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Role of Glucose-6 Phosphatase, Glucokinase, and Glucose-6 Phosphate in Liver Insulin Resistance and Its Correction by Metformin

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ABSTRACT. We investigated the role of glucose-6 phosphatase (Glc6Pase), glucokinase (GK), and glucose-6 phosphate (Glc6P) in liver insulin resistance, an early characteristic of type 2 diabetes, and its correction by metformin. We determined hepatic glucose production (HGP) by tracer dilution, and enzyme activities and substrate concentrations after saline or insulin perfusions during euglycemic clamps in rats fed: 1) a standard hyperglucidic diet (S); 2) a high-fat diet (HF); and 3) a high-fat diet and treated with the oral antidiabetic metformin (HF/Met). Basal HGP was similar in the 3 groups: 75 ± 8, 65 ± 9.5 and 71 ± 3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (means ± SEM, $N = 5$) in S, HF and HF/Met rats, respectively. Upon insulin perfusion at 240 pmol/hr, HGP was decreased by 35% in S rats (49 ± 4.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.01$ vs. basal) and 65% in HF/Met rats (23 ± 10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.01$ vs basal), whereas it was not decreased in HF rats (60 ± 12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), revealing insulin resistance. GK activity was lower (by 65%, $P < 0.01$) in HF and HF/Met rats (0.8 ± 0.1 and 0.9 ± 0.1 U/g liver, respectively) than in S rats (2.4 ± 0.3 U/g). Microsomal Glc6Pase activity was lower (by 35%, $P < 0.01$) in HF and HF/Met rats (0.25 ± 0.01 and 0.27 ± 0.02 $\mu mol \cdot min^{-1} \cdot$ mg prot $\cdot^{-1}$, respectively) than in S rats (0.39 ± 0.03 $\mu mol \cdot min^{-1} \cdot$ mg prot $\cdot^{-1}$). Glc6P concentration was decreased by insulin perfusion at 480 pmol/hr in S and HF/Met rats ($P < 0.05$ vs. saline), but not in HF rats, in agreement with insulin resistance in the latter group. However, the differential inhibitions of HGP by insulin could not be ascribed to the variations in Glc6P concentrations. Metformin was present in the liver at a concentration of 27 ± 2 nmol/g wet tissue and was not detected in the plasma. These results strongly suggest that the regulation of HGP by insulin additionally involves short-term regulatory mechanism(s) of Glc6Pase, occurring *in vivo*, and lost under *in vitro* conditions. These might be impaired in HF rats, in keeping with insulin resistance of HGP, and restored by metformin. BIOCHEM PHARMACOL **55**;8:1213–1219, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. glucose-6 phosphatase; glucokinase; glucose-6 phosphate; hepatic glucose production; insulin resistance; metformin

Because of its privileged position in glucose metabolism, Glc6Pase† constitutes a major determinant of liver glucose output. It has long been believed that the substrate flux through Glc6Pase is controlled by the concentration of its substrate (Glc6P) only, and that Glc6Pase does not constitute a regulatory element in the short-term control of glucose metabolism by itself [1]. This former concept has recently been challenged by several groups [recently reviewed in refs. 2 and 3]. For example, it has been strongly suggested that inhibition of Glc6Pase activity is involved in glycogen synthesis in the rat liver perfused with L-proline [4] and in the rebound of glycogen in the liver of 72 hr-fasted rats [5]. We have shown that liver Glc6Pase activity is inhibited during the postprandial period in rats [6]. It has also been suggested that acute inhibition of Glc6Pase is involved in the action of insulin to suppress HGP during hyperinsulinic euglycemic clamp in rats [7].

The second crucial determinant of HGP is the substrate flux through GK. It has been emphasized that basal HGP is correlated with the ratio of Glc6Pase to GK in both normal and insulin-deficient diabetic (pancreatectomized) rats [8]. Increased HGP in diabetic animals thus seems to be the consequence of increased Glc6Pase activity and decreased GK activity. Despite the fact that basal HGP is increased in insulin-deficient rats, it is suppressed as efficiently as in normal rats by insulin perfusion [8].

On the other hand, there is compelling evidence that HGP is less efficiently suppressed by insulin in type 2 diabetic patients than in normal individuals, a phenomenon referred to as hepatic insulin resistance [9]. It has been suggested that an increased substrate flux through Glc6Pase might be one key metabolic feature in this phenomenon [10, 11]. This could implement either an increased amount of the enzyme or the loss of putative regulatory mechanisms

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† *Abbreviations:* Glc6Pase, glucose-6 phosphatase; Glc6P, glucose-6 phosphate; GK, glucokinase; HK, hexokinases; HGP, hepatic glucose production; HF, high-fat; HF/Met, high-fat/metformin; NIDDM, non insulin-dependent diabetes mellitus; S, standard.

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of its activity, or possibly an increase in the concentration of Glc6P. Alternatively, a decrease in GK activity might also account for abnormal regulation of HGP in type 2 diabetic patients, as suggested from studies in GK-deficient patients with maturity-onset diabetes of the young [12] and in transgenic mice with partial disruption of the GK gene [13].

The goal of this work has been to gain a better understanding of the respective roles of these parameters in hepatic insulin resistance. We studied three groups of rats in terms of insulin sensitivity of HGP, liver Glc6Pase and GK activities, and of hepatic Glc6P levels. Control rats were fed a standard hyperglucidic diet. A second group of rats was fed a HF diet for 3 weeks, a diet expected to induce hepatic insulin resistance [14]. The third group, fed the same HF diet for three weeks, was treated by metformin during the last week. Met has been reported to decrease basal HGP in NIDDM patients [refs. 15–18, 19 as a recent review], an effect which has been suggested to account for the improvement of hepatic insulin sensitivity [17].

MATERIALS AND METHODS

Animals and Diets

Male Sprague–Dawley rats (IFFA CREDO) were housed in individual cages in a temperature-controlled room (22–23°) with a 12 hr light–dark cycle, with free access to food and water. Rats were fed isocalorically matched diets (315 kJ/d, corresponding to the normal caloric requirement of rats) either rich in starch (standard diet) (15 rats) or in carthame oil (HF diet, rich in ω-6 fatty acids) (30 rats), for 21 days. The composition of diets was very similar to those in ref. 24. On a dry weight basis, the composition of the standard (S) diet was (starch/glucose, 58%; proteins, 23%; lipids, 5%; cellulose, 6%; mineral salts, 7%; vitamins, 1%), and that of the HF diet was (starch/glucose, 25%; proteins, 25%; carthame oil, 36%; cellulose, 6%; mineral salts, 7%; vitamins, 1%). Food consumption was monitored in order to add any spillage to the ration of the next day, but it did not occur. Half of HF-fed rats (15 rats) were given Met (LIPHA Lyonnaise Internationale Pharmaceutique) mixed to food, at a dose of 50 mg/kg of body weight, for the last 7 days.

Hyperinsulinic Euglycemic Clamps

Rats with no access to food for 6 hr were anesthetized using a single injection of pentobarbital (7 mg/100 g of body weight). Polyethylene catheters were placed in the left carotid artery for blood sampling, and in the controlateral jugular vein for insulin (or saline) and [3-^{3}H]glucose (Isotopchim) perfusions at a constant rate. Blood glucose was monitored every 5 min during perfusions, using a Glucometer II (Bayer Diagnostics). Within each diet group (15 rats), rats were divided into 3 groups of 5 rats. One group received saline perfusion, while the other two received insulin perfusion at two different rates (240 pmol/hr and 480 pmol/hr). In rats perfused with insulin (Lilly France S.A.), glucose (1.67 mmol/L) was also infused in the jugular vein at adapted rates to maintain glycemia at its basal starting value, as previously described [6]. Body temperature was maintained at *ca.* 37.5° by means of a heating blanket monitored by a rectal probe. After insulin perfusion for 60 min, a laparotomy was performed to allow full access to the liver. A liver lobe was freeze-clamped *in situ* between cooled steel blocks (−196°), weighed and stored at −80°. The remaining fresh liver was rapidly removed, weighed, and used for the purification of microsomes, as previously described [20]. Blood was collected for the determinations of plasma glucose, insulin concentrations, and the specific radioactivity of glucose. In rats treated by Met, jejunum and skeletal muscles were additionally sampled for assay of Met content of the tissue. This protocol was approved by a local ethics committee for animal experimentation.

Assay of Enzymes and Metabolites

GK and HK were assayed at 37° and pH 7.3 using 12,000 g supernatants of liver homogenates prepared from freeze-clamped samples, as described by Bontemps *et al.* [21]. Glc6Pase was assayed at 37° and pH 7.3 using a complexometric assay of P_i produced from Glc6P and intact microsomes isolated from fresh livers. The assay procedure has been described in detail elsewhere [22, 23]. Glc6P and glycogen were determined using samples of freeze-clamped livers, as described by Lang and Michal [24] and Keppler and Decker [25], respectively.

Other Methods and Calculations

Plasma glucose concentration was determined by the method of Bergmeyer *et al.* [26]. Plasma immunoreactive insulin concentration was assayed by radio-immunoassay [27]. Plasma [3-^{3}H]glucose radioactivity was counted after $ZnSO_4$/$Ba(OH)_2$ protein precipitation and evaporation to dryness to eliminate tritiated water. Protein was measured by Lowry's method [28] using bovine serum albumin as a standard. Met was assayed in liver, skeletal muscle, jejunum and plasma serum, as previously described [29].

HGP was estimated by means of dilution of infused [3-^{3}H] glucose. The rate of appearance of glucose R_a, equal to the rate of disappearance R_d at the steady-state of isotopic enrichment of plasma glucose (which was reached in less than 30 min under our conditions), was calculated at the end of the clamp using the Steele's equation: [3-^{3}H]glucose infusion rate/isotopic enrichment of plasma glucose. HGP was obtained from R_a by subtracting the unlabelled glucose infusion rate.

Statistical analyses were performed by ANOVA. When significance was established, the differences between the individual groups of data were tested by Fisher's test [30].

TABLE 1. Glycemic parameters during hyperinsulinic euglycemic clamp

Diet	Insulin perfused	Final insulin	Basal glucose	Final glucose
	pmol/hr	pmol/L plasma	mmol/L plasma	
Standard	saline	168 ± 17	8.3 ± 0.2	8.8 ± 0.4
	240	357 ± 35	7.4 ± 0.3	7.8 ± 0.3
	480	637 ± 63	7.9 ± 0.3	7.4 ± 0.5
High-fat	saline	119 ± 14	8.1 ± 0.3	8.2 ± 0.4
	240	343 ± 28	7.4 ± 0.6	8.4 ± 0.5
	480	540 ± 77	7.8 ± 0.7	7.7 ± 0.3
High-fat/Met	saline	112 ± 14	8.4 ± 0.4	8.6 ± 0.3
	240	350 ± 17	6.9 ± 0.2	6.7 ± 0.3
	480	623 ± 56	7.8 ± 0.7	8.3 ± 0.4

Values are means ± SEM ($N = 5$).

RESULTS

Irrespective of the diet given, rats were matched on a weight basis: 325 ± 6 g (S), 334 ± 5 g (HF), and 320 ± 5 g (HF/Met) (means ± SEM, $N = 15$, NS). They were also comparable in terms of plasma glucose concentration before perfusions and of insulinemia after either saline or insulin perfusions (Table 1). Basal HGP (saline perfusion) was similar in the three groups: 75 ± 8, 65 ± 9.5 and 71 ± 3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (means ± SEM, $N = 5$, NS) in S, HF and HF/Met rats, respectively. After insulin perfusion at a rate of 240 pmol/hr, HGP was significantly decreased by *ca.* 35% in S rats (49 ± 4.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.05$ vs basal HGP), was not decreased in HF rats (60 ± 12 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, NS vs basal HGP), and was decreased by approximately 65% in HF/Met rats (23 ± 10 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < 0.01$ vs basal HGP). At a higher insulin perfusion rate (480 pmol/h), HGP was similarly suppressed in the three groups (Fig. 1).

Glc6Pase and GK were assayed in isolated microsomes and in homogenates, respectively, after saline and insulin perfusions. Glc6Pase V_{max}, assayed in intact microsomes from rats perfused with saline, was lower by *ca.* 35% in HF and HF/Met rats: 0.25 ± 0.01 and 0.27 ± 0.02 $\mu mol \cdot min^{-1} \cdot mg\ prot \cdot {}^{-1}$ (means ± SEM, $N = 5$, NS) as compared to S rats (0.39 ± 0.03 $\mu mol \cdot min^{-1} \cdot mg\ prot \cdot {}^{-1}$ ($P < 0.01$ vs both HF diets) (Fig. 2). Glc6Pase Vmax was also determined after detergent treatment of microsomes (0.5% cholate for 20 min at 4°). Glc6Pase V_{max} was increased under these conditions, as previously reported [5, 22, 23]. The differences according to diets were very similar, however: 0.39 ± 0.02 and 0.36 ± 0.03 $\mu mol \cdot min^{-1} \cdot mg\ prot \cdot {}^{-1}$ in HF and HF/Met rats, respectively (means ± SEM, $N = 5$, NS) vs 0.6 ± 0.05 $\mu mol \cdot min^{-1} \cdot mg\ prot \cdot {}^{-1}$ in S rats ($P < 0.01$ vs both HF diets). There was no effect of diet on Glc6Pase K_ms either in intact microsomes or in detergent-treated microsomes. Glc6Pase was also assayed in the homogenates from freeze-clamped pieces of liver [6]. The differences in V_{max} regarding diets were very similar. Within each diet group, insulin perfusions had no effect on the kinetic parameters of Glc6Pase (data not shown). This is in agreement with previous results from us and others [6, 7]. GK V_{max}, determined in liver homogenates from rats perfused with saline, was lower by 65% in HF and HF/Met rats (0.8 ± 0.1 and 0.9 ± 0.1 U/g of liver, respectively, means ± SEM, $N = 5$, NS) as compared to S rats (2.4 ± 0.3 U/g liver, $P < 0.01$ vs both HF groups). On the other hand, there was no effect of diet on liver HK V_{max} (Fig. 2). There was no effect of diet on GK Kms (*ca.* 7–8 mM in all cases). Within each diet group, insulin perfusions had no effect on the GK kinetic parameters (not shown). This resulted in a significant doubling of the Glc6Pase/GK Vmax ratio in HF and HF/Met rats, as compared to S rats (Fig. 2).

When considering a given condition of perfusion (either saline or insulin perfusions), there was no significant difference regarding the Glc6P concentrations among the

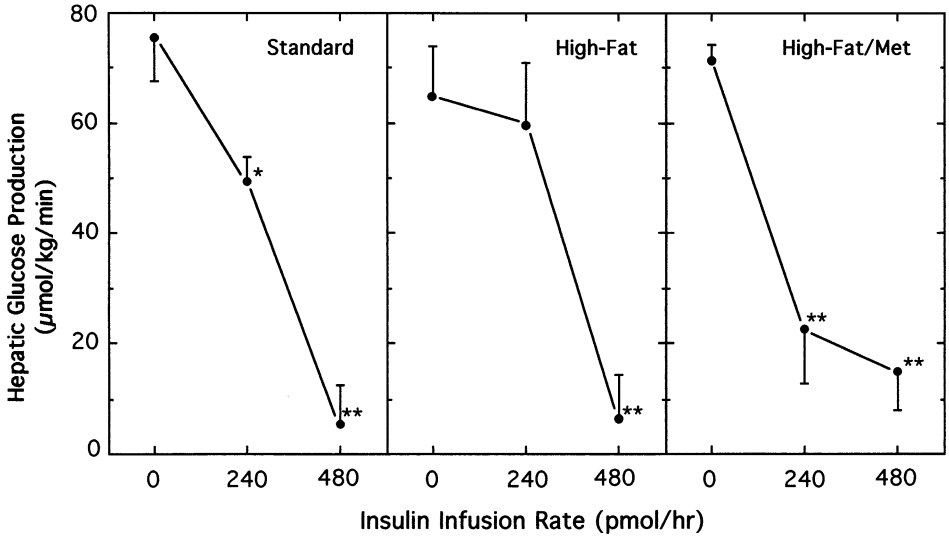

FIG. 1. Effect of diet on the inhibition of hepatic glucose production by insulin perfusion. Data are given as means ± SEM (N = 5). Significantly different from basal HGP. *$P < 0.05$. **$P < 0.01$.

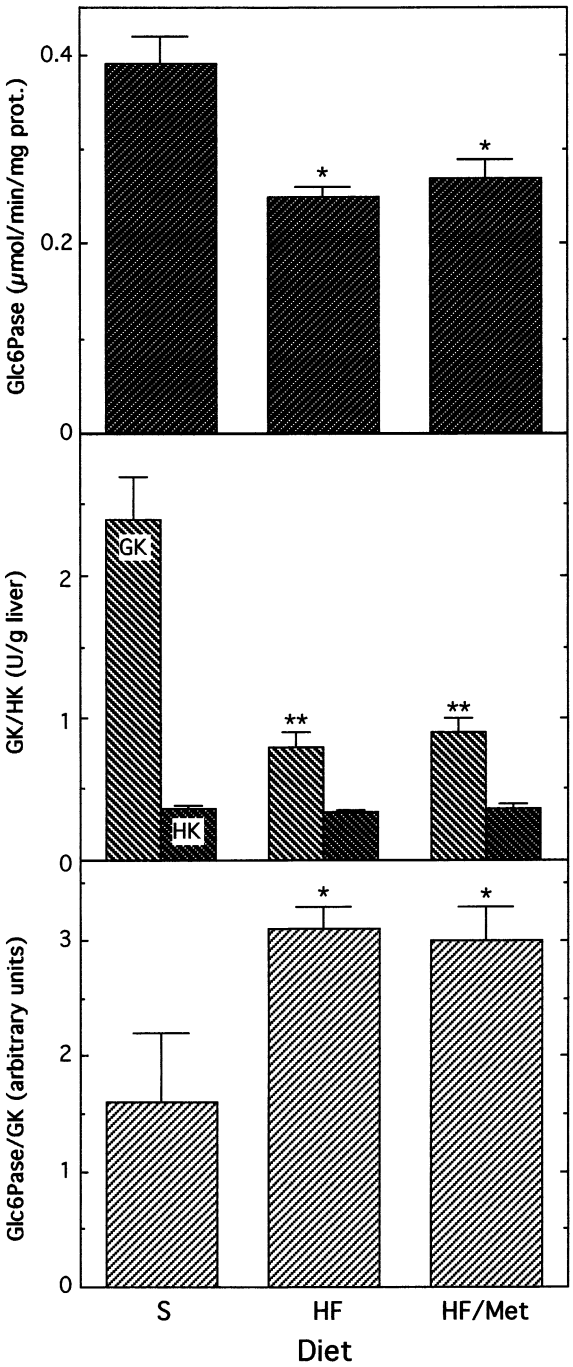

FIG. 2. Effect of diet on microsomal Glc6Pase (upper panel) and GK (mid panel) total activities. Data are given as means ± SEM ($N = 5$) of V_{max} determined at 37° and pH 7.3. The relative ratio of Glc6Pase to GK is given in lower panel. ⋆$P < 0.05$ and ⋆⋆$P < 0.01$.

diet groups (Table 2). However, when considering each diet group individually, insulin perfusion at 480 pmol/hr had a significant lowering effect on the Glc6P concentration in S and HF/Met rats (50 and 48% vs saline, respectively, $P < 0.05$). In contrast, it had no significant lowering effect on the same parameter in HF rats (23% vs saline, NS) (Table 2). Insulin perfusion had no significant effect on the liver glycogen content within each group

TABLE 2. Effect of insulin perfusion on hepatic Glc6P and glycogen

Diet	Insulin perfused	Glc6P	Glycogen
	pmol/hr	nmol/g	mg/g
Standard	saline	184 ± 34	40 ± 3
	240	138 ± 21	42 ± 2
	480	92 ± 11*	46 ± 5
High-fat	saline	175 ± 17	28 ± 3
	240	135 ± 24	35 ± 4
	480	134 ± 16	31 ± 3
High-fat/Met	saline	210 ± 20	33 ± 4
	240	188 ± 21	28 ± 3
	480	109 ± 11*	37 ± 4

*Significantly different from saline perfusion. Values are means ± SEM ($N = 5$).

(Table 2). The absence of effect of insulin on the liver glycogen content during euglycemic clamps is a well-established fact and is in agreement with previous unpublished results from this laboratory and with other published studies [31]. Therefore, we pooled the data obtained within each diet group to yield a better evaluation of the effect of diet on liver glycogen content. The liver glycogen content was lower by 30% in HF and HF/Met rats [31 ± 2 and 32 ± 3 mg/g of wet liver (means ± SEM, $N = 15$)] as compared to S rats [43 ± 2 mg/g of wet liver ($P < 0.01$ vs both other groups)].

The concentration of metformin in tissues from HF-Met rats was 27 ± 2 nmol/g in the liver (wet weight), 29 ± 3 nmol/g in the skeletal muscle, and 57 ± 6 nmol/g in the jejunum (means ± SEM, $N = 5$). Metformin was not detected in the plasma.

DISCUSSION

To address the question of the role of Glc6Pase and GK in hepatic insulin resistance at the molecular level, we studied three groups of rats exhibiting varying degrees of insulin sensitivity. HGP and its inhibition by insulin were determined during hyperinsulinemic euglycemic clamps at plasma insulin concentrations matching those which could be attained in the portal vein of rats in the postprandial period [6, 32]. No difference could be evidenced regarding either basal HGP measured under saline perfusion or HGP determined under insulin at a high perfusion rate. However, HGP was not suppressed under insulin at a low perfusion rate in HF rats, thereby revealing hepatic insulin resistance, whereas it was significantly suppressed by 35% and 65% in S and HF/Met rats, respectively, under very similar conditions of final insulinemia (see Table 1).

It must be emphasized that HF rats share the HGP features of a majority of type 2 diabetic patients, in which HGP is not (or is only moderately) increased in the basal

state, less efficiently suppressed by insulin within the physiological range of concentration, and totally suppressed at high insulinemia [9]. In addition, in contrast to insulin-deficient rats whose liver is very depleted in glycogen, the liver of HF rats contains significant glycogen stores. These stores are lower by 30% than those of S rats 7–8 hr after food removal, in good concordance with the glycogen stores of NIDDM patients in regard to controls in the postabsorptive state [33]. Therefore, HF rats may represent a good model to approach the metabolic mechanisms taking place in the liver of type 2 diabetics. Moreover, it has been suggested that the inhibitory effect of metformin on HGP in NIDDM patients [15–18] is mediated through an improvement in hepatic insulin sensitivity [17]. Our results, obtained with rats fed the high-fat diet and treated with metformin doses close to those given to treat NIDDM patients [16–18], allow us to certify a major improving action of the drug on the insulin sensitivity of HGP in insulin-resistant states. Very interestingly, the effect of metformin treatment was shown here to take place under conditions where the drug was not detected in the plasma and was found at a concentration of 27 nmol/g in the liver, i.e. far below the concentrations required to observe direct effects in the perfused rat liver or isolated hepatocytes (usually 10^{-4}–10^{-3} mol/L) [19, 34, 35].

The first explanation which might be put forward to account for hepatic insulin resistance in HF rats is an alteration in Glc6Pase and/or GK total activities. The most dramatic change relates to GK activity, lower by 65% in both groups of rats fed the HF diet as compared to the group fed the S diet. This is in keeping with the observation that weaning suckling rats to an HF diet reduces the postweaning increase in GK activity by *ca.* 60% [36]. Since the GK gene is induced by insulin [37], the decreased GK activity is in keeping with the fact that HF feeding induces liver insulin resistance. It is noteworthy that Glc6Pase activity was also lower by 35% in both groups fed the HF diet in contrast to the group fed the S diet. Since insulin has a suppressive effect on the expression of Glc6Pase activity [2, 38], this decreased Glc6Pase activity is surprising in a context of liver insulin resistance. It should be mentioned that previous studies have reported that liver Glc6Pase activity is increased by high-fat diet in rat [39]. However, the increase was very small when food was given on an isocalorically matched basis, such as provided here, and not *ad lib*. [40]. The discrepancy with our results could be due to the nature of dietary fats. It has indeed been shown that rat liver Glc6Pase activity was decreased by fat diets enriched in polyunsaturated ω-6 fatty acids (such as that given here), as compared to isocalorically matched fat diets rich in saturated fats [40, 41]. Although Glc6Pase activity was decreased in HF and HF/Met rats, the ratio of Glc6Pase V_{max} to GK V_{max} was increased by a factor of 2 with regards to S rats. This is in keeping with insulin resistance of HGP. However, this parameter taken individually has few repercussions on basal HGP, which takes into account other important parameters such as the respective K_m values and substrate concentrations of both enzymes, and above all additional mechanisms of regulation of Glc6Pase activity taking place *in situ*, but not retained in isolated microsomes [2–7]. In addition, it may not account by itself for hepatic insulin resistance, since it was similar in insulin-resistant HF rats and in insulin-sensitive HF/Met rats.

It must be mentioned that the decreased Glc6Pase activity in HF rats, in contradiction with the expected increased expression of the gene in the insulin resistant state, constitutes a protection opposing the decreased activity of GK. From Michaelis–Menten kinetics, it is easy to calculate that if Glc6Pase activity were increased by a factor of 2 in HF rats, as has been reported in the insulin-deficient pancreatectomized [8] or streptozotocin-treated [38] rats, this should result, all other parameters being equivalent, in a basal HGP increased by a factor of 2.3 in HF rats. The decreased expression of Glc6Pase gene could therefore represent a crucial adaptative mechanism to the decreased expression of the GK gene in a situation of insulin resistance, hindering a large increase in basal HGP. It is tempting to speculate that the loss of this protective mechanism in type 2 diabetes could account at least in part for the metabolic staging of the illness from diabetes without increased basal HGP to diabetes with increased basal HGP [42].

The second possibility which might account for insulin resistance of HGP could be an increased concentration of Glc6P, which would result in an increased flux through Glc6Pase by substrate effect. In S and HF/Met diet groups, insulin had a significant decreasing effect on the hepatic Glc6P concentration at the high perfusion dose. This is in agreement with previous results [7, 31]. This might likely be accounted for by a stimulation of glycolysis [31] and/or by an inhibition of gluconeogenesis, since insulin has no effect on the glycogen cycle in euglycemia [31 and Minassian and Mithieux, unpublished results]. In contrast, the latter dose did not significantly lower hepatic Glc6P concentration in HF rats (Table 2). Since HGP under this condition was comparable to that in the other two groups (see Fig. 1), one may assume that this might not result from a lower utilization by Glc6Pase. Therefore, it might be due to either a lesser inhibition of gluconeogenesis by insulin or a lesser stimulation of glycolysis, or both, either of these phenomena being indicators of hepatic insulin resistance. However, the salient observation is that, at the low insulin perfusion rate (240 pmol/hr), the Glc6P concentration was not higher in insulin-resistant HF rats than in insulin-sensitive HF/Met rats, whereas the differences in the effect of insulin on HGP are obvious (see Fig. 1 and Table 2). Moreover, the Glc6P concentration tended to be higher in HF/Met rats than in HF rats (188 ± 21 vs 135 ± 24 nmol/g, $P = 0.09$), whereas HGP in the former was 3 times lower than in the latter (23 ± 10 vs 60 ± 12 $\mu mol \cdot kg^{-1} \cdot min^{-1} \cdot$, $P < 0.05$). It is therefore evident that there is no parallelism between HGP and the Glc6P concentration. Even if it is difficult to assign a significance to Glc6P concentration at a given time, since it is the result of the combination of

several provision and utilization pathways (see above), that it tended to be higher in HF/Met rats than in HF rats is in agreement with an inhibition of Glc6Pase flux, taking place in the former and not (or less) in the latter. Furthermore, since total Glc6Pase activity (V_{max}) and K_{ms} were comparable in both groups, if no inhibition of Glc6Pase activity by insulin took place, the HGP should only be dependent on the Glc6P concentration and therefore, should be equal or slightly higher in HF/Met rats than in HF rats. However, the contrary is true. This definitely confirms that additional regulatory mechanisms are involved in the control of the substrate flux through Glc6Pase *in vivo*, and are crucial in determining the effect of insulin on HGP. We could not evidence any alteration of the kinetic parameters (K_m and V_{max}) of Glc6Pase upon insulin perfusion in either group. This is in keeping with previous data suggesting that these regulatory mechanisms are highly labile, being lost under *in vitro* conditions of assay [2–7]. Our results constitute a further strong support to the existence of such mechanisms and emphasize the crucial role that they could play in hepatic insulin resistance. Their nonoccurrence might explain the impaired effect of insulin on HGP in HF rats. The beneficial effect of metformin on insulin sensitivity of HGP in HF/Met rats might be to restore them.

In summary, we have shown that: 1) hepatic insulin resistance in HF rats is associated with marked changes in the total activities of the two enzymes involved in the control of HGP: the dramatic decrease in GK activity is partially compensated by a decrease in Glc6Pase activity, preventing HGP from increasing under basal insulinemia conditions; 2) the impairment of insulin to inhibit HGP in insulin-resistant rats is not dependent on an increased concentration of Glc6P; and 3) short-term regulatory mechanisms of Glc6Pase are involved in the effect of insulin on HGP: these mechanisms might be impaired in insulin-resistant states, and restored by metformin treatment.

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