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Erratum to: "Glucose utilization is suppressed in the gut of insulin-resistant high fat-fed rats and is restored by metformin" [Biochem. Pharmacol. 72 (2) (2006) 198–203]

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ABSTRACT

It has been recently suggested that the small intestine (SI) has the capacity to contribute to endogenous glucose production (EGP), in addition to the liver and kidney. The aim of this work was: (1) to estimate the role of SI glucose fluxes in glucose homeostasis in insulin resistance states (induced by high-fat (HF) feeding); (2) to assess the effect of metformin, an anti-diabetic molecule, on these fluxes. Rats were fed for 6 weeks on a HF-diet, supplemented or not with metformin (HF-Met) at a daily dosage of 50 mg/kg during the last week. We combined arterio-venous glucose balance measurements and isotopic dilution techniques to separate basal intestinal glucose uptake (IGU) and release (IGR). Contrary to what was observed in control starch-fed rats, IGU was negligible in HF-fed rats: 0.6 ± 2.4 μmol/kg min (mean ± S.E.M., $n = 9$). It was restored to a level close to that of control rats in the HF-Met group: 13.0 ± 6.7 μmol/kg min (mean ± S.E.M., $n = 9$, $p < 0.05$ compared to the non-treated group). Similarly, IGR was close to zero in HF-fed rats (-3.8 ± 2.6 μmol/kg min), but was significant in HF-Met rats (7.4 ± 4.4 μmol/kg min, $p < 0.05$ compared to non-treated rats). These data strongly suggest that the impairment of glucose uptake in the SI might be a crucial feature of insulin resistance states and that a key beneficial effect of metformin in these situations might be to restore a normal glucose metabolism in this tissue.

1. Introduction

Insulin resistance is a common key feature in obesity, metabolic syndrome and type 2 diabetes. Among the numerous metabolic functions that may be affected in insulin resistance states, increased endogenous glucose production (EGP) is recognized to constitute a crucial step in the metabolic staging of the illness towards impaired glucose tolerance and frank diabetes [1,2]. Until recently, the liver and kidney were considered to be the only organs capable of releasing endogenous glucose into the blood, because they were the only tissues known to express the key glucose-6 phosphatase (Glc6Pase) gene. In contradistinction to this dogma, we reported that the small intestine is actually a third gluconeogenic organ, expressing Glc6Pase [3], and able to contribute to EGP in insulinopenia states [4–6]. Interestingly, the regulatory genes

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of gluconeogenesis, i.e. Glc6Pase and phosphenolpyruvate carboxykinase-cytosolic form (PEPCK-C), are suppressed by insulin in the SI as in the liver [3,7]. While intestine glucose production was not detectable in the post-absorptive state in the normal rat [4], the hypothesis that SI gluconeogenesis might be induced in insulin resistance states, thus representing a potential additional source of glucose able to account at least in part for insulin resistance of EGP, therefore appeared attractive. We also previously reported that metformin, a widely used oral antidiabetic agent known to decrease basal EGP in type 2 diabetic patients [8–10], suppresses EGP and Glc6Pase activity in the liver of insulin-resistant high fat (HF)-fed rats [11,12]. A putative action of metformin on glucose production in the SI was thus a relevant hypothesis.

There was another reason to focus on the possible role of the SI in insulin resistance. Actually, the gut is known to be an important quantitative site of glucose disposal, accounting for about 20% of the total glucose turnover of the whole body [4,13,14]. Metformin has also been strongly suggested to have a major impact on glucose uptake by the gut [15–17]. However, the latter effect may appear questionable nowadays, since it was evidenced from 2-deoxyglucose uptake, an approach that is prone to pitfalls in those tissues expressing Glc6Pase [18]. In line with a key action on the gut, metformin is known to preferentially accumulate in this tissue [11,17].

In our previous works dealing with glucose fluxes in the gut, we separated intestinal glucose release and uptake using a combination of tracer dilution and arterio-venous balance techniques [4,5]. This approach helped us to raise several questions in this work. On the one hand, we sought to know whether an intestinal component of EGP and/or any other alteration of intestinal glucose fluxes (i.e. glucose disposal) did take place in insulin resistance states in the rat. On the other hand, we wondered whether metformin might have a beneficial effect on these parameters. Since we previously showed that the rat fed on a HF-diet for 6 weeks is a valuable model of insulin resistance of EGP and is very useful in the study of the action of metformin on the liver [11,12], we used this model to raise these questions at the intestinal level.

2. Experimental procedures

2.1. Animals and diets

Male Sprague–Dawley rats were obtained from Charles River (L'Arbresle, France). They were fed for 6 weeks on a control starch-diet or a HF-diet as previously described [11,12]. The HF-diet was composed as follows: 24% starch/glucose, 25% casein, 36% carthame oil, 6% cellulose, 7% mineral salts, 1% vitamins, 1% choline, mass basis. The starch-diet differed by its content in starch/glucose (50%), protein (17%), lipid (6%) and cellulose (12%). In the metformin-treated group, the drug was given for the last week, mixed with the food, at a dose of 50 mg kg^{-1} day^{-1}. Food was withdrawn 5 h before the experiment. Water was available ad libitum. All experiments were performed in anesthetized animals (pentobarbital 6 mg/100 g of body mass, with temperature monitored with a rectal probe and maintained at 37.5 °C using a heating blanket). Our protocols were performed according to the rules of our local ethics committee for animal experimentation.

2.2. Determination of intestinal and total glucose fluxes

Rats in the postabsorptive state were anesthetized, fitted with catheters (in the right jugular vein for glucose tracer infusion and in the left carotid artery for blood sampling) and infused with [3-^{3}H] glucose as previously described [4,5]. After infusion for 10 min, a laparotomy was performed. The inferior mesenteric circulation was stopped by ligatures, to exclude the blood flow coming from the cecum and the colon. After 90 min, a time lapse where a steady state of glucose specific activity (SA) was obtained [4], blood was gently sampled simultaneously in the carotid artery and in the portal vein, using a catheter inserted into a superior mesenteric vein and pushed up to the junction with the pancreaticoduodenal vein. Total EGP was obtained from the [3-^{3}H] glucose infusion rate and the SA of arterial glucose [4,12]. The fractional extraction (FX) represents the fraction of [3-^{3}H] glucose removed by the SI. It was calculated as ([3-^{3}H] glucose SA_{artery} × glucose $concentration_{artery}$) − ([3-^{3}H] glucose SA_{vein} × glucose $concentration_{vein}$)/([3-^{3}H] glucose SA_{artery} × glucose $concentration_{artery}$). The intestinal glucose uptake (IGU) was determined from this FX, the arterial plasma glucose and the intestinal blood flow (IBF) as: IGU = IBF × glucose $concentration_{artery}$ × FX. The determination of IBF using radiolabeled microsphere technique was previously described [4]. The intestinal glucose balance (IGB) is the net result of both the uptake and release of glucose by the SI. It was calculated from the difference between the arterial and venous glucose concentration and IBF, as IBF × (glucose $concentration_{artery}$ − glucose $concentration_{vein}$). Finally, the intestinal glucose release (IGR) was derived from IGB and IGU by resolving: IGB = IGU − IGR. All results were expressed in μmol/kg min.

2.3. Other determinations

Determinations of plasma glucose concentration and glucose SA were described previously [4,5]. Plasma insulin and glucagon concentrations were determined by radioimmunoassay [11,12]. Enzyme activities and metabolite contents were determined in a liver lobe and a small intestine sample (proximal jejunum). The latter were removed just after the final blood sampling, by freezing in situ at −196 °C using a pair of tongs pre-cooled in liquid nitrogen. The procedures for the quantification of enzyme activities under conditions of maximal velocity from liver and intestine samples were previously described [3–7]. Glycogen and glucose-6 phosphate contents were determined according to the procedure described by Keppler and Decker [19].

3. Results

3.1. Effect of metformin on intestinal glucose fluxes in HF-fed rats

Body weight, plasma insulin and glucagon concentrations were comparable in control and metformin-treated animals (Table 1). These data were similar to those previously reported

Table 1 – Effect of metformin (Met) on glycemic parameters in HF-rats

Diet/treatment	Body weight (g)	Insulin (pmol/L)	Glucagon (ng/L)	Plasma glucose concentration (mmol/L)		[3-^{3}H]glucose-specific activity (dpm/μmol)	
				Artery	Vein	Artery	Vein
Starch-fed	303 ± 4	304 ± 42	584 ± 52	9.3 ± 0.6	8.6 ± 0.5[a]	10 539 ± 681	10 738 ± 610
HF	323 ± 13	261 ± 40	612 ± 61	10.3 ± 0.6	10.1 ± 0.6	12 933 ± 374	13 147 ± 349
HF ± Met	326 ± 20	252 ± 35	618 ± 64	9.2 ± 0.5	9.0 ± 0.6	14 960 ± 1025	14 416 ± 801[a]

Data are the means ± S.E.M. ($n = 9$).
[a] Different from value in artery, $p < 0.05$ (Student's *t*-test for paired data).

and discussed [12]. They will not be discussed here. Basal EGP was lower (−15%) in metformin-treated HF-fed animals than in untreated HF-fed rats (Table 2). This result is in good agreement with our previous study on the effect of metformin in HF-rats [12]. The SI blood flow (6.22 ± 0.59 mL/min in HF-fed rats) was not different from that previously determined in post-absorptive rats fed on a classical starch-diet [12] and was not altered by metformin treatment.

In control starch-fed rats, the specific activity (SA) of plasma glucose was similar in the portal vein and in the artery (Table 1). This fact indicates that no newly synthetized (unlabelled) glucose was released by the SI in this nutritional situation, in agreement with previous results [12,20]. There was a significant decrease in plasma glucose concentration in the portal blood, reflecting glucose utilization by the intestine. These data allowed us to calculate significant FX, IGU and IGB values in these rats, although IGR was not different from zero (Table 2).

In untreated HF-rats, as in control starch-fed rats, the arterial and venous plasma glucose SA were not different from each other (Table 1), also indicating that no release of glucose took place from the SI. Contrary to what was observed in starch-fed rats, there was no significant difference between the arterial and venous plasma glucose concentration (Table 1). Consequently, both the IGB and the FX calculated from these data were close to zero in these rats (Table 2). This highlights that the removal of glucose from the blood by the SI was not detectable. Accordingly, the IGU calculated from these data was not different from zero (Table 2). Consistent with glucose SA data (see above), the IGR calculated from IGB and IGU was also not different from zero (Table 2).

In metformin-treated animals, contrary to the untreated HF-fed group, the venous plasma SA was lower than the arterial plasma glucose SA (Table 1). This indicates that newly synthesized glucose was released by the SI. As in untreated HF-rats, the venous plasma glucose concentration was not

Table 2 – Effect of metformin (Met) on intestinal glucose fluxes in HF-rats

Diet/treatment	FX	IGB	IGU	IGR	EGP
Starch-fed	0.06 ± 0.01[a]	16.3 ± 6.0[a]	12.9 ± 5.5[a]	−3.4 ± 2.7	72.9 ± 4.5
HF	0.00 ± 0.01	4.4 ± 1.9	0.6 ± 2.4	−3.8 ± 2.6	64.4 ± 3.4
HF ± Met	0.06 ± 0.02[a]	5.6 ± 4.2	13.0 ± 6.7[a]	7.4 ± 4.4[a]	54.2 ± 3.7[a]

Data are the means ± S.E.M. ($n = 9$). IGB, IGU, IGR and EGP are expressed as μmol/kg min.
[a] Different from HF value, $p < 0.05$ (Student's *t*-test for unpaired values).

Table 3 – Enzymatic activities involved in liver and intestinal glucose metabolism in HF-rats treated or not with metformin (Met)

Enzyme	Liver		Small intestine	
	HF	HF-Met	HF	HF-Met
Glc6Pase	10.7 ± 0.5	8.4 ± 0.6[a]	2.5 ± 0.2	2.3 ± 0.1
PEPCK-C	2.9 ± 0.1	2.8 ± 0.2	0.23 ± 0.06	0.21 ± 0.03
HK	ND	ND	0.97 ± 0.05	0.97 ± 0.12
Glutaminase				
(Pi)	1.8 ± 0.1	1.7 ± 0.2	5.2 ± 0.7	5.4 ± 0.6
(Malate)	0.13 ± 0.1	0.07 ± 0.03	1.6 ± 0.2	1.6 ± 0.3
ALAT	14.2 ± 1.5	16.1 ± 2.5	50.8 ± 2.1	46.4 ± 7.2
Glycerokinase	2.6 ± 0.1	2.8 ± 0.2	0.35 ± 0.08	0.36 ± 0.11

Data are the means ± S.E.M. ($n = 9$). All enzyme activities are expressed as μmol of substrate transformed per minute per gram of wet tissue.
[a] Different from HF-value, $p < 0.05$ (Student's *t*-test for unpaired values). ND, not determined; Glc6Pase, glucose-6 phosphatase; PEPCK-C, phosphoenopyruvate carboxykinase-cytosolic; HK, hexokinase; ALAT, alanine aminotransferase.

different from the arterial one. Consequently, the IGB was not different from zero. However, the SA data allowed us to calculate a fractional extraction of glucose (FX) significantly higher than that found in HF-rats, and similar to that found in control starch-fed animals (Table 2). Accordingly, the IGU calculated from the FX was significantly higher than that determined in untreated animals, and comparable to that of starch-fed rats (Table 2). As a consequence, the IGR derived from IGB and IGU was substantial, and significantly higher than that calculated in both untreated HF-fed rats and starch-fed rats (Table 2).

3.2. *Effect of metformin on liver and intestinal enzyme activities in HF-fed rats*

We then tried to know whether these marked modifications of SI glucose fluxes could be explained by alterations of key metabolites – or of the expression of regulatory enzymes – of SI glucose metabolism [4,5]. The liver enzymes were assayed in parallel. It is to be noted that none of these enzymes was altered by metformin-treatment in the SI (Table 3). On the contrary, there was a ≈20% decrease in the sole Glc6Pase activity in the liver of metformin-treated rats, whereas the other enzymes were not affected (Table 3). This is in line with our previous results [12]. The intestinal glucose-6 phosphate content was not different in HF-fed rats (54 ± 6 nmol/g wet tissue) and in their counterparts treated by metformin (42 ± 8 nmol/g). In the same way, glycogen content was not detectable in both groups (lower than 10 μg/g of wet tissue).

4. Discussion

In our previous works [11,12], we have shown that the rat fed on a high-fat diet is a convenient model of insulin resistance of EGP. In addition, it is extremely useful to specify the mechanism of action of active drugs like metformin on basal EGP, a key parameter altered in type 2 diabetic patients [1,2,8–10]. Our first hypothesis was that an intestinal component of EGP might take place in insulin-resistant states, accounting at least in part for the dysregulations of EGP. On the contrary, SI glucose production did not take place in HF-rats. Moreover, the decrease in EGP observed in metformin-treated animals was concomitant with the onset of an additional (albeit weak) glucose production by the SI. This could a priori appear paradoxical. However, one should take into account that Glc6Pase activity was attenuated in the liver of HF-fed metformin-treated rats (see Table 3), a process previously shown to decrease hepatic glucose production and to improve the suppression of EGP by insulin [11,12]. Taking into account this participation of the SI to EGP in metformin-treated rats, our results even suggest that the suppression of glucose production in non-intestinal tissues, i.e. the liver (and/or the kidney) might be quantitatively stronger than the global impact of the drug on total EGP. This is in keeping again with the inhibition of the liver Glc6Pase and with our previous observation that a major effect of metformin in HF rats was a dramatic accumulation of hepatic glucose-6 phosphate and of glycogen stores [12]. Taken together, these data indicate: (1) that SI gluconeogenesis does not contribute to basal EGP in insulin-resistance states, at least in the states induced by lipid-enriched diets; (2) that a global improvement at the level of basal EGP, i.e. herein the beneficial suppressing effect of metformin, may take place even if a low contribution to glucose production originates from the SI.

It must be stressed here that one has to be cautious regarding the quantification of intestinal fluxes in the approach used here. Moreover, relevant questions were recently raised in a comprehensive critical analysis of our works [21]. We [4] and others [21–23] previously emphasized that a critical data set in this type of determination relates to the estimation of glucose SA in the vein and artery. Partly because of the high blood flow through the SI, differences in glucose SA are difficult to determine with accuracy. This is especially crucial to obviate the reality of intestinal glucose production, when it takes place. Actually, in the absence of an increase in glucose concentration at the exit of the organ (which is the case here because the release does not exceed the uptake), the decrease in glucose SA in the portal blood is the unique evidence suggesting the occurrence of glucose release. In defence of this methodology, portal and arterial glucose SA are obtained from the same animal. Therefore, statistical analyses of the data may involve paired test analyses [4,5,20]. This permits one to evidence differences in portal and arterial glucose SA even if the means are very close and obtained from scattered data sets (see Table 1), because the sense of the variation is tested and not the mean. Actually, the glucose SA was lower in the vein than in the artery in all but one (in which it was virtually equal) HF-fed rats treated with metformin (the difference was significant). On the contrary, there was no privileged sense of variations (which were within the range of accuracy of the methodology) of glucose SA in the two other groups (differences were not significant). Another point is that these small differences have to be multiplied or divided by other parameters – which are themselves inaccurate to some extent (e.g. blood flow) – to calculate glucose fluxes. One must therefore be aware that, even when differences are significant, the final fluxes calculated constitute estimations rather than definitive values from a quantitative point of view.

Another unexpected observation from our data was a dramatic decrease (i.e. virtual total suppression) in glucose uptake by the SI in HF-fed rats. Glucose indeed is generally considered as a crucial substrate for the SI [13,14]. This is in line with the accepted view that the SI is an important site of glucose disposal in the normal situation [4,13,14]. However, we should keep in mind that glucose is only weakly oxidized in the SI and that the bulk of the enterocyte energy requirement may be accounted for by ketone bodies (for about 50%), glutamine (about 35%) and free fatty acids (8–10%) [24,25]. It is thus likely that the SI should not lack energy substrates in the context of high fat feeding. A key result is that metformin treatment might restore SI glucose uptake in HF-rats at a level comparable to that determined in control starch-fed rats (see Table 2). This constitutes a sound in vivo validation of previous results [15,16]. The latter indeed were obtained from a 2-deoxyglucose technique, an approach now known to strongly underestimate the actual

glucose uptake in those tissues expressing Glc6Pase. Again, it may appear paradoxical that the possible onset of SI glucose release might be concomitant with the restoration of a normal glucose uptake. However, one must keep in mind that glutamine is the main, if not the sole, precursor of glucose in the SI [4,6,20]. We previously emphasized the specificities of glutamine metabolism in the SI. The latter actually involves alanine aminotransferase (ALAT), a two-substrate enzyme functioning with pyruvate as a second substrate [6,13]. A normal SI glutamine utilization thus appears strongly dependent on a continuous feeding of the pyruvate pool. This is a major function of the sustained anaerobic glucose metabolism in the SI [26]. When this metabolism is impaired, as in HF-rats, the key cataplerotic function of PEPCK-C may serve to feed the pyruvate pool via the pyruvate kinase [6,14,26,27]. This may thus divert PEPCK-C from its putative implication in the gluconeneogenic pathway. In contrast, when glucose utilization is restored, as in metformin-treated animals, the feeding of the pyruvate pool from glucose might be favoured, thus freeing PEPCK from this function to the benefit of its gluconeogenic role. Since gluconeogenesis mainly proceeds from glutamine in the SI, the occurence of glucose utilization with gluconeogenesis seems thus highly relevant. In strong agreement with this rationale, the presence of glucose markedly stimulates the transamination of glutamine in enterocytes [14,28]. It must be mentioned that a recent breakthrough in the understanding of the intracellular mechanism of action of metformin has been the demonstration of its strong effect of inhibition of the mitochondrial respiratory chain at the level of complex I [29]. This might be a key mechanism diverting pyruvate (a substrate utilized at the level of complex I) from oxidation to the benefit of its anaerobic metabolism. This might also favour cell respiration from fatty acids (available in excess in HF-fed rats), being oxidized at the level of complex II.

It must be pointed out that metformin promotes very different mechanisms in the liver and the SI. In the liver, we previously reported (and confirm here) that the drug suppresses Glc6Pase activity and hepatic glucose release, causing the accumulation of both Glc6P and glycogen. No such effects occur in the SI. On the contrary, metformin restores both the flux of substrate through Glc6Pase and a normal turnover of the pathways involved in glucose utilization and glucose production, precluding any accumulation of metabolic intermediates. These different mechanisms might explain why the identification of a general mechanism of action of the drug has so far remained elusive [17]. Even if much remains to be understood regarding metformin and its various effects, the results reported here strongly suggest that the impairment of glucose metabolism in the SI might be a crucial feature of insulin resistance states, and that a key beneficial effect of metformin in these situations might be to restore a normal glucose utilization in this tissue.

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