

EFFECT OF FASTING ON THE RATES OF LACTATE TURNOVER AND OXIDATION IN RATS

A. FREMINET*, L. LECLERC, M. GENTIL and C. POYART
INSERM U 27, 42, rue Desbassayns de Richemont, 92150 Suresnes, France

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1. Introduction

The decrease in the rates of glucose turnover and oxidation during fasting is well documented [1–5]. It was of interest to know if the same phenomenon occurred for the rates of lactate turnover and oxidation and the extent of such a decrease so that the contribution of lactate to gluconeogenesis *in vivo* could be estimated in these conditions. It has been reported that lactate turnover as measured with the [^{14}C]lactate perfusion technique decreases during fasting in sheep [6] but does not vary in obese human patients [7]. Recently, the study of the effects of functional hepatectomy and of insulin on blood lactate concentration led to the conclusion that the flux of lactate from peripheral tissues to the liver is doubled after a 24 h fasting-period in rats [8]. Because of these conflicting results, probably due to methodological and species differences, the effect of fasting on the rates of lactate production have been measured in rats. This work shows: a reduction of the rates of lactate turnover and oxidation; a larger decrease in the rate of lactate oxidation compared to the rate of lactate turnover which may be assigned to a greater lactate conversion to glucose if the latter is expressed as a fraction of the rate of lactate turnover.

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.

*To whom correspondence should be addressed.

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 (280–340 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 detail [9,10]. After an equilibrating period of 30 min following anaesthesia and surgery which allows a normal acid-base and oxygenation status of the animals, L-[U- ^{14}C]lactate was infused into a jugular vein at a constant rate during 120 min. A priming dose was injected at the beginning of the perfusion. The priming dose/injection rate ratio was 10. Arterial blood (0.2–0.3 ml) was sampled directly in weighed tubes and $^{14}\text{CO}_2$ in expired gases was trapped in hyamine hydroxide during two minute collecting periods.

2.1. Calculations

In steady state conditions, the rate of lactate turnover $\mu\text{moles}\cdot\text{min}^{-1}$ is obtained by the ratio of the rate of [^{14}C]lactate infusion ($n\text{Ci}\cdot\text{min}^{-1}$) to the mean lactate specific activity ($n\text{Ci}\cdot\mu\text{moles}^{-1}$). The fraction of lactate turnover oxidized is computed as the ratio of the $^{14}\text{CO}_2$ activity ($n\text{Ci}\cdot\text{min}^{-1}$) in expired gases [10] to the rate of tracer infusion ($n\text{Ci}\cdot\text{min}^{-1}$). Then the rate of oxidation ($\mu\text{moles}\cdot\text{min}^{-1}$) is obtained from this value and the turnover rate and can also be given as a fraction of the total carbon dioxide production of the animal.

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

Condition	Body weight (g)	$\dot{V}O_2$ (ml·min ⁻¹)	$\dot{V}CO_2$ (ml·min ⁻¹)	RQ	[L] (mM)	[G] (mM)	Lactate turnover rate (μmoles·min ⁻¹)	Lactate turnover oxidized (%)	Lactate oxidation rate (μmoles·min ⁻¹)
C (n = 8)	331 8 ^a	4.8 0.1	3.9 0.1	0.81 0.03	0.69 0.03	6.4 0.2	18.7 0.6	51.3 2.3	9.6 0.4
F ₂₄ (n = 4)	320 6	4.5 0.1	3.3 0.1	0.75 0.01	0.60 0.04	5.0 0.1	11.4 0.4	42.8 1.7	4.9 0.3
F ₄₈ (n = 3)	293 7	4.2 0.1	3.0 0.05	0.70 0.01	0.52 0.05	4.6 0.2	9.9 0.9	35.0 1.3	3.5 0.3
F ₇₂ (n = 3)	298 5	3.9 0.05	2.8 0.05	0.70 0.01	0.48 0.04	4.3 0.1	8.6 0.5	31.5 1.6	2.7 0.1
FR (n = 3)	318 10	4.5 0.1	3.8 0.1	0.84 0.01	1.13 0.05	6.6 0.1	20.3 2.0	53.1 1.1	10.8 1.4

^a Mean ± SEM n = number of experiments.

3. Results

Table 1 shows body weight values and respiratory parameters measured during the experiments. For the same pH (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 lactate metabolism. Lactataemia and glycaemia decrease during fasting. Lactate turnover rate is reduced after the different periods of fasting. The percentage of lactate turnover oxidized decreases from 50% in fed conditions to 30% after a 72 h fasting-period which leads to a larger decrease of lactate oxidation rate than that of lactate turnover. After refeeding, for a normal glycaemia, lactataemia is more elevated than in normal fed animals and the rates of lactate turnover and oxidation are increased compared to the controls but the fraction of lactate turnover oxidized does not change. The effect of fasting on the rates of lactate turnover and oxidation expressed per kg of metabolic weight (body weight to the power 0.75 [10]) is represented in fig.1. The reduction of the rate of lactate turnover is important: 38% at 24 h, 42% at 48 h, 50% at 72 h and the rate of

oxidation is even more reduced: 40% at 24 h, 60% at 48 h, 70% at 72 h. After refeeding there is a 12% and a 16% increase of lactate turnover and lactate oxidation rates respectively.

4. Discussion

During fasting the rate of lactate turnover decreases. This observation can be explained by the reduction of glycolysis occurring during fasting due to the well known decrease of glycogen stores and of

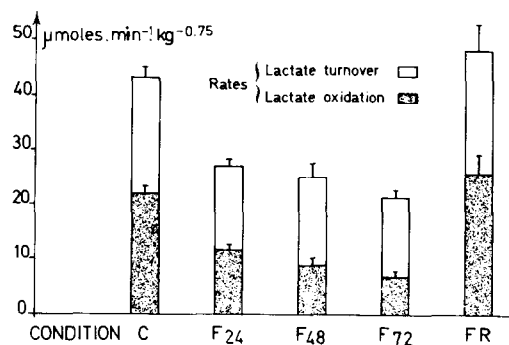


Fig.1. Effect of fasting and refeeding on the rates of lactate turnover and oxidation in rats.

hepatic glucose production [2,11,12]. This is in agreement with data reported in sheep [6] but the reduction after a 24 h fasting-period is more pronounced in rats (-38%) than in sheep (-29%). This fact is probably due to the relatively more severe fasting period for rats than for sheep and possibly to species differences. These results do not confirm the observation by Kreisberg et al. [7] who found no change or a very slight decrease of the rate of lactate turnover (-7%) in 5 out of 6 human obese patients starved for 7 days.

As the fraction of lactate turnover oxidized decreases during fasting the rate of lactate oxidation is more reduced than the rate of lactate turnover. The same observation was reported for glucose metabolism [1]. The 'glucose-fatty acids cycle' can explain this fact through the inhibition of pyruvate dehydrogenase by free fatty acids [12-14]. An inverse relationship was demonstrated between the fraction of glucose turnover oxidized and blood free fatty acids levels [1-15]. The reduced concentration of insulin during fasting contributes also to the decrease of pyruvate dehydrogenase activity [2,16]. In these conditions, pyruvate carboxylase and PEP carboxykinase are stimulated and gluconeogenesis is enhanced [14,17]. As the percentage of lactate to the carbon dioxide production decreases from a value of 16% in control to 6% in 72 h fasted rats, one may postulate that there is a larger participation of lactate to glucose synthesis in these conditions.

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, it is apparent that the fractional participation of lactate turnover to gluconeogenesis increases from a value of 49% in controls to 70% in 72 h fasted rats despite the reduction of the absolute value of this rate. In fed animals $21 \mu\text{mol}\cdot\text{min}\cdot\text{kg}^{-0.75}$ of lactate are converted to glucose compared to $15 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-0.75}$ after 72 h of fast. With a figure of 60% reduction of the rate of glucose turnover during a 24 h fasting-period in the rat as reported by Heath and Corney [3], one may calculate that the participation of lactate to glucose synthesis would be more than doubled if expressed as a fraction of glucose turnover rate.

During refeeding the rate of lactate turnover increases as lactataemia is elevated as previously

observed [9,10]. The percentage of lactate oxidized is identical to that of control animals and the rate of lactate oxidation explains 21% of the carbon dioxide production. As in these conditions it is likely that the rate of glucose synthesis from lactate is decreased or abolished, lactate is certainly utilized to glycogenesis and/or lipogenesis either as a 3 carbons skeleton precursor or as a reducing equivalent carrier [18].

As mentioned in the introduction, Blackshear, Holloway and Alberti have studied the effect of functional hepatectomy on blood glucose and gluconeogenic substrates concentrations in fed and fasted rats [8]. They found that lactate accumulates more and glucose decreases less in fasted than in fed rats. They conclude that the flux of lactate from peripheral tissues to the liver is doubled after 24 h of starvation but such a result is not in agreement with the decrease of lactate turnover observed by others [6] and by the present work. These conflicting results are more likely due to the very different experimental conditions and to a deep hormonal stimulation following an important surgical procedure as used by Blackshear et al. in their animals [8]. The rate of lactate production is probably increased in these conditions but a drastic reduction of lactate utilization cannot be eliminated due to the absence of gluconeogenesis in hepatectomized animals. As in fasted animals, lactate is mainly directed to gluconeogenesis in the liver, this would explain the larger accumulation of lactate observed in fasted as compared to fed animals. In these conditions, it is difficult to conclude that the flux of lactate from peripheral tissues to the liver is doubled in fasted animals.

In conclusion, this study confirms that the rates of lactate turnover and oxidation are decreased during fasting. One question remains unanswered; whether the difference between the rate of lactate turnover and the rate of lactate oxidation represents the rate of gluconeogenesis from lactate *in vivo*. This problem is under investigation in our laboratory.

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