Relationship between the resting metabolic rate and hepatic metabolism in rats: effect of hyperthyroidism and fasting for 24 hours

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ABSTRACT

We have examined the relationship between the changes in resting metabolic rate (RMR) and those in hepatic metabolism induced by hyperthyroidism and fasting for 24 h. We found that hyperthyroidism induced a significant increase in RMR, while fasting for 24 h reduced RMR in euthyroid but not in hyperthyroid rats. We have also measured oxygen consumption in isolated hepatocytes from euthyroid and hyperthyroid rats, fed or fasted for 24 h. Hyperthyroidism induced an increase in oxygen consumption in rat liver cells; fasting for 24 h increased respiratory rates in isolated liver cells from euthyroid but not from hyperthyroid rats.

The findings showed that hyperthyroidism and fasting for 24 h have opposite effects on RMR but similar effects on hepatic metabolism. The results also indicated that the increase in RMR found in hyperthyroid rats is partly due to an increase in hepatic metabolism, while no correlation exists between variations in resting and hepatic metabolism induced by 24-h fasting.

Journal of Endocrinology (1992) **135**, 45–51

INTRODUCTION

It is well known that the state of the thyroid and the nutritional state of an animal influence the resting metabolic rate (RMR) (Guernsey & Edelman, 1983; Rothwell & Stock, 1983; Dauncey, 1990). It has been suggested that the variations in RMR induced by changes in thyroid and/or nutritional state occur through a modulation of energy metabolism in the various tissues. There is, however, little information about the tissues in which these variations in energy expenditure occur, about the quantitative contribution of these tissues and about the mechanisms underlying the variations. The liver is one of the most likely candidates as, although it constitutes only about 4% of the total body weight, it consumes about 20% of the total oxygen used by the animal (Ma, Nadeau & Foster, 1987; Seifter & Englard, 1988). In addition, it has been shown that the nutritional state of the animal induces several modifications in the liver. In fact, it has been shown that starvation induces a decrease in liver mass, liver protein content and liver RNA content (Cascarano, Migler & Wilson, 1978;

Goodman & Ruderman, 1980), as well as a decrease in liver protein synthesis (Burrin, Britton & Ferrell, 1988). Our previous results have also shown that extensive modifications are induced by various thyroid states in hepatic mitochondria. In fact, we have found that the state of the thyroid influences the synthesis of mitochondrial DNA (Liverini, Martino, Barletta *et al.* 1976), RNA (Gadaleta, Barletta, Galdarazzo *et al.* 1972) and proteins (Goglia, Liverini, Lanni & Barletta, 1988), mitochondrial lipid composition and lipid/protein ratio, as well as mitochondrial respiration and ATP production (Iossa, Barletta & Liverini, 1991*a*; Iossa, Liverini & Barletta, 1991*b*). It was therefore of interest to investigate the relationship between the changes in RMR and the changes in hepatic metabolism which take place when energy utilization is stimulated, such as during hyperthyroidism (Guernsey & Edelman, 1983; Oppenheimer, Schwartz, Lane & Thompson, 1991), or decreased, such as during fasting (Hayashi, 1983; Ma & Foster, 1986). In addition, we studied RMR and hepatic metabolism when the mechanisms which stimulate energy expenditure or conserve energy are

in opposition to one another, such as in hyperthyroid fasted rats.

MATERIALS AND METHODS

Animals

After a period of acclimatization in the laboratory, male Wistar rats of the same weight (about 200 g) were divided into two groups. Euthyroid rats were reared at 24 °C for 12 days, while hyperthyroidism was induced in the other group of rats by subcutaneous injections of $3,3',5$-tri-iodothyronine (T_3; 50 µg/100 g body weight) on 3 alternate days. Hyperthyroid rats were used 24 h after the final injection. All rats were kept in individual grid-bottomed cages under an artificial light cycle of 12 h light:12 h darkness. Water and food were available *ad libitum*. Fasted rats were deprived of food for 24 h before they were killed (24-h fasted). Food intake was measured each day (at 10.00 h). The intake measurements were corrected for spillage. Thyroid state was monitored by measuring serum free T_3 levels according to the method of Romelli, Pennisi & Vancheri (1978).

Measurement of resting metabolic rate

RMR was measured between 11.00 and 11.30 h in fed and 24-h fasted rats with an oxygen consumption monitor (Columbus Instruments, Columbus, OH, U.S.A.) in a chamber at 24 °C. Although most of the rats became quiet after about 30 min in the chamber, all were allowed to adapt to the conditions for a minimum of 1 h before beginning the measurements. RMR in each animal was obtained over a period of at least 10 min when the rat remained quiet.

Preparation and incubation of liver cells

Rat liver cells were prepared as described by Seglen (1974), except that the rat was anaesthesized by i.p. administration of chloral hydrate (40 mg/100 g body weight). The hepatocytes were washed and suspended in a medium containing 120 mmol NaCl/l, 5 mmol KCl/l, 50 mmol Hepes/l, pH 7·4, 1 mmol KH_2PO_4/l, 1·2 mmol $MgSO_4$/l and 2% fatty acid-free bovine serum albumin. The final cell suspension was counted with trypan blue in order to assess viability (routinely >90%).

Hepatocyte oxygen consumption was measured polarographically, with a Clark-type electrode maintained in a chamber at 37 °C. Aliquots corresponding to about 10^6 viable cells were incubated in the above suspension buffer with different substrates, at the concentrations reported in the Tables.

Circulating substrate concentrations

Blood was collected via the abdominal aorta. Serum samples were stored at -20 °C until the time of analysis. Glucose, total cholesterol, triglycerides and free fatty acids (FFA) were determined with enzymatic kits (Boehringer–Mannheim Biochemia, Milan, Italy).

Materials

T_3, collagenase (type IV), lactate, hexanoate and ouabain were purchased from Sigma Chemical Co., St Louis, MO, U.S.A. Chloral hydrate was purchased from Serva Feinbiochemica, Heidelberg, Germany. All other reagents used were of the highest purity commercially available.

Statistics

Data are given as means ± s.e.m. Two-way or multi-way analysis of variance was used to determine significant differences among means, while differences between individual means were examined by two-tailed Student's *t*-test, when a significant F value was obtained.

RESULTS

Serum T_3 levels, food intake, body growth rate and weight loss in euthyroid and hyperthyroid fed and fasted rats

The serum free T_3 levels are given in Table 1. Administration of T_3 induced hyperthyroidism, with 3·5-fold

TABLE 1. Serum free tri-iodothyronine (T_3) levels, food intake and weight loss in euthyroid and hyperthyroid fed and fasted rats. Values are means ± s.e.m. of ten different experiments

	Serum T_3 levels (ng/l)	Food intake (g/day per 100 g body wt)	Weight loss (% body wt)
Euthyroid fed	4·1 ± 2·5	14 ± 1	
Euthyroid fasted	2·0 ± 0·1*		8·2 ± 0·7
Hyperthyroid fed	13·9 ± 1·2*	15 ± 1	
Hyperthyroid fasted	14·1 ± 1·3†		9·2 ± 0·7

*$P < 0.0001$ compared with euthyroid fed rats; †$P < 0.0001$ compared with euthyroid fasted rats (two-way analysis of variance followed by two-tailed Student's *t*-test).
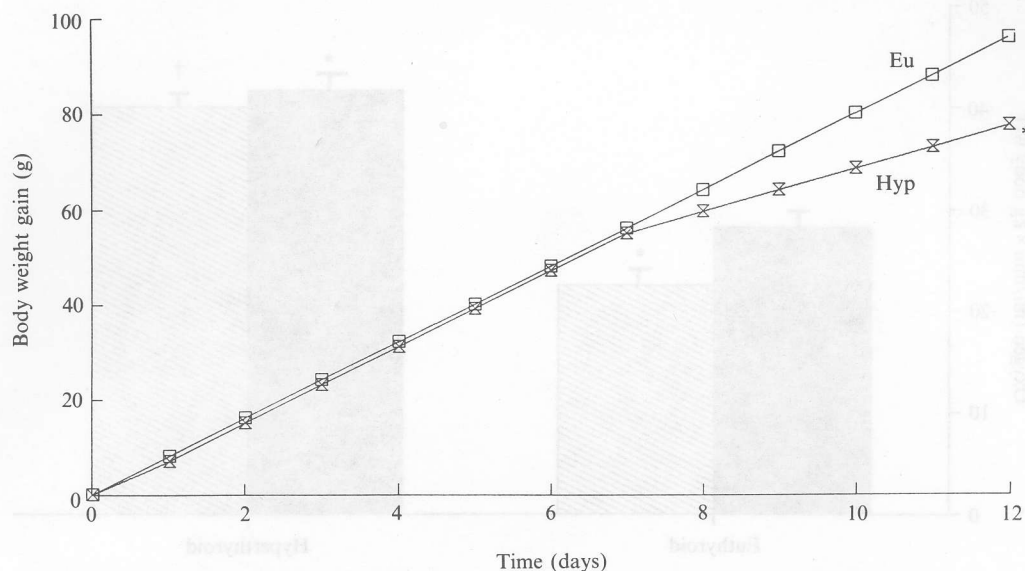
FIGURE 1. Effect of hyperthyroidism on body weight gain. Values are the means of ten different experiments. Twenty rats were kept at 24 °C for 7 days, and body weight gain was assessed every day. Starting from day 7, half of the rats were made hyperthyroid (Hyp) with s.c. injections of tri-iodothyronine (50 µg/100 g body weight) on 3 alternate days before death. *$P < 0.05$ compared with euthyroid rats (Eu) (two-tailed Student's t-test).

higher levels of serum free T_3 in hyperthyroid fed and fasted rats than in euthyroid rats. Free T_3 decreased by about 50% in euthyroid fasted animals, in agreement with previous data obtained after longer periods of starvation (Hayashi, 1983; Ikeda, Ohtani, Hoshino *et al.* 1991).

Food intake was not affected by T_3 treatment (Table 1), in agreement with recent work by Oppenheimer *et al.* (1991), showing that the increase in food intake caused by hyperthyroidism occurred only after 6 days of treatment. Hyperthyroid rats uniformly continued to gain weight, although at a diminished rate, at about 3 g/day compared with the euthyroid rats at 8 g/day (Fig. 1).

Fasting for 24 h resulted in body weight loss of about 8% in both euthyroid and hyperthyroid rats (Table 1).

Effect of hyperthyroidism and/or 24-h fasting on blood substrate concentrations

In the fed rats hyperthyroidism induced a significant increase in FFA (+49%) and a significant decrease in triglycerides (−50%) (Table 2).

Glucose concentrations significantly decreased in response to 24-h fasting in both euthyroid and hyperthyroid rats, while FFA concentrations significantly increased only in euthyroid animals (Table 2).

Effect of hyperthyroidism and/or 24-h fasting on RMR

Hyperthyroidism induced a significant increase (+47%) in RMR, in agreement with previous results (Guernsey & Edelman, 1983; Oppenheimer *et al.* 1991). On the other hand, RMR significantly

TABLE 2. Effect of hyperthyroidism on blood substrate concentration in fed and 24-h fasted rats. Values are means ± s.e.m. of ten different experiments

	Glucose (g/l)	Free fatty acids (µmol/l)	Cholesterol (mg/l)	Triglycerides (g/l)
Euthyroid fed	2·13 ± 0·20	540 ± 23	820 ± 50	1·03 ± 0·16
Euthyroid fasted	1·14 ± 0·04*	1043 ± 80*	950 ± 100	1·22 ± 0·20
Hyperthyroid fed	1·70 ± 0·11	807 ± 27*	700 ± 70	0·54 ± 0·03*
Hyperthyroid fasted	1·35 ± 0·11†	894 ± 99	800 ± 80	0·60 ± 0·06‡

*$P < 0.001$ compared with euthyroid fed rats; †$P < 0.05$ compared with hyperthyroid fed rats; ‡$P < 0.01$ compared with euthyroid fasted rats (two-way analysis of variance followed by two-tailed Student's t-test).
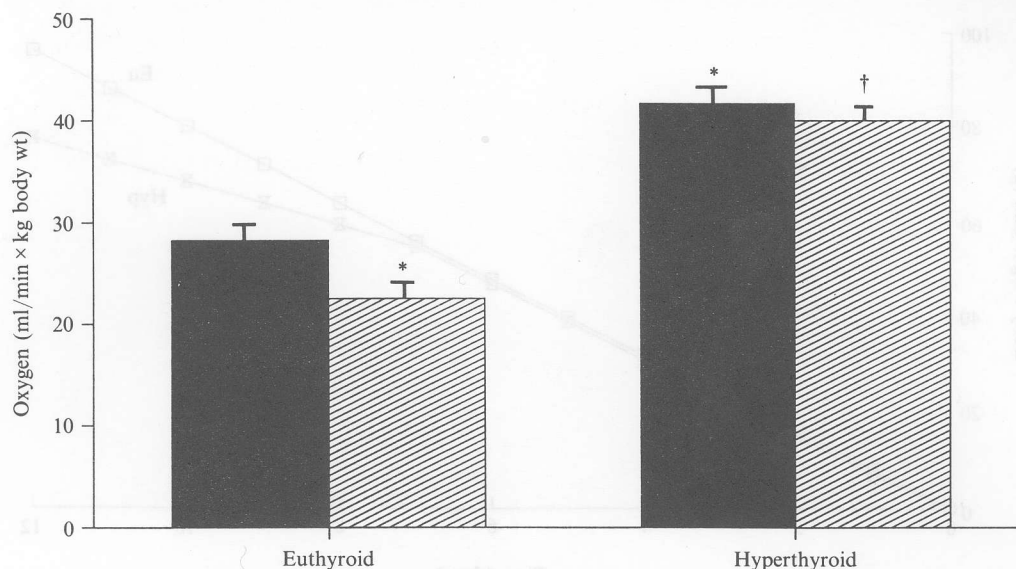
FIGURE 2. Effect of hyperthyroidism and fasting for 24 h on resting metabolic rate (RMR). Values are the means ± S.E.M. of ten different experiments. RMR was measured in euthyroid and hyperthyroid rats, fed (solid bars) or fasted (hatched bars) for 24 h, with an oxygen consumption monitor in a chamber at 24 °C, for a period of at least 10 min when the rat remained quiet. *$P < 0.001$ compared with euthyroid fed rats; †$P < 0.0001$ compared with euthyroid fasted rats (two-way analysis of variance followed by two-tailed Student's t-test).

decreased in response to 24-h fasting in euthyroid but not in hyperthyroid rats (Fig. 2).

Effect of hyperthyroidism and/or 24-h fasting on oxygen consumption in isolated hepatocytes

The oxygen consumption in isolated hepatocytes from euthyroid and hyperthyroid rats, fed or fasted for 24 h, was measured under different conditions of stimulation of mitochondrial respiration (Table 3).

First, when the cells were provided with additional substrate, namely hexanoate, a significant increase in oxygen consumption in comparison with the respective basal state occurred in all the groups. A further

significant increase in the respiration rates of the cells from all the groups was achieved by adding lactate to the incubation medium containing hexanoate, since lactate is a gluconeogenic substrate which increases ADP availability. In the fed state, liver cells from hyperthyroid rats exhibited significantly higher rates of respiration than those from euthyroid rats (Table 3). Fasting for 24 h induced a significant increase in hexanoate- and hexanoate + lactate-stimulated respiration in euthyroid but not in hyperthyroid rats. However, the respiratory rates of hyperthyroid fasted rats remained higher than those of euthyroid fasted rats.

We also measured oxygen consumption in the presence and absence of 1 mmol ouabain/l and the results

TABLE 3. Effect of hyperthyroidism and fasting for 24 h on oxygen consumption in isolated hepatocytes. Values are expressed as nmol O_2/min × 10^6 cells. Data are the means ± S.E.M. of ten different experiments

Additions	Hyperthyroid fed rats	Hyperthyroid fasted rats	Euthyroid fed rats	Euthyroid fasted rats
(1) None	22·7 ± 1·6†	22·4 ± ± 2·0‡	13·0 ± 0·5	12·0 ± 0·5
(2) Hexanoate	33·6 ± 1·7†‖	31·4 ± 2·7‡§	17·4 ± 0·6‖	22·0 ± 1·9*‖
(3) Hexanoate + lactate	42·2 ± 1·9†‖	40·4 ± 2·6‡§	22·0 ± 1·4‖	29·0 ± 1·6†§

*$P < 0.05$ compared with euthyroid fed rats; †$P < 0.01$ compared with euthyroid fed rats; ‡$P < 0.02$ compared with euthyroid fasted rats; §$P < 0.05$ comparing value 2 with value 1, or value 3 with value 2; ‖$P < 0.01$ comparing value 2 with value 1, or value 3 with value 2 (multi-way analysis of variance followed by two-tailed Student's t-test).
Isolated liver cells were incubated at 37 °C as described in Materials and Methods. Final substrate concentrations were: hexanoate 4 mmol/l; lactate 10 mmol/l.

are shown in Fig. 3. The specific Na/K-ATPase inhibitor, ouabain, significantly decreased oxygen consumption in all the groups. The Na/K-dependent oxygen consumption significantly increased in hyperthyroid fed rats, while fasting for 24 h had no effect in euthyroid and hyperthyroid rats.

DISCUSSION

In this study we have tried to establish a correlation between RMR and hepatic metabolism in the hyperthyroid and 24-h fasting state.

We found that hyperthyroidism significantly increased RMR in intact animals, while 24-h fasting significantly decreased it (Fig. 2). This reduction in RMR after 24-h fasting is not attributable to the absence of the contribution of food processing and storage (thermic effect of food) as the measurements were made in conditions in which the thermic effect of food is also excluded in fed animals, as shown by Ma & Foster (1986) and it must therefore be considered to be of adaptive value. Whether the decrease in free T_3 levels found after 24-h fasting in euthyroid animals (Table 1) could be considered as part of the metabolic adaptation serving to reduce RMR is at present a debatable question. The present study seems to support this suggestion as we have found that no

variation in RMR occurs after 24-h fasting in hyperthyroid rats (Fig. 2). It should be noted that our finding is different from that obtained by Wimpfheimer, Saville, Voirol et al. (1979) using longer periods of starvation (3–5 days) and lower T_3 doses than those used in the present work.

To test whether the changes in RMR found in intact rats subjected to T_3 treatment or 24-h fasting are associated with variations in oxygen consumption in the liver, we have measured oxygen consumption in isolated hepatocytes from euthyroid and hyperthyroid fed and fasted rats.

The examination of our results shows that basal respiration is increased in hyperthyroid rats (Table 3); the observed increase may be due to an improvement in substrate supply, which would be consistent with the observed increase in blood FFA concentration which takes place in the hyperthyroid state (Table 2). In addition, our results show that a significant increase in hexanoate- and hexanoate + lactate-stimulated respiration occurs in hyperthyroid rats (Table 3). The enhanced metabolic rate of isolated hepatocytes from hyperthyroid rats is in good agreement with our previous work which showed an increased liver mitochondrial mass as well as an increased lipid oxidative capacity in isolated mitochondria from the livers of hyperthyroid rats (Iossa et al. 1991b). On the whole, our present results
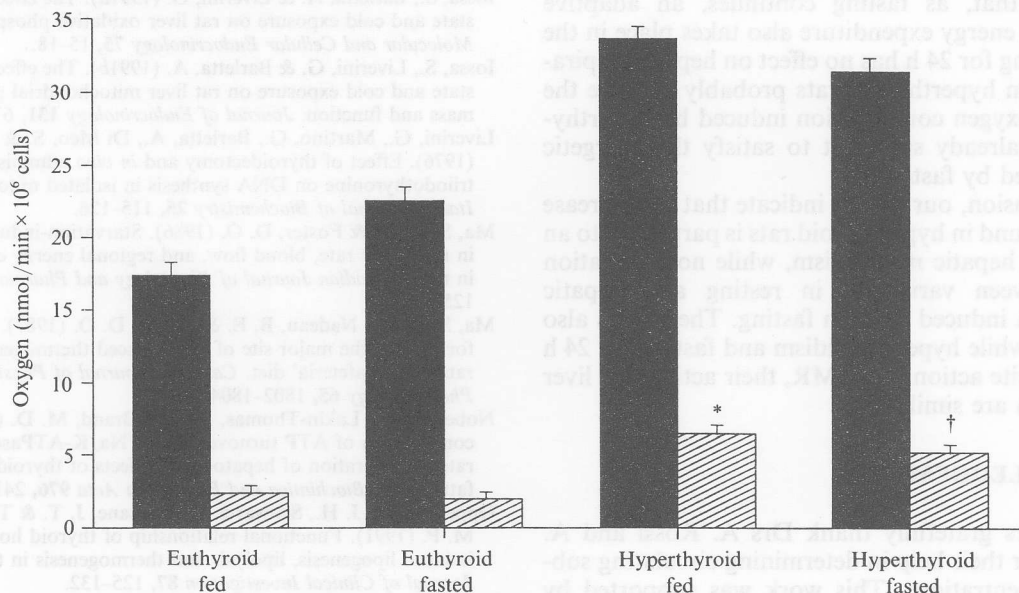

FIGURE 3. Effect of hyperthyroidism and fasting for 24 h on Na/K pump-dependent oxygen consumption of isolated hepatocytes. Values are the means ± S.E.M. of ten different experiments. The Na/K pump-dependent oxygen consumption (hatched bars) was obtained from the difference between the total oxygen consumption (solid bars) measured in the presence of 4 mmol hexanoate/l and that measured for 10 min in the presence of 4 mmol hexanoate/l + 1 mmol ouabain/l. *$P < 0.05$ compared with euthyroid fed rats; †$P < 0.05$ compared with euthyroid fasted rats (two-way analysis of variance followed by two-tailed Student's t-test).

suggest that an increase in ATP turnover occurs in the liver of hyperthyroid rats, as also indicated by the increased Na/K transport across the liver plasma membrane (Fig. 3) associated with the hyperthyroid state. Our results also show, in agreement with others (Clark, Brinkman, Filsell *et al.* 1982; Nobes, Lakin-Thomas & Brand, 1989), that about 25% of the increase in oxygen consumption of hepatocytes isolated from hyperthyroid rats is due to the enhanced activity of Na/K transport, a contribution which is much lower than that found in liver slices (Guernsey & Edelman, 1983).

In addition, our findings show that 24-h fasting significantly increased hepatic metabolic rates (Table 3) in euthyroid but not in hyperthyroid rats. The increase in the oxygen consumption of isolated hepatocytes from euthyroid rats after 24-h fasting (Table 3) can be readily explained when taking into account the fact that hepatic metabolism switches to gluconeogenic activity in response to fasting-induced hypoglycaemia (Table 2) (Seifter & England, 1988; Slaunwhite, 1988), thus increasing ADP availability which in turn stimulates mitochondrial respiration. The above modifications give rise to an increase in metabolic activity in the liver cells, at least in the first phase of adaptation to fasting. In fact, it has been shown that hepatic oxygen consumption per cell decreases after 3 days of fasting (Burrin *et al.* 1988), indicating that, as fasting continues, an adaptive decrease in energy expenditure also takes place in the liver. Fasting for 24 h has no effect on hepatic respiratory rates in hyperthyroid rats probably because the increased oxygen consumption induced by hyperthyroidism is already sufficient to satisfy the energetic needs caused by fasting.

In conclusion, our results indicate that the increase in RMR found in hyperthyroid rats is partly due to an increase in hepatic metabolism, while no correlation exists between variations in resting and hepatic metabolism induced by 24-h fasting. The results also show that while hyperthyroidism and fasting for 24 h have opposite actions on RMR, their actions on liver metabolism are similar.

ACKNOWLEDGEMENTS

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