Initial glucose kinetics and hormonal response to a gastric glucose load in unrestrained post-absorptive and starved rats

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A gastric [$U\text{-}^{14}\text{C}$]glucose load (4.8 mg/g body wt.) was delivered to unrestrained post-absorptive or 30 h-starved rats bearing peripheral and portal vein catheters and continuously perfused with [$3\text{-}^3\text{H}$]glucose, in order to compare their metabolic and hormonal responses. In the basal state, portal and peripheral glycaemia were less in starved rats than in rats in the post-absorptive period ($P < 0.001$), whereas blood lactate was similar. Portal insulinaemia ($P < 0.05$) and portal glucagonaemia ($P < 0.005$) were lower in starved rats, but insulin/glucagon ratio was higher in post-absorptive rats ($P < 0.005$). The glucose turnover rate was decreased by starvation ($P < 0.005$). After glucose ingestion, blood glucose was similar in post-absorptive and starved rats. A large portoperipheral gradient of lactate appeared in starved rats. Portal insulinaemia reached a peak at 9 min, and was respectively 454 ± 68 and 740 ± 65 $\mu\text{-units/ml}$ in starved and post-absorptive rats. Portal glucagonaemia remained stable, but was higher in post-absorptive rats ($P < 0.05$). At 60 min after the gastric glucose load, 30 % of the glucose was delivered at the periphery in both groups. The total glucose appearance rate was higher in starved rats ($P < 0.05$), as was the glucose utilization rate ($P < 0.05$), whereas the rate of appearance of exogenous glucose was similar. This was due to a non-suppressed hepatic glucose production in the starved rats, whereas it was totally suppressed in post-absorptive rats. At 1 h after the glucose load, the increase in both liver and muscle glycogen concentration was greater in starved rats. Thus short-term fasting induces an increased portal lactate concentration after a glucose load, and produces a state of liver insulin unresponsiveness for glucose production, whereas the sensitivity of peripheral tissues for glucose utilization is unchanged or even increased. This might allow preferential replenishment of the peripheral stores of glycogen.

INTRODUCTION

The fasting state has been widely used in the rat in order to study the hepatic or peripheral response to a glucose load (Newgaard *et al.*, 1983, 1984; Scofield *et al.*, 1985; Holness *et al.*, 1986, 1988a,b; Holness & Sugden, 1987) and the underlying biochemical mechanisms. However, from a physiological viewpoint, it must be pointed out that in the rat even a short-term fasting period corresponds to a large catabolic drain and large metabolic adaptations, since the energy turnover in such a small animal is extremely high. For instance, its glucose turnover rate expressed per kg body wt. in the post-absorptive state is 10-fold higher than in man in the same situation (Bier *et al.*, 1977; Smadja *et al.*, 1988). In the rat, a 24–48 h starvation period is concomitant with a marked decrease in glucose turnover rate (Issad *et al.*, 1987), complete exhaustion of hepatic glycogen stores and activation of gluconeogenesis (Bois-Joyeux *et al.*, 1986), a marked increase in plasma non-esterified fatty acids and blood ketone bodies (Goodman *et al.*, 1980), a large decrease in plasma insulin and an increase, although more controversial, in plasma glucagon concentrations (Seitz *et al.*, 1976; Goodman *et al.*, 1980; Balks & Jungermann, 1984; Bois-Joyeux *et al.*, 1986). Peripheral metabolism seems to be also profoundly altered, with a marked decrease in blood glucose utilization in oxidative muscles (Issad *et al.*, 1987) and an increased sensitivity to insulin as assessed with the euglycaemic-hyperinsulinaemic clamp (Pénicaud *et al.*, 1985) or in studies *in vitro* (Le Marchand-Brustel & Freychet, 1979; Brady *et al.*, 1981; Stirewalt *et al.*, 1985). Thus the hormonal and metabolic responses to an exogenous glucose load could be markedly different in a post-absorptive and a starved rat.

In order to document this problem, we have studied metabolic

and hormonal parameters of unrestrained post-absorptive and 30 h-starved rats bearing a chronic portal-vein catheterization in the basal state and after a gastric glucose load.

MATERIALS AND METHODS

Animals

Female rats weighing 250–300 g were used. They were housed in individual wire cages at 23 °C with light from 07:00 h to 19:00 h. They had free access to water and chow pellets, unless otherwise indicated.

Surgical procedure

The surgical procedures have been described in detail elsewhere (Smadja *et al.*, 1988). Briefly, under ether anaesthesia, a silastic catheter was inserted into the portal vein and two silastic catheters were respectively positioned into the superior vena cava via the right jugular vein and in the inferior vena cava through a lumbar vein. A gastrostomy tube was introduced into the fundus of the stomach. The catheters were fixed to the top of the skull and occluded with a metal plug.

Experimental protocol

Metabolic and hormonal responses were evaluated in rats after a [$U\text{-}^{14}\text{C}$]glucose load (4.8 mg/g body wt., 40 μCi) delivered through the gastrostomy tube after the 5th post-operative day when the rat had recovered from the surgical procedure (Smadja *et al.*, 1988). In the post-absorptive control group ($n = 8$) rats were studied after 6–7 h of fasting (i.e. 08:00 h to 14:00–15:00 h). In the starved group ($n = 8$), rats were starved for 30 h and studied at 14:00–15:00 h. At 1 h before the experiments, silastic

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tubes were connected to the portal vein, peripheral veins and gastric tube for blood sampling and infusion studies. The changes in glucose and lactate concentrations in the portal and peripheral blood were monitored without disturbing the rat. Portal plasma insulin and glucagon were also studied. At the end of the experiment, rats were anaesthetized with pentobarbital, and livers and various muscles were quickly removed and frozen in liquid N_2 for subsequent determination of glycogen. Liver and muscle glycogen concentrations were also evaluated after 6 h ($n = 6$) and 30 h ($n = 6$) of fasting in rats bearing a portal-vein catheter.

Glucose kinetics were assessed by tracer studies and the calculations were performed as described by Steele (1959) and Saccà *et al.* (1981). In order to obtain valid values (Steele, 1959), it is necessary to determine in which volume glucose entering the system is instantaneously mixed (rapidly mixing pool). This was determined as previously described (Smadja *et al.*, 1988), and was 25 % of body weight in our experimental conditions. Since $[U-^{14}C]$ glucose was used to label the gastric glucose load, and since it is a reversible tracer, it was necessary to check to what extent $[^{14}C]$ glucose recycling contributed to the $[^{14}C]$ glucose appearance in blood. Thus, in preliminary experiments, the gastric glucose load was labelled with both $[U-^{14}C]$ glucose and $[3-^3H]$ glucose, and the recycling rate of $[U-^{14}C]$ glucose was evaluated. It was 5 % in starved rats and 10 % in post-absorptive rats (Smadja *et al.*, 1988). These rates were taken into account for the evaluation of the appearance rate of the exogenous glucose (Ra_{EG}) at the periphery and the calculation of the hepatic glucose production (Ra_{HG}).

Determination of blood substrates, plasma pancreatic hormones and liver glycogen concentrations

Blood samples were immediately deproteinized with $HClO_4$ (6 %, w/v) and centrifuged at 16000 g for 2 min; 25 μ l of the neutralized supernatant were used for determination of blood glucose by the glucose oxidase method (Boehringer, Mannheim, Germany). Blood lactate was measured by the method of Hohorst (1963) in 25 μ l of the neutralized supernatant. Portal blood samples (150 μ l) were collected in chilled tubes containing 10 % (v/v) of Iniprol (200000 peptidase-inhibitor units/ml; Choay, Paris, France). They were centrifuged at 4 °C and stored at -20 °C for subsequent determination of insulin and glucagon by radioimmunoassay. Plasma insulin was determined by a technique described previously (Girard *et al.*, 1973), and pancreatic plasma glucagon by a radioimmunological method using 30 K antiserum from Dr. R. H. Unger (Dallas, TX, U.S.A.). The liver glycogen concentration was determined as described by Chan & Exton (1976).

Measurement of glucose specific radioactivity

A sample of neutralized $HClO_4$ supernatant (100 μ l) was evaporated to dryness at 70 °C to remove 3H_2O . The dry residue was redissolved in 0.2 ml of water, which was passed through an ion-exchange resin column (I.D. 5 mm) containing 2.5 cm of Dowex AG1 X8 (formate form) and 2.5 cm of Dowex AG 50W X8 (H^+ form) to remove ^{14}C -labelled metabolites: 1.3 ml of water was then passed through the column. Then 1 ml of the eluate was sampled, and the radioactivity was measured after

Fig. 1. Portal (a) and peripheral (b) blood glucose and portal (c) and peripheral (d) blood lactate in starved (●) and control (○) rats before and after the gastric glucose load (4.8 mg/g body wt.), indicated by the arrow

*, **, ***, ****, difference significant at respectively $P < 0.05$, $P < 0.025$, $P < 0.005$ and $P < 0.001$.

addition of 9 ml of Unisolve I (Koch-Light, Colnbrook, Bucks., U.K.) with a Betamatic I counter (Kontron, Montigny, France) with double channel and automatic correction for quenching and spill-over of ^{14}C into the ^3H channel.

Statistics

Results are presented as means $\pm$ S.E.M. Significance of difference was assessed by the unpaired *t* test.

RESULTS

The pre-operative body weight of the rats was 282 ± 12 g in the starved group and 261 ± 10 g in control rats. At the time of experiments, body weight was 261 ± 8 g in starved rats and 266 ± 9 g in control rats. Fasting induced in rats a body-weight loss of 24 ± 2 g.

Under basal conditions the portal and peripheral blood glucose concentrations were significantly lower in starved rats ($P < 0.01$) than in control rats (Figs. 1*a* and 1*b*). After the gastric glucose load, there was no significant difference in portal and peripheral blood glucose concentration between both groups (Figs. 1*a* and 1*b*).

Portal and peripheral blood lactate concentrations were similar in starved and control rats (Figs. 1*c* and 1*d*). After the gastric glucose load, in the portal blood, lactate concentration increased markedly in starved rats after 12 min onwards ($P < 0.005$) (Fig. 1*c*); in the peripheral blood, lactate concentrations were similar in starved and control groups (Fig. 1*d*).

Portal plasma insulin (Fig. 2*a*) was lower in starved than in control rats (24 ± 4 versus 83 ± 10 $\mu\text{-units/ml}$, $P < 0.05$). After the gastric glucose load, plasma insulin concentrations were lower in starved rats during the first 12 min and similar thereafter to the concentrations achieved in control rats (Fig. 2*a*). Under basal conditions, portal plasma glucagon (Fig. 2*b*) was 113 ± 17 pg/ml in starved rats and 273 ± 48 pg/ml in control rats ($P < 0.005$). After the gastric glucose load, plasma glucagon levels remained lower in starved rats than in control rats. In the basal state, the plasma insulin/glucagon molar ratio was respectively 4.42 ± 0.69 in starved rats and 6.29 ± 0.49 in control rats ($P < 0.05$) (Fig. 2*c*). There was a transient increase in this ratio after the gastric glucose load ($P < 0.05$) (Fig. 2*c*).

Liver and muscle glycogen concentrations are given in Table 1. In the basal state, liver glycogen was lower in starved rats, as was the glycogen concentration in diaphragm. At 1 h after the glucose load, there was a small but significant increase in glycogen concentration in the liver of fasting rats, a significant increase in the soleus of post-absorptive rats and a large increase in the soleus and the diaphragm of fasting rats. At 3 h after the glucose load, the increase in hepatic glycogen was substantial in both starved and post-absorptive rats (Table 1).

In the basal state, the glucose turnover rate was decreased by starvation (starved rats, 15.1 ± 1.2 mg/min per kg; control rats, 20.1 ± 1.3 mg/min per kg) (Fig. 3). After the gastric glucose load, the total (exogenous plus endogenous) glucose appearance rate (R_a) increased less in control rats than in starved rats ($P < 0.05$) (Fig. 3*a*). The appearance rate of the exogenous glucose in the systemic circulation ($R_{a_{\text{EG}}}$) (Fig. 3*b*) was similar in starved and control rats, and reached a plateau by 12 min. Since there was only a marginal liver glycogen synthesis during the first 1 h of the experiment, $R_{a_{\text{EG}}}$ curves allowed evaluation of the amount of glucose absorbed during the 60 min of the study, which was 1.4 ± 0.1 mg/g body wt. in starved rats and 1.6 ± 0.2 mg/g body wt. in control rats. The glucose disappearance rate (R_d) (Fig. 3*c*) increased steadily in both groups, although after 20 min the rate was higher in starved rats than in control rats ($P < 0.05$), despite similar plasma insulin levels (Fig. 2*a*). Hepatic glucose production

Fig. 2. Portal plasma insulin (a) and glucagon (b) and insulin/glucagon ratio (c) in starved (●) and control (○) rats, before and after the gastric glucose load, indicated by the arrow

*, **, ***, ****, difference significant at respectively $P < 0.05$, $P < 0.025$, $P < 0.005$ and $P < 0.001$.

Table 1. Glycogen concentrations in liver and muscles of post-absorptive and starved rats in the basal state and 1 or 3 h after a glucose load (4.8 mg/g body wt.)

Results are means $\pm$ S.E.M. of 5–6 determinations: **, ***, difference significant at $P < 0.01$ and $P < 0.001$ respectively compared with the basal state.

Rats	State	Glycogen concn. (mg/g wet wt.)				
		Liver	Heart	Diaphragm	Soleus	Extensor digitorum longus
Post-absorptive	Basal	8.4 $\pm$ 3.0	2.7 $\pm$ 0.7	3.8 $\pm$ 0.4	3.3 $\pm$ 0.2	2.9 $\pm$ 0.6
	1 h glucose	7.3 $\pm$ 3.0	2.4 $\pm$ 0.5	4.4 $\pm$ 0.2	5.4 $\pm$ 0.2**	2.3 $\pm$ 0.3
	3 h glucose	17 $\pm$ 1**	—	—	—	—
Starved	Basal	0.2 $\pm$ 0.1	2.6 $\pm$ 0.3	2 $\pm$ 0.2	3.2 $\pm$ 0.1	1.7 $\pm$ 0.2
	1 h glucose	3.3 $\pm$ 0.7***	3.0 $\pm$ 0.3	8.8 $\pm$ 0.5***	7.4 $\pm$ 0.6***	1.2 $\pm$ 0.8
	3 h glucose	12 $\pm$ 2***	—	—	—	—

(Ra_{HG}) was not suppressed by the glucose load in starved rats, whereas it (Ra_{HG}) was totally inhibited in control rats at the end of the experiment (Fig. 3d).

DISCUSSION

Basal state

Glucose turnover rate was decreased by starvation. The decrease in glucose utilization can be a consequence of the decreased blood glucose, the decreased insulin concentration as well as utilization of lipid-derived substrates, especially in working muscles (Issad *et al.*, 1987).

As expected (Goodman & Ruderman, 1979; Pénicaud *et al.*, 1985), portal insulin was decreased by starvation. However, portal glucagon was also markedly decreased by starvation. In the anaesthetized rat, peripheral plasma glucagon has been shown to increase (Seitz *et al.*, 1976; Bois-Joyeux *et al.*, 1986) or to remain unchanged after starvation (Balks & Jungermann, 1984), whereas portal plasma glucagon was increased in the study by Balks & Jungermann (1984). These discrepancies may well arise from the different experimental protocols, and especially from the fact that, in our study, portal blood sampling was performed without anaesthesia or immediate surgery, two stressful situations which might artefactually alter glucagon

Fig. 3. Total glucose appearance rate (Ra) (a), exogenous glucose appearance rate (Ra_{EG}) (b), glucose utilization rate (Rd) (c) and hepatic glucose production (Ra_{HG}) (d) in starved (●) and control (○) rats in the basal state and after the gastric glucose load (4.8 mg/g body wt.)

*, **, ***, ****, difference significant at respectively $P < 0.05$, $P < 0.025$, $P < 0.005$ and $P < 0.001$.

Table 2. Metabolic responses to a gastric glucose load (4.8 mg/g body wt.) in post-absorptive and starved rats

The data are integrated over a 60 min period for both the basal and the glucose-load conditions. In the basal state, hepatic glucose production is equal to overall glucose utilization (glucose turnover rate). Results are means $\pm$ S.E.M. for 7–8 determinations: *, ***, difference statistically significant at $P < 0.05$ and $P < 0.001$ respectively between post-absorptive and starved rats.

Rats	Glucose load				
	Basal state Glucose turnover rate (mg/kg body wt.)	Exogenous glucose absorbed as glucose (mg/kg body wt.)	Intestinal lactate production (mg of glucose equivalent/ kg body wt.)	Hepatic glucose production (mg/kg body wt.)	Glucose utilization (mg/kg body wt.)
Post-absorptive	1209 $\pm$ 75	1600 $\pm$ 100	118 $\pm$ 12	571 $\pm$ 47	2216 $\pm$ 210
Starved	906 $\pm$ 72*	1400 $\pm$ 100	352 $\pm$ 23***	1321 $\pm$ 79***	2628 $\pm$ 208

secretion. A lower portal glucagon during short-term fasting is not totally unexpected, since meal-induced gastrointestinal-peptide secretions, which are glucagon stimulators, are lacking during fasting (Brady *et al.*, 1981).

However, since the insulin-glucagon ratio is lower during fasting (Fig. 2c), it should allow, as proposed by Unger (1971), the necessary adaptations of hepatic and peripheral metabolism, increased gluconeogenesis (Kraus-Friedman, 1984) and decreased glucose utilization (Pénicaud *et al.*, 1985; Issad *et al.*, 1987).

Glucose absorption after the glucose load

After the gastric glucose load, the peripheral-blood glucose concentration was higher in the control than in the starved rats during the first 12 min of the experiment, whereas blood glucose in the portal vein was similar in the two groups. This, together with the similar rate of appearance in the blood of exogenous glucose, suggests that gastric and intestinal glucose absorption is not decreased after short-term starvation and that the difference observed in peripheral glycaemia during the early phase of the glucose load is probably related to the lower basal glycaemia in starved rats.

Intestinal lactate production

In post-absorptive rats, and as previously described (Smadja *et al.*, 1988), a positive small porto-peripheral gradient of lactate appeared progressively during the glucose load, to reach a value of 0.3 mmol/l. These results are at variance with those of Rich-Denson & Kimura (1988), who showed a negligible production of [14 C]lactate from absorbed [14 C]glucose. It must be pointed out, however, that their results are based exclusively on radioactive data. In the starved rats this gradient was 4-fold higher, despite similar peripheral lactate concentrations in the two groups (Fig. 1d). Although we have not determined directly the portal blood flow, it can be roughly estimated in each group from the exogenous glucose absorption rate and the porto-peripheral glucose gradient. By using these values, the amount of glucose converted into lactate by the intestine can be computed and is 118 $\pm$ 12 mg in post-absorptive rats and 352 $\pm$ 23 mg in starved rats during the 60 min study, respectively 7 and 25% of the glucose absorbed as such (Table 2). Since a similar amount of glucose was absorbed in the two groups, it suggests a profound modification of the intestinal metabolism of glucose in starved rats, with an increased proportion of glycolytic products escaping oxidation. This might indicate that the mechanisms which operate in muscles during fasting (oxidation of lipid-derived substrate) and which lead to glucose recycling might also be effective in the intestine. The increased lactate production by the intestine could

contribute to the non-suppressed hepatic gluconeogenesis, and later on to the replenishment of hepatic glycogen.

Hepatic glucose metabolism

The early increase in the glucose appearance rate (R_a) in starved and post-absorptive rats was related to an early transient rise in hepatic glucose production, as shown in other situations (Rohner-Jeanrenaud *et al.*, 1986; Smadja *et al.*, 1988). This transient increase can be related to a similarly transient increase in portal glucagon concentration, which could activate the glucose production pathways. However, an increase in the splanchnic blood flow (Hernandez *et al.*, 1986; Lebrec & Girod, 1986), exerting a 'glucose washing' effect on the liver, cannot be excluded.

At 60 min after the glucose load, hepatic glucose production was totally suppressed in post-absorptive rats, whereas it was not inhibited at all in starved rats, despite similar portal plasma insulin and blood glucose and a higher insulin/glucagon ratio in starved rats (Figs. 1b, 2a and 2c). In previous studies on starved rats (Lang *et al.*, 1986; Huang & Veech, 1988), hepatic glucose production was also only partially decreased by a glucose load or a glucose infusion. In a study by Newgaard *et al.* (1983), glucose production in starved rats was inhibited 2 h after the beginning of the gastric glucose infusion, but, owing to the protocol used (the primed continuous infusion of the glucose tracer was begun 90 min after the beginning of the gastric glucose infusion), the immediate response of the liver could not be inferred from the experiment. Moreover, these rats were much younger (100–150 g) than in other studies and they were maintained before the experiment on a high-sucrose diet, two factors which can modify hepatic glucose metabolism. Studies using the euglycaemic-hyperinsulinaemic clamp technique to test the liver sensitivity of starved rats have yielded conflicting results: anaesthetized starved rats were shown to be insulin-resistant (Pénicaud *et al.*, 1985), whereas in conscious rats no insulin-resistance was found (Kruszynska & McCormack, 1989). The present study confirms that in physiological conditions the liver of fasting rats is indeed insulin-resistant. Moreover, the liver insulin-resistance is not linked to a higher plasma glucagon concentration.

A small but detectable increase in hepatic glycogen 1 h after the glucose load was seen only in the starved rats. This might be due to the fact that hepatic glycogen concentration was not totally depleted in the liver of post-absorptive rats (Table 1) and that hepatic glycogen synthesis is more efficient when an active gluconeogenesis is present (Newgaard *et al.*, 1983, 1984).

The absence of a high rate of glycogen synthesis after a glucose load in starved rats might seem unusual, and could cast some doubts on the portal-catheter model. However, in the studies by

Holness *et al.* (1986) and Holness & Sugden (1987), 180–220 g rats maintained on a standard chow diet, starved for 48 h and then receiving 3.6 mg of glucose/g body wt. (4.8 mg/g body wt. in our study), show no glycogen synthesis during the first 30 min and a modest increase, 3–5 mg/g liver wet wt., after 1 h, which compares favourably with our value (3.1 mg/g liver wet wt.). Similarly, in the study by Pallardo & Williamson (1989), 200 g rats starved for 48 h and refed with chow diet had an increase of 3.9 mg/g liver wet wt. in 90 min. Finally, the fact that rats bearing a portal catheter are able to accumulate substantial amounts of glycogen 3 h after a single glucose load (the present study) and have normal post-prandial hepatic glycogen concentrations (Smadja *et al.*, 1988) clearly shows that the portal catheter does not interfere with hepatic glucose metabolism.

Glucose utilization during the glucose load

The rate of glucose utilization (*Rd*) tends to be higher in starved rats than in post-absorptive rats, despite the presence of a similar peripheral blood glucose concentration and similar portal insulin levels. Moreover, determination of peripheral plasma insulin 40 min after the load indicated lower plasma insulin concentrations in starved rats (100 ± 18 μ -units/ml) than in post-absorptive rats (150 ± 20 μ -units/ml) ($P < 0.05$). This suggests that peripheral tissues of starved rats are not insulin-resistant (Kruszynska & McCormack, 1989) and are possibly even hypersensitive to insulin. Studies *in vitro* (Le Marchand-Brustel & Freychet, 1979; Brady *et al.*, 1981; Stirewalt *et al.*, 1985) and *in vivo* (Pénicaud *et al.*, 1985) have indeed shown in skeletal muscles of starved rodents that insulin-sensitivity was increased as a consequence of an increased insulin binding and a higher effectiveness of insulin on post-binding steps. This increased sensitivity is underlined by the fact that a larger amount of glycogen is deposited in the diaphragm and the soleus of starved rats (Table 1), two oxidative muscles which have been shown to be more sensitive to the action of insulin than are less oxidative muscles such as the extensor digitorum longus (James *et al.*, 1985).

Conclusion

The response to a gastric glucose load in post-absorptive and starved rats is summarized in Table 2, where glucose absorption, intestinal lactate production, hepatic glucose production and peripheral glucose utilization, integrated over the 60 min experiment and compared with a 60 min basal period, are given. From these data, it is clear that a short-term fasting profoundly alters glucose-stimulated homeostatic mechanisms. The non-suppressed gluconeogenesis in the starved rat, backed up by the increased intestinal lactate production, allows provision of glucose for the glucose drain by peripheral tissues linked to replenishment of glycogen stores.

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