EFFECT OF FASTING ON THE CORI CYCLE IN RATS

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1. Introduction

In a recent study, we have reported decreased lactate turnover and oxidation rates in fasted rats [1]. Concomitantly, the rate of total glucose recycling (Glucose→X→Glucose: TGR), estimated as the difference between the true ([2-^{3}H]glucose) and the apparent ([U-^{14}C]glucose) rates of glucose turnover, was lowered after 24, 48 and 72 h of fast [2]. In normal fed rats, the Cori cycle (Glucose→Lactate→Glucose: CC) represents 20 to 30% of TGR [3]. Results published by others on the variation of CC during fasting in rats are conflicting [4–8]; these discrepancies being due either to different technical approaches or to a confusion between TGR and CC. Therefore, the aim of the present report is to estimate the fractionnal participation of CC to TGR in fasted rats. To do so, previous data [1,2] were tested with a more sophisticated analytical method [3]. This study shows that during fasting CC is only slightly reduced and accounts entirely for TGR.

2. Material and methods

White male Sherman rats were maintained on a commercial standard diet (R 98. CNRZ. 45 000 Orleans-la-Source, France) until the fasting period. Five groups of animals 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 animals had free access to water until the experiments which were performed in the morning at 8:30 AM. The experimental procedure and set up has been previously described in full details [1,3,9]. After an equilibrating period of 30 min following anaesthesia and surgery which allows a normal blood acid-base and oxygenation status of the animals, a solution of [U-^{14}C]lactate or of [2-^{3}H]- and [U-^{14}C]glucose was infused at a constant rate during 120 min. Arterial blood (0.2–0.3 ml) was sampled directly in weighed tubes containing perchloric acid, then lactate and glucose specific activities were determined. $^{14}CO_2$ in expired gases was trapped in hyamine hydroxide during two-minute collecting periods for counting.

2.1. *Calculations*

In steady state conditions, the rate of glucose or lactate turnover (mg·min^{-1}) is obtained by the ratio of the rate of tracer infusion (nCi·min^{-1}) to the mean glucose or lactate specific activity (nCi·mg^{-1}). The [2-^{3}H]glucose tracer leads to the measurement of the true rate of glucose turnover (RGT) and the [U-^{14}C]glucose tracer to that of the apparent rate of glucose turnover (RG). The difference between these two rates is taken as the best estimate of total glucose recycling [10–12]. The fraction RG or of the lactate turnover oxidized is calculated as the ratio of the $^{14}CO_2$ activity (nCi·min^{-1}) in expired gases [9] to the rate of [^{14}C]glucose or [^{14}C]lactate infusion (nCi·min^{-1}). Then the rates of oxidation (mg·min^{-1}) are obtained from these values and the respective turnover rates.

Glucose-lactate interconversion rates are calculated according to the two-compartment model of Depocas and De Freitas [13] as recently reported [3]. Lactate specific activity derived from glucose and

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glucose specific activity derived from lactate are measured in [^{14}C]glucose and [^{14}C]lactate experiments in different animals. In these experiments it is assumed that the metabolic state of the animals is comparable and the results obtained in either type of tracer infusion are used to calculate the rates of glucose–lactate interconversions in an ideal single animal. This technique leads however to a potential maximal relative error of about 25%. Therefore results will be given only as means of pooled data. The rate of glucose conversion to lactate is given either in absolute value or as a fraction of RG (A). Similarly, lactate conversion to glucose is also given in absolute value or as a fraction of lactate turnover (B). The fractional value of CC is A × B. The product of this fraction to RGT or RG leads to a maximal or a minimal estimation of CC, but for the clearness of this report only the maximal value will be presented. (for discussion see [3]). Absolute rate values are calculated per kg metabolic weight (body weight to the power 0.75) as previously discussed [9,14].

3. Results

Data for body weight, respiratory parameters and rates of glucose and lactate turnover obtained in this series of experiments have been reported in earlier communications [1,2]. For the sake of clearness, values of glucose and lactate turnover are shown again in table 1 (Part A). Part B in table 1 gives values

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

	n (Lactate perfusion/glucose perfusion)	Condition	C (10 + 10)	F_{24} (4 + 5)	F_{48} (3 + 4)	F_{72} (3 + 4)	FR (3 + 4)
(A)	[b]Glucose turnover rate (3H)	mg·min^{-1} per kg$^{0.75}$	10.7	5.2	4.9	4.2	9.8
	(RGT)		0.3[a]	0.4	0.1	0.2	0.3
	[b]Glucose turnover rate (^{14}C)	mg·min^{-1} per kg$^{0.75}$	6.6	3.5	3.5	3.1	6.7
	(RG)		0.3	0.3	0.1	0.2	0.2
	[c]Lactate turnover rate	mg·min^{-1} per kg$^{0.75}$	4.1	2.4	2.2	1.8	4.3
	(RL)		0.1	0.1	0.2	0.1	0.4
(B)	Rate of glucose conversion to lactate	mg·min^{-1} per kg$^{0.75}$	3.1	1.8	1.7	1.6	2.8
	Rate of lactate conversion to glucose	mg·min^{-1} per kg$^{0.75}$	1.2	1.0	1.2	0.9	0.8
(C)	[b]Glucose turnover oxidized	% of RG	48	32	29	30	52
	Glucose going to lactate	% of RG	47	51	49	52	42
	Glucose coming from lactate	% of RG	18	29	34	29	12
(D)	[c]Lactate turnover oxidized	% of RG	51	43	35	32	53
	Lactate going to glucose	% of RG	29	42	55	50	19
	Lactate coming from glucose	% of RG	76	75	77	88	65

[a]Mean ± SEM. [b]Already published [2]. [c]Already published [1].

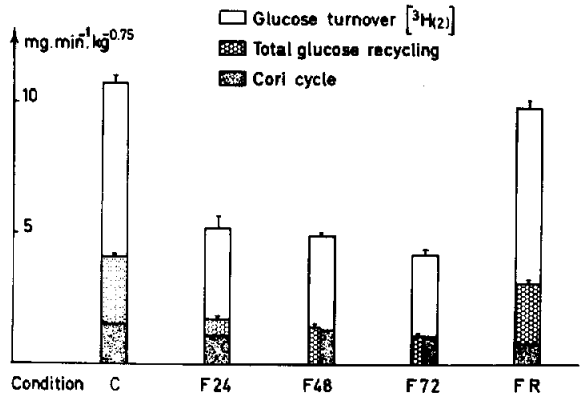

Fig.1. Effect of fasting and refeeding on the rates of true glucose turnover, total glucose recycling and the Cori cycle in rats.

for glucose–lactate interconversions rates. The rate of glucose conversion to lactate is decreased by 40 to 50% during fasting whereas the rate of lactate conversion to glucose is reduced by only 15 to 25%. Part C in table 1 shows these rates as fractions of RG and part D as fractions of lactate turnover rate. The respective fraction of glucose and lactate turnover oxidized are also presented in table 1. In fractional terms, lactate and glucose oxidation decrease, glucose conversion to lactate does not vary and gluconeogenesis from lactate is almost doubled. It is noteworthy that refeeding produces a reduction of glucose production from lactate. Fig.1 shows the variations of RGT, TGR and CC in this series. Whereas TGR is decreased during fasting by 60–75%, CC is diminished by only 15–30% which explains that after 48 and 72 h of fast CC accounts for all TGR. In faster-refed animals, CC is lowered to half its control value.

4. Discussion

The main feature of the present study is the observation that CC accounts completely for TGR during fasting. TGR is considerably lowered whereas CC is maintained or only slightly decreased during fasting and its fractional participation to glucose production is increased from 10–20% in fed to 25–35% in fasted rats.

The variation of CC during fasting may appear at variance with the few results published by others in rats. For example, Baker et al. [4,5], using a compartmental analysis of [^{14}C]glucose specific activity in blood after the injection of [U-^{14}C]glucose, reported that 45–50% of glucose turnover are recycled in fed conditions and that this rate is decreased by a factor 2 or 3 and represents 30% of glucose turnover after a 16–21 h fasting period. On the opposite Von Holt et al. [6], from the ^{14}C-atoms randomization in the glucose molecule after an injection of [6-^{14}C]glucose, calculated that CC represents 12% of glucose turnover in fed animals and that this rate is doubled and represents 50% of glucose turnover after 15 h of fast. With the same methodology, Ashmore et al. (1961) [7] reported that no more than 10% of glucose are recycled in 24 h fasted animals. Lastly, Dunn et al. [8], estimating CC as the difference between the rates of glucose turnover measured with [6-^{3}H] and [6-^{14}C] glucose, reported that CC represents 25% of glucose turnover in fed animals and does not vary after 24 h of food deprivation. As stated above these scattered values are likely due to methodological and/or technical differences. Thus, Baker's values for glucose recycling represent more probably TGR than CC even though [^{14}C]glucose tracer was used. In fact, their data are close to that reported for TGR in the present study. The results of Ashmore et al. [7] and of Von Holt et al. [6] are not in agreement with ours except for the increase of the fractional participation of CC to glucose turnover observed during fasting for the latter. The stability and the value of CC during fasting reported by Dunn et al. [8] agree with our observation but the stability of glucose turnover reported in these conditions is not in accordance with common observations [2].

As discussed elsewhere [3], the two-compartment model used in this study probably underestimates lactate conversion to glucose in fed rats. However the identity found between CC and TGR after 48 and 72 h of fast suggests that the estimation of gluconeogenesis from lactate is satisfactory in these conditions. Nevertheless, when using the data presented in table 1 (Part D) it is apparent that only 80–90% of lactate utilization are explained by oxidation and gluconeogenesis during fasting. If we assume that the difference between the rate of

lactate turnover and the rate of lactate oxidation represents the rate of gluconeogenesis from lactate there is a 15 to 40% underestimation of this rate in these conditions. It is more difficult to discuss the data obtained in the control and the fasted-refed animals as in these conditions lactate can be used in other pathways than oxidation and gluconeogenesis such as glycogenesis and/or lipogenesis [15,16].

An important point from this study is the identity found between TGR and CC during fasting. This would indicate that the important reduction of TGR during fasting is due to the suppression of the successive futile cycles occuring during glycolysis in the liver and the kidney [17]. This is not surprising as in these conditions, the flux of substrates is more or less completely oriented towards gluconeogenesis in the liver and the kidney. These futile cycles are important in fed rats and increase in fasted-refed animals playing certainly an important role in the regulation of metabolism [18].

The uncertainties regarding the interpretation of isotopic data in the study of intermediary metabolism in vivo lead to two questions: (i) What is the extent of lactate recycled through a pyruvate-dicarboxylic acids-phosphoenolpyruvate cycle in the liver and the kidney [19] especially in control and fasted-refed animals? (ii) What is the relative importance in vivo of the 'crossing-over' of carbons from respiration of gluconeogenesis and vice-versa in these organs [20]?

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