EFFECT OF FASTING ON GLUCOSE RECYCLING IN RATS

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1. Introduction

The rate of glucose recycling through lactate, known as the Cori cycle (glucose → lactate → glucose), has been estimated in vivo by the ^{14}C atoms randomization in the glucose molecule after an injection of [1-^{14}C]- or [6-^{14}C]glucose and is generally given as a fraction of glucose turnover [1–3]. It should be pointed out that glucose turnover determined with [^{14}C]glucose (a reversible isotopic tracer) is underestimated because of the recycling phenomenon and represents actually the apparent rate of glucose turnover [4–6]. Recently the use of [2-^{3}H]glucose (an irreversible non recycling tracer) have been advocated to measure glucose turnover which represents more precisely the true rate of glucose turnover [4–6]. The difference between these two rates may be considered as the best estimate of total glucose carbon recycling (glucose → X → glucose).

In fed rats, glucose recycling measured by the randomization technique is 12% of the apparent rate of glucose turnover [2] but it can reach 30–50% after a 15–18 h period of fast whereas the apparent glucose turnover rate decrease [1,2]. However there are no data concerning the effect of fasting on the true rate of glucose turnover and on total glucose recycling in rats except those reported by Dunn et al. 1967 [7] who did not find any difference between fed and fasted animals. The aim of the present work was to study the effect of fasting and refeeding on total glucose recycling. It is shown that glucose recycling decreases during fasting.

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2. Materials and methods

White male Sherman rats were maintained on a commercial standard diet (R 98 CNRZ 45000 Orléans la Source France) until the fasting period. Five groups of rats were studied: fed or controls (C), 24 h fasted (F_{24}), 48 h fasted (F_{48}), 72 h fasted (F_{72}), and 24 h after refeeding following a 72 h fasting period (FR). The variation of body weight during such fasting periods was recorded in preliminary experiments and the duration of the fast was established for each animal according to its pre-fast body weight so that the experiments could be performed on a series of animals of similar weight (300–340 g). Each experiment was performed in the morning at 8.30 a.m. The experimental procedure and set up has been previously described in full details [8,9]. After an equilibrating period of 30 min following anaesthesia and surgery which allows a normal acid-base and oxygenation status of the animals, a mixture of [2-^{3}H]- and [U-^{14}C]glucose was infused into a jugular vein at a constant rate during 120 minutes. A priming dose was injected at the beginning of the perfusion. The priming dose-injection rate ratio was 80. Arterial blood (0.2–0.3 ml) was sampled directly in weighed tubes and $^{14}CO_2$ in expired gases was trapped in hyamine hydroxide during two minutes collecting periods for counting.

2.1. *Calculations*

In steady state conditions, the rate of glucose turnover (mg·min^{-1}) is obtained by the ratio of the rate of tracer infusion (nCi.min^{-1}) to the mean glucose specific activity (nCi·mg^{-1}). The [2-^{3}H]glucose tracer leads to the measurement of the true rate of

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glucose turnover and the [U-^{14}C]glucose tracer leads to that of the apparent rate of glucose turnover. The difference between these two rates is taken as the best estimate of total glucose recycling which can also be expressed as a fraction of the true rate of glucose turnover. The fraction of the apparent rate of glucose turnover oxidized is computed as the ratio of the $^{14}CO_2$ activity ($nCi \cdot min^{-1}$) in expired gases [9] to the rate of [U-^{14}C]glucose infusion ($nCi \cdot min^{-1}$). Then the rate of oxidation ($mg \cdot min^{-1}$) is obtained from this value and the apparent rate of glucose turnover. The results are expressed per kg of metabolic weight (body weight to the power 0.75 [9]).

3. Results

Table 1 shows body weight and respiratory parameters measured during the experiments. For the same pHa (7.41 ± 0.01), oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) decrease during fasting and the respiratory quotient is reduced from 0.81 to 0.70. After refeeding these values return to control conditions. Table 1 shows also the results concerning glucose metabolism. Glycaemia and the rates of glucose turnover and oxidation decrease during fasting. The true rate of glucose turnover is decreased by 61% after 72 h of starvation and the apparent rate of glucose turnover is reduced by 53% for the same period. As the percentage of glucose turnover oxidized decrease from a value of 51% in controls to 31.5% after 72 h of fast, the reduction of the glucose oxidation rate is more important than that of the apparent rate of glucose turnover (−72% after 72 h of fast). After refeeding the true rate of glucose turnover is lower (−8%) and the rate of glucose oxidation is higher (+ 9%) than in control animals whereas the apparent rate of glucose turnover does not differ from that of fed animals. Fig.1 represents the effect of fasting on the rate of glucose recycling expressed either in $mg \cdot min^{-1}$ per $kg^{0.75}$ or as a fraction of the true rate of glucose turnover. Both representations show an important decrease of approx. 75% for the rate of recycling or 42% when expressed as a fraction. After refeeding glucose recycling is lessened when compared to that of controls (−24%).

Table 1
Effect of fasting and refeeding on respiratory parameters and glucose metabolism in rats

Condition	Body weight	$\dot{V}O_2$	$\dot{V}CO_2$	R_Q	[G]	Glucose turnover rate $_3$H	Glucose turnover rate ^{14}C	Glucose turnover oxidized	Glucose oxidation rate
	g	$ml \cdot min^{-1}$			mM	$mg \cdot min^{-1}$ per $kg^{0.75}$		%	$mg \cdot min^{-1}$ per $kg^{0.75}$
C	328	4.8	3.9	0.81	6.7	10.7	6.6	48.2	3.2
(n = 10)	8	0.1	0.1	0.02	0.2	0.3	0.3	1.0	0.1
F_{24}	323	4.6	3.3	0.72	5.1	5.2	3.5	31.9	1.1
(n = 5)	7	0.1	0.05	0.02	0.4	0.4	0.3	1.5	0.1
F_{48}	311	4.0	2.8	0.70	4.9	4.9	3.5	28.7	1.0
(n = 4)	13	0.1	0.05	0.005	0.3	0.1	0.1	0.7	0.1
F_{72}	327	3.9	2.8	0.70	4.8	4.2	3.1	29.8	0.9
(n = 4)	11	0.05	0.05	0.005	0.3	0.2	0.2	0.8	0.1
FR	325	4.7	4.0	0.85	7.3	9.8	6.7	51.7	3.5
(n = 4)	8	0.1	0.1	0.01	0.2	0.3	0.2	1.7	0.2

Mean ± SEM; n = number of experiments.

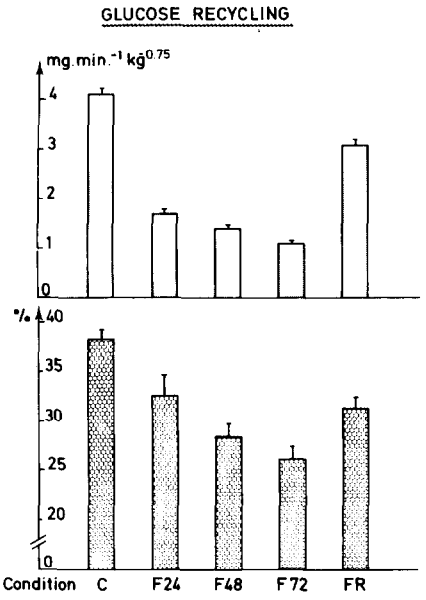

Fig.1. Effect of fasting and refeeding on glucose recycling. The rate of glucose recycling is the difference between the true and the apparent rates of glucose turnover (upper panel) and can be expressed as a fraction of the true rate of glucose turnover (lower panel).

4. Discussion

The observed decrease of the apparent rate of glucose turnover confirms well known data [2,10,13] and can be explained by the reduction of glycolysis occurring during fasting due to the decrease of glycogen stores and to the reduction of insulin concentration. The reduction of the rate of glucose oxidation is similar with other findings [10,12] and is an application of the 'glucose-fatty acid cycle' in vivo through the inhibition of pyruvate dehydrogenase by the increase of free fatty acids utilization [14,15] and by the decreased insulin concentration [11,17]. In these conditions, pyruvate carboxylase and PEP-carboxykinase are stimulated and gluconeogenesis is enhanced [16,18].

The reduction of the true rate of glucose turnover is at variance with data reported by Dunn et al. (1967) [7] who did not observe any change after 24 h of starvation in rats but it is to note that these authors used [6-^{3}H]glucose which cannot be considered as a true irreversible tracer [6]. The reduction of total glucose recycling during fasting has not been reported up to now. This fact may be surprising as it has been shown that glucose recycling measured with the randomization technique increases if expressed as a fraction of the apparent rate of glucose turnover [1,2]. As stated in the introduction this methodology leads to the estimation of the Cori cycle (glucose → lactate → glucose) but does not take into account the successive futiles cycles occuring in the liver and the kidney [19]. We have recently reported that in fed rats the Cori cycle represents only 20–30% of total glucose recycling [20]. Thus it is possible that during fasting there is no variation of the Cori cycle but that the decrease of the overall glucose recycling is due to the reduction or disparition of these futile cycles as, in these conditions of fasting, the flux of substrates is more or less completely oriented to gluconeogenesis in the liver and the kidney. This is supported by the fact that when using a figure of 50% for the apparent rate of glucose turnover recycled after a 24 h fasting period as shown by others [1,2] the rate of the Cori cycle estimated in our experimental conditions would be $3.7 \times 0.5 = 1.85$ mg·min^{-1} per kg$^{0.75}$, which is similar to the rate of total glucose recycling (see fig.1). As it has been shown in a previous study [21] that the rate of glucose synthesis from lactate decreases during fasting, this interpretation of our results should be confirmed by the direct measurement of the Cori cycle during fasting.

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