Effects of fasting on tissue glucose utilization in conscious resting rats

Major glucose-sparing effect in working muscles

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The effects of fasting on glucose metabolism in the conscious resting rat were studied. Fasting decreased whole-body glucose utilization by 40 %. The fast induced a decrease in glucose utilization in muscles which are constantly working even in the resting state, i.e. heart, diaphragm and postural muscles. No modification was observed in other tissues.

INTRODUCTION

During fasting, a number of hormonal and metabolic adaptations occur to stimulate endogenous glucose production through glycogenolysis and gluconeogenesis and to spare glucose for tissues which have an absolute requirement for this substrate. These adaptations include a decreased plasma insulin concentration and increased concentrations of lipid-derived substrates, i.e. non-esterified fatty acids and ketone bodies. The decreased plasma insulin could theoretically decrease glucose utilization in insulin-dependent tissues (liver, muscles and adipose tissue essentially), whereas the increased plasma non-esterified fatty acids and ketone bodies could allow sparing of glucose in tissues which are well equipped for their oxidation. The glucose-sparing effect of lipid-derived substrates has been clearly demonstrated *in vitro* in heart and diaphragm (Randle *et al.*, 1964), but remains controversial in skeletal muscles (Zorzano *et al.*, 1985). Whereas Rennie & Holloszy (1977) have shown that oleate inhibited glucose uptake in perfused rats hindquarters, Berger *et al.* (1976) and Ruderman *et al.* (1980) did not observe any effect of elevated concentrations of non-esterified fatty acids or ketone bodies on glucose utilization in similar preparations. The difficulty in showing a clear effect of non-esterified fatty acids on glucose utilization may be linked to the experimental conditions used (poor tissue oxygenation, type of muscle studied). Moreover, the tissues quantitatively important *in vivo* in the sparing of glucose during starvation have never been precisely determined. The only work of which we are aware (Ferré *et al.*, 1985) was performed on animals anaesthetized with pentobarbital and showed a modest decrease in glucose utilization in skeletal muscles and white adipose tissue. Since pentobarbital anaesthesia considerably decreases glucose utilization in postural red muscles (Pénicaud *et al.*, 1987), i.e. in those that could be the most affected by lipid-derived substrate oxidation, the effect of starvation on glucose utilization by these tissues could have been considerably underestimated.

The aim of the present work was thus to compare glucose utilization in various tissues in conscious post-absorptive and 48 h-starved rats.

MATERIALS AND METHODS

Animals

Female Wistar rats (10 weeks old) were housed individually in plastic cages with light on from 07:00 to 19:00 h and an ambient temperature of 23 °C. They had free access to water and chow pellets (content, in terms of energy: carbohydrate 62 %, fat 5 %, protein 33 %). Under light diethyl ether anaesthesia, a catheter was implanted in the right heart atrium via the jugular vein and driven subcutaneously to the top of the skull. The catheter was fixed with cement (Texton; S. S. White, Paris, France) and occluded with a metal plug after a flush of heparinized (200 i.u./ml) saline solution. Animals were allowed to recover until their weight reached its pre-operative value. This took 5–7 days.

Then the food was removed at 10:00 h and the animals were studied 48 h later (fasted rats). The control group was studied at 10:00 h, i.e. in the post-absorptive state, after food had been removed 3 h previously. At 1 h before the beginning of the experiments, the catheter was lengthened to reach injection and blood-sampling devices.

Measurement of glucose turnover rate

A tracer dose of [3-³H]glucose (30 µCi; CEA, Saclay, France) in 200 µl of 0.9 % NaCl was injected as a bolus through the jugular catheter in both groups of rats. The catheter was then immediately flushed with 500 µl of 0.9 % NaCl. Blood samples (50 µl) were then taken through the same catheter at 1, 3, 5, 10, 20, 40, 75 and 90 min after the [3-³H]glucose injection, and were immediately deproteinized in 250 µl of ZnSO₄ (2.51 %, w/v) and 250 µl of Ba(OH)₂ (2.63 %, w/v) and centrifuged (2 min, 16000 g). Glucose specific radioactivity was determined as follows: glucose concentration was

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measured in the supernatant with a glucose oxidase kit (Boehringer, Meylan, France). Another sample of the supernatant was evaporated to dryness to remove $^3\text{H}_2\text{O}$, redissolved in 200 μl of water, and $[3\text{-}^3\text{H}]\text{glucose}$ was determined in a liquid-scintillation spectrometer (β eta-Matic I; Kontron, Vélizy, France). The glucose turnover rate was calculated as the ratio:

$$[3\text{-}^3\text{H}]\text{Glucose injected} / \int_0^\infty \text{glucose specific radioactivity}$$

At time 90 min, the specific radioactivity of glucose was less than 5% of the 1 min value in both groups of rats. It was extrapolated to infinity by a semi-logarithmic plot.

Measurement of glucose utilization index on individual tissues

A tracer dose of 2-deoxy $[1\text{-}^3\text{H}]\text{glucose}$ (30 μCi ; CEA) in 200 μl of 0.9% NaCl was injected as a bolus through the jugular catheter in both groups of rats. The catheter was then immediately flushed with 500 μl of 0.9% NaCl. Blood samples (50 μl) were then taken through the same catheter at time 1, 3, 5, 10, 20, 40 and 60 min after the 2-deoxy $[1\text{-}^3\text{H}]\text{glucose}$ injection. Blood and tissue samples were treated as previously described (Ferré *et al.*, 1985). The 2-deoxyglucose method in fact measures the rate of glucose phosphorylation in a given tissue. Since glucose utilization is in steady state during the course of the experiments, and since no glucose can be utilized without prior phosphorylation in the tissues studied, glucose phosphorylation rate is equivalent to glucose utilization rate. Glucose utilization by each tissue was calculated as described by Ferré *et al.* (1985), except that the value found was not corrected by the discrimination factor (lumped constant) for 2-deoxyglucose in glucose metabolic pathways. Therefore the results should be taken as an index of glucose utilization rather than an absolute value (Hom *et al.*, 1984). Results are expressed per mg wet wt., since the weights and protein contents of the tissues studied are only marginally affected by 48 h starvation (Goodman & Ruderman, 1980).

Plasma insulin and circulating substrate concentrations

At the end of the 2-deoxy $[1\text{-}^3\text{H}]\text{glucose}$ experiments, a larger blood sample was taken. A portion was deprotein-

ized in 6% (w/v) HClO_4 and centrifuged (2 min, 16000 g). The neutralized supernatant was used for the determination of blood ketone bodies (Williamson *et al.*, 1962). Another blood sample was immediately centrifuged at 4°C, and the plasma was kept frozen for subsequent determination of insulin with a radioimmunoassay kit, with an antibody against human insulin and human insulin as standard (Oris Industrie, Gif-sur-Yvette, France). Non-esterified fatty acids were also determined in the plasma with an enzymic kit (NEFA C; Biolyon, Dardilly, France).

Statistics

Results are presented as means $\pm$ S.E.M. for the numbers (n) of determinations. Statistical significance was determined by Student's unpaired t test.

RESULTS AND DISCUSSION

Body weights

At the time of the experiment, mean body weight was 223 ± 25 g ($n = 11$) in control rats and 189 ± 4 g ($n = 11$) in 48 h-fasted rats.

Glucose turnover studies

Whole-body glucose turnover rate in the post-absorptive conscious rats was 17.6 ± 1.2 mg/min per kg body wt. (Table 1). After a fast of 48 h, the glucose turnover rate was 11 ± 1 mg/min per kg. Studies in pentobarbital-anaesthetized fed and 48 h-fasted Wistar rats have been performed in similar experimental conditions (Ferré *et al.*, 1985). In those studies, the glucose turnover rate was 8.4 mg/min per kg in fed anaesthetized rats and 5.2 mg/min per kg in 48 h-fasted anaesthetized rats. Thus, in conscious rats, a fast of 48 h induces a decrease in glucose turnover rate of 6.6 mg/min per kg, whereas glucose turnover decreases by only 3.2 mg/min per kg in anaesthetized rats. This suggests that an important part of the glucose-sparing effect associated with fasting occurs in tissues in which glucose utilization is affected by anaesthesia.

Glucose utilization index in individual tissues

In an attempt to determine which tissues were involved in the decrease in glucose utilization observed in the fasting state, the 2-deoxyglucose technique (Ferré *et al.*, 1985) was used in post-absorptive control and fasted conscious rats. Glucose utilization indexes were determined in brain, white adipose tissue and muscles (Table 2).

Muscles. Muscles were classified into two categories, according to their activity (Pénicaud *et al.*, 1987): (1) muscles which are working in conscious resting rats, i.e. heart, diaphragm and postural muscles (soleus and adductor longus); (2) muscles which do not work in conscious resting rats (tibialis anterior, epitrochlearis, extensor digitorum longus). It should be remembered that working muscles are mainly composed of oxidative fibres, whereas resting muscles are primarily composed of glycolytic fibres (Ariano *et al.*, 1973; Green *et al.*, 1984).

In the post-absorptive state, glucose utilization was much higher in working muscles than in non-working muscles, confirming previous results (Pénicaud *et al.*, 1987). After a fast of 48 h, glucose utilization was

Table 1. Glucose kinetics, plasma insulin, plasma non-esterified fatty acid and blood ketone-body concentrations in post-absorptive control or 48 h-fasted rats

Results are means $\pm$ S.E.M. for six determinations; * difference statistically significant ($P < 0.01$) when compared with control rats.

Rats ...	Control	Fasted
Blood glucose (mg/ml)	0.96 ± 0.04	$0.70 \pm 0.03^*$
Glucose turnover rate (mg/min per kg)	17.6 ± 1.2	$11 \pm 1^*$
Plasma insulin ($\mu\text{units/ml}$)	25 ± 5	$6.3 \pm 1.4^*$
Blood β -hydroxy-butyrate (mM)	0.20 ± 0.02	$1.54 \pm 0.08^*$
Blood acetoacetate (mM)	0.13 ± 0.02	$0.41 \pm 0.04^*$
Plasma non-esterified fatty acids (mM)	0.26 ± 0.04	$0.69 \pm 0.05^*$

Table 2. Glucose utilization index in tissues of post-absorptive control or 48 h-fasted conscious rats

Results are means $\pm$ S.E.M. for six determinations: *difference statistically significant ($P < 0.01$) when compared with control rats.

Rats ...	Glucose utilization index (mg/min per g wet wt.)	
	Control	Fasted
Working muscles		
Soleus	44.0 $\pm$ 7.5	8.6 $\pm$ 0.8*
Adductor longus	28.0 $\pm$ 3.0	7.5 $\pm$ 0.8*
Diaphragm	36.5 $\pm$ 4.3	14.9 $\pm$ 3.5*
Heart ventricle	83.3 $\pm$ 12	8.2 $\pm$ 1.5*
Non-working muscles		
Extensor digitorum longus	5.3 $\pm$ 0.7	3.9 $\pm$ 0.8
Tibialis anterior	5.9 $\pm$ 1.0	4.1 $\pm$ 1.0
Epitrochlearis	6.4 $\pm$ 1.0	4.0 $\pm$ 0.8
Other tissues		
White adipose tissue (periovarian)	1.6 $\pm$ 0.2	1.2 $\pm$ 0.8
Brain	26.1 $\pm$ 1.0	24.5 $\pm$ 1.7

considerably decreased in working muscles, whereas it was only slightly decreased in non-working muscles.

The decrease in glucose utilization observed in working muscles could be related first to the lower plasma insulin concentrations found in the fasted rats (Table 1). However, studies *in vitro* have shown that insulin-sensitivity was increased in skeletal muscles of fasted rodents, as a consequence of an increased insulin binding and a higher efficiency of insulin on post-binding steps (Le Marchand-Brustel & Freychet, 1979; Brady *et al.*, 1981; Stirewalt *et al.*, 1985). If this occurs also *in vivo*, it will minimize the effect of decreased plasma insulin on glucose utilization in these muscles.

Furthermore, studies *in vivo* (James *et al.*, 1985), like those *in vitro* (Challiss *et al.*, 1983), have shown that changes in insulin concentrations from 5 to 50 μ units/ml produce minor effects on glucose utilization in skeletal muscles. These observations suggest that the decreased plasma insulin concentrations are not solely responsible for the lower glucose utilization observed in working muscles of fasted rats. In that range of plasma insulin concentrations, insulin action on muscles could be indirect, by increasing the rate of adipose-tissue lipolysis and the availability of lipid-derived substrates. Indeed, non-esterified fatty acids, acetoacetate and β -hydroxybutyrate are considerably increased after a fast of 48 h (Table 1). The finding that glucose utilization is particularly decreased in muscles which possess a high capacity for non-esterified fatty acid and ketone-body utilization suggests that the glucose-fatty acid cycle (Randle *et al.*, 1964) is indeed operative, not only in heart and diaphragm, but also in skeletal postural muscles. That postural skeletal muscles are implicated in the decrease in glucose utilization observed during fasting is substantiated by the finding that, in a situation where these muscles are not working (anaesthesia) (Pénicaud *et al.*, 1987), the decrease in glucose turnover rate induced by fasting is much less (see above) (Ferré *et al.*, 1985).

From the studies initiated by Randle *et al.* (1964), it can be inferred that muscles which possess a high capacity for oxidizing fatty acids and ketone bodies, i.e. those composed of fast- and slow-twitch oxidative fibres, can participate in glucose sparing when concentrations of lipid-derived substrates are elevated. From a quantitative point of view, however, it is clear that the glucose-sparing effect is more efficient in muscles which utilize large amounts of glucose, i.e. working muscles. In the resting state, those muscles are heart, diaphragm and skeletal postural muscles such as soleus and adductor longus.

It might be possible that in other physiological situations (exercise) muscles composed of fast-twitch oxidative fibres such as the extensor digitorum longus can contribute to the glucose-sparing effect observed during starvation. Indeed, Rennie *et al.* (1976) have shown a glucose-sparing effect of increased plasma non-esterified fatty acid concentrations on glycogen depletion in fast-twitch oxidative muscles during exercise.

White adipose tissue. Unlike in muscles, a decrease in plasma insulin from 25 to 6 μ units/ml is likely to affect glucose utilization in white adipose tissue (Green & Newsholme, 1979; Thomas *et al.*, 1979; Challiss *et al.*, 1983). Moreover, lipogenesis is inhibited in white adipocytes of fasted rats (Owens *et al.*, 1979). Thus one could expect that a 48 h fast would decrease the glucose utilization rate in white adipose tissue. However, in our experiments, glucose utilization in periovarian white adipose tissue remains unaltered by a 48 h fast. Even if we take into account the fact that the lipid content decreases by 20% after 48 h starvation in the adipose tissue (results not shown), the difference does not reach significance. Since the rate of glucose utilization in adipose tissue is very low even in the fed state, we are at the limit of reliability of the technique used. This might conceal subtle differences in glucose utilization in adipose tissue after a 48 h fast.

Brain. In this study, as well as in studies by Hawkins *et al.* (1971) and Ruderman *et al.* (1974), glucose utilization by the brain was not modified after a fast of 48 h. Although Ferré *et al.* (1985) have observed a small decrease in glucose utilization in the brain of 48 h-fasted anaesthetized rats, they have calculated that brain glucose utilization represents a marginal fraction of total glucose utilization (5% in the fed state and 4% in the fasted animals). Thus the brain cannot take an important part in the glucose-sparing effect observed in the fasted rats.

Conclusion

This study shows that an important glucose-sparing effect occurs in resting conscious fasted rats. It takes place principally in working muscles in which glucose utilization is normally elevated (i.e. heart, diaphragm, and postural muscles).

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