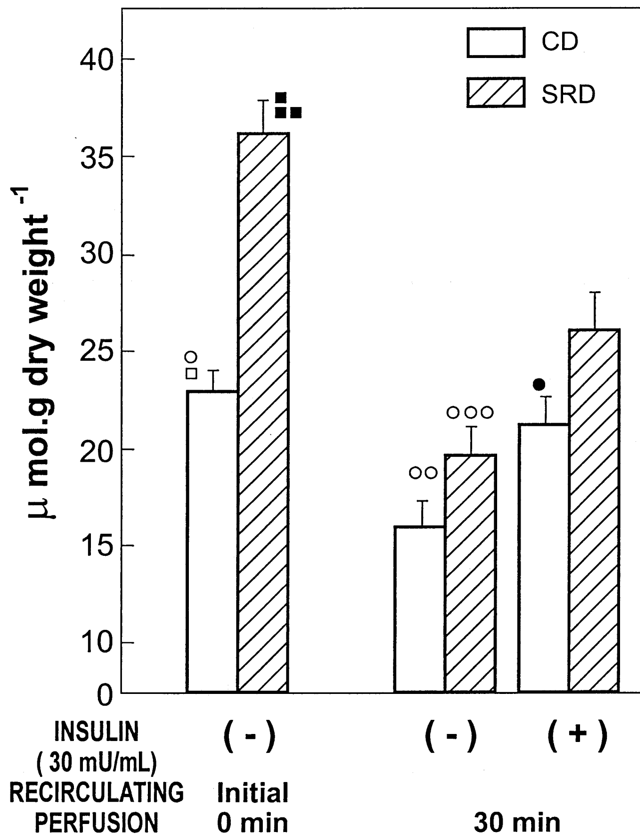

Figure 1 Triglyceride contents on perfused hearts of rats fed a sucrose-rich diet (SRD) or control diet (CD). Hearts were perfused with a Krebs Henseleit bicarbonate buffer (pH 7.4) containing glucose 5.5 mmol/L (CD) or 8.5 mmol/L (SRD). After 5 min of nonrecirculating perfusion, the hearts were switched to a recirculating type and perfused during 30 min in the absence or presence of insulin. Insulin (30 mU/mL) was present in the perfusate throughout the 30 min experimental period. Perfusion was carried out as described in Materials and methods. At the end of the perfusion, heart tissues were instantly frozen in liquid nitrogen, powdered, and assayed for triglyceride contents. Initial time (0 min) represent the heart's triglyceride levels at the beginning of the recirculating perfusion period. Values are expressed as mean ± SEM. Six animals were used in each experimental group. ○–$P < 0.05$ CD vs. SRD at the beginning of recirculating perfusion. ○○–$P < 0.05$ CD (− insulin) vs. CD (+ insulin) at 30 min time point. ○○○–$P < 0.05$ SRD (− insulin) vs. SRD (+ insulin) at 30 min time point. ●–$P < 0.05$ CD (+ insulin) vs. SRD (+ insulin) at 30 min time point. □–$P < 0.05$ CD at the beginning of the recirculating perfusion vs. CD (− insulin) at 30 min time point. ■–$P < 0.05$ SRD at the beginning of the recirculating perfusion vs. SRD (− insulin) at 30 min time point. ■■–$P < 0.05$ SRD at the beginning of the recirculating perfusion vs. SRD (+ insulin) at 30 min time point.

values (mean ± SEM, $n = 6$) were as follows: 0.88 ± 0.01 in CD rats and 0.89 ± 0.01 in SRD rats. The levels of Cr and PCr (and thus the PCr/Cr ratio) were also found to be within the normal range in both groups of animals. Values (mean ± SEM, $n = 6$) for PCr/Cr were 1.25 ± 0.12 in CD rats and 1.22 ± 0.10 in SRD rats. Values obtained from perfused hearts of SRD or CD rats in the presence of insulin for all these parameters were not statistically different (data not shown).

Figure 1 shows that the initial triglyceride content in the hearts of SRD-fed rats at the beginning of recirculating perfusion was higher than control, but declined rapidly up to 30 minutes after perfusion in the absence of insulin. The rate and the total amount of triglyceride disappearance were much less pronounced in the CD-fed rats. Furthermore, residual triglyceride content in the hearts of both dietary groups were similar after 30 minutes of perfusion. When insulin was added to the perfusion medium, the rate and total amount of triglyceride disappearance were reduced in perfused hearts of both CD- and SRD-fed rats (*Figure 1*). At the 30-minute time point, the residual triglyceride content in the hearts of rats fed a SRD was higher than that in animals fed a CD. However, over the 30-minute period of recirculating perfusion, the rate of triglyceride disappearance (lipolysis) in SRD-fed rats was still significantly higher than in rats fed a CD.

The impaired glucose uptake observed in the perfused hearts of SRD-fed rats was accompanied by a significant decline of glucose oxidation (estimated from the activation state of PDHc[30]) (*Table 2*). Values of PDHa and PDH kinase activities obtained at the end of the perfusion period were similar to those recorded in the nonperfused hearts of SRD-fed rats (see *Table 2* footnote). To investigate whether the addition of insulin to the perfusion medium could normalize the PDHa and PDH kinase activities, we determined the activities of both enzymes in the perfused heart at the 30-minute time point of the experimental period. Although insulin improved PDHa and PDH kinase activities, and thereby glucose oxidation, in hearts from SRD-fed rats, the hormone was still unable to normalize them. No effect of insulin on PDHa and PDH kinase activities were observed in perfused hearts from CD-fed rats. Perfusion of hearts from both SRD- and CD-fed rats under the present experimental conditions did not change the level of PDHt activity.

Discussion

Myocardial glucose metabolism can be affected by many different factors. Among them are competing substrates and hormonal milieu. The present studies, which used isolated perfused hearts from rats fed a SRD, show new features of myocardial carbohydrate metabolism in these animal model, including: (1) glucose uptake was impaired and its utilization reduced when glucose was used as the only exogenous substrate; (2) glucose oxidation (estimated from the activation state of PDHc) was depressed mainly due to an increase of PDH kinase and a decrease of PDHa activities, and (3) although the addition of insulin in vitro to the perfusion medium improved the above parameters, it was unable to normalize them during the perfusion time. These findings suggest that the impaired peripheral glucose utilization (insulin resistance, mainly in skeletal muscle) observed in vivo by the euglycemic-hyperinsulinemic clamp[31] is also present in the isolated heart muscle of rats fed a SRD.

Glucose plays an important role in myocardial energy metabolism, providing ATP through both glycolysis and oxidation in the citric cycle. The reduced glucose uptake and the enhanced release of lactate in the hearts of SRD-fed rats perfused with glucose alone is accompanied by a significant decrease of both glycogen stores and PDHa activity. Altered glucose homeostasis (hyperglycemia) and

Table 2 Pyruvate dehydrogenase (PDH) and pyruvate dehydrogenase kinase (PDH kinase) activities in isolated perfused hearts of rats fed a sucrose-rich diet (SRD) or control diet (CD)

Diet	Additions to the perfusion medium			
	Glucose* (mmol/L)	Insulin (mU/mL)	PDHa[†] (% of PDHt)	PDH kinase[‡] ($K.min^{-1}$)
CD	5.5	–	61.0 ± 4.2[§]	0.96 ± 0.10[a]
CD	5.5	+	69.0 ± 2.8	0.89 ± 0.05[a]
SRD	8.5	–	22.0 ± 2.1	2.90 ± 0.43[b]
SRD	8.5	+	39.0 ± 4.3	1.65 ± 0.12[c]
		Two-way ANOVA (2 × 2)[‖]		
Diet			S	S
Insulin			S	S
Diet × insulin			NS	S
Residual mean square			71.4	0.22

Hearts were perfused with a Krebs Henseleit bicarbonate buffer (pH 7.4) containing glucose as shown. After 5 min of nonrecirculating perfusion, the hearts were switched to a recirculating type and perfused during 30 min in the absence or presence of insulin. Insulin (30 mU/mL) was present in the perfusate throughout the 30 min of the experimental period. Perfusion was carried out as described in Materials and methods. At the end of the perfusion, heart tissues was instantly frozen in liquid nitrogen, powdered, and extracted for assays of PDH and PDH kinase activities.
*Initial glucose concentration.
†PDHa expressed as percentage of total PDH activity (basal activity × 100/total activity).
‡PDH kinase activity was assayed as determining the adenosine triphosphate-dependent inactivation of PDH activity as a function of time. $K.min^{-1}$ was calculated from the first order kinetic constant. Values of PDHa (% of PDHt) and PDH kinase activities ($K.min^{-1}$) in nonperfused heart were as follows (mean ± SEM, $n = 6$): 68.6 ± 2.6 in CD vs. 21.0 ± 3.1 in SRD and 0.97 ± 0.03 in CD vs. 3.10 ± 0.15 in SRD respectively.
§Values are expressed as mean ± SEM. Six animals were used in each experimental group.
‖Analysis of variance (ANOVA): Effects significant (S; $P < 0.05$) or not significant (NS). Values in each column that do not share the same superscript were significantly different ($P < 0.05$) when one mean at a time was compared by Scheffe's test.

increased plasma free fatty acids levels were also observed in the SRD-fed rats. This appeared together with a decreased PDHa activity in cardiac muscle.[16] This suggests that chronically SRD-fed rats' hearts may have adapted to reduced utilization of glucose oxidation as a source of ATP production, and instead preferentially use free fatty acids. Moreover, if the increased availability and oxidation of free fatty acids were responsible for the observed reduced glucose uptake and oxidation, then the metabolizable portion of the increased triglyceride levels within the myocytes should provide the endogenous source of free fatty acids under the present experimental conditions. As expected,[32,33] our results show a decrease in overall myocardial triglyceride contents in CD-fed rats' hearts perfused in the absence of fatty acids. However, a more accelerated lipolysis (higher rate of triglyceride breakdown) was recorded in the perfused hearts of SRD-fed rats.

Decreased flux through PDH complex is associated with lower PDHa levels. The inhibition of PDHc limits oxidation of pyruvate derived from glycolysis.[13,34] Our studies show both low PDHa and significantly increased PDH kinase activities in the perfused hearts of SRD-fed rats. These results suggest that increased oxidation of fatty acids, which increases the mitochondrial acetyl-CoA/CoA and NADH/NAD^+ concentration ratios stimulating PDH kinase activity, would be the mechanism responsible for the impaired glucose oxidation in the hearts of rats chronically fed a SRD. Increased glycogen and glucose-6-phosphate concentrations observed in the nonperfused hearts of these rats together with the high plasma free fatty acids levels are consistent with the operation of the glucose-fatty acid cycle in this tissue.[13]

Regarding the effect of insulin, the hormone stimulates both glucose uptake and lactate production and increases the net glycogen concentration in hearts of rats fed a CD perfused with glucose. In addition, glycogen levels are higher than those measured in nonperfused hearts.

On the other hand, the uptake of glucose, lactate production, and glycogen stores (net glycogen contents) are insulin sensitive in the hearts of SRD-fed rats, because all these metabolic variables were also increased by perfusion with insulin. However, the responsiveness of glucose to the hormone was significantly reduced compared with that obtained from the hearts of CD-fed rats. Although a significant increase of glycogen levels was observed due to the action of insulin in cardiac muscle of SRD-fed rats, the values obtained at the end of perfusion were still lower than those measured in the nonperfused heart of this dietary group (*Table 1*).

The addition of insulin in isolated perfused hearts increases the levels of glucose-6-phosphate and activates the enzyme glycogen synthase by increasing the I form.[35] Recently, Klimes et al.[36] reported an impaired response of glycogen synthase activity to insulin in vitro in the cardiac muscle of rats fed a SRD for a short period of time (3–4 weeks). In this regard, we recently showed[16] that the different metabolic and hormonal milieu present in rats fed a SRD for short (3–4 weeks) or long (15–30 weeks) periods could play an important role in cardiac muscle fuel utilization. For instance, although high citrate levels were observed in heart muscle in both periods, glycogen and glucose-6-phosphate were increased only after a long-time feeding. At present, we are unaware of any publication about the activity of glycogen synthase in the hearts of rats fed a SRD for an extended period.

On the other hand, reduced uptake of glucose in vivo at the levels of glut1 and glut4 was observed in hearts of streptozotocin diabetic rats.[37] Garvey et al.[38] also showed that impaired glucose utilization in diabetic rats is the result of a decrease in glucose transport activity that could be

reversed by insulin therapy. The present results show impaired glucose uptake and metabolism in perfused hearts of SRD-fed rats. This occurs at high levels of insulin (maximal stimulating doses) and low pressure, conditions under which the effect of the hormone on glucose uptake is maximal. Although the increased glucose-6-phosphate concentration could contribute to the impaired glucose uptake, we cannot discard the possibility that altered glucose transporters and/or their activity might also play an important role in glucose utilization. Moreover, a possible defect in the coupling process of the insulin receptor to the intracellular signal generation also could contribute to the present findings.

Regardless of an increase of glucose availability by insulin, glucose oxidation was still impaired (PDHa activity only reached half of the values observed in control heart, and the PDH kinase activity was significantly increased). This suggests that the heart continues to preferentially use fatty acids as a source of energy. In agreement with this, our results show that lipolysis from the endogenous triglyceride storage was still accelerated in the presence of insulin, whereas in the control hearts this metabolic pathway was of minor importance. Wu et al.[14] showed that either re-feeding starved rats or insulin treatment of diabetic rats brings to normal values the increased amount of PDK4 protein and PDK4 mRNA abundance while restoring the PDH kinase activity in heart muscle. In view of the above, the increased activity of PDH kinase present in the hearts of SRD-fed rats and the latency of the reactivation of the PDHc on insulin action might indicate that a longer time is required to down-regulate the PDH kinase isoenzyme and consequently reactivate the PDH complex. In addition, insulin did not modify either the proportion of the active form of PDHc or PDH kinase activities in the hearts of CD-fed rats perfused in the presence of the hormone. The values reached at the end of the perfusion were similar to those of nonperfused hearts. These results suggest that both enzyme activities were at or near the maximum and minimum levels, respectively, becoming insensitive to further modulation under these experimental conditions. In agreement with our results, Caterson et al.[39] showed no effect of insulin on the heart PDHa activity in normal fed rats perfused in the presence of glucose.

In brief, the present results suggest that at least two different mechanisms may contribute to insulin resistance and impaired glucose metabolism in the perfused hearts of SRD-fed animals: (1) reduced basal and insulin-stimulated glucose uptake and its utilization, and (2) increased availability and oxidation of lipids (low PDHa and high PDH kinase activities), which in turn decrease glucose uptake and utilization. Thus, our data support the use of this nutritional experimental model to study how impaired glucose homeostasis, hypertriglyceridemia, and increased plasma free fatty acid levels could contribute to heart tissue malfunction.

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