# Modulation of β-oxidation and proton conductance pathway of brown adipose tissue in hypo- and hyperinsulinemic states

J. Seydoux, E.R. Trimble*, F. Bouillaud+, F. Assimacopoulos-Jeannet°, S. Bas†, D. Ricquier+, J.P. Giacobino† and Girardier, L.

*Département de Physiologie and †Département de Biochimie Médicale, Centre Médical Universitaire, 1211 Genève 4, *Institut de Biochimie Clinique and °Laboratoire de Recherches Métaboliques, avenue de la Roseraie, 1205 Geneva, Switzerland* and *+Laboratoire de Physiologie comparée (L.A. 307 du CNRS), Université P.M. Curie, 4 place Jussieu, 75230 Paris Cédex 05, France*

Received 5 November 1983

The metabolic capacity of interscapular brown adipose tissue of hypoinsulinemic (diabetic) rats is decreased and a reduced β-oxidative capacity contributes to this metabolic alteration. It was thus of interest to compare, in diabetic and in chronically (8 days) insulin-infused rats, the β-oxidative capacity and indices of the thermogenic state (GDP-binding and 32000 $M_r$ protein) in this tissue. Mitochondrial GDP-binding and 32000 $M_r$ protein were both decreased in diabetic rats compared to appropriate controls and markedly increased as was also the β-oxidative capacity in hyperinsulinemic rats.

*Brown adipose tissue* *Mitochondria* *Guanine nucleotide binding* *32000* $M_r$ *protein* *Reduced equivalent* β-Oxidation

## 1. INTRODUCTION

Brown adipose tissue (BAT) is now recognized as the main effector of regulatory thermogenesis in rodents [1]. It plays an important role in cold-induced thermogenesis and under conditions where diet-induced thermogenesis occurs [2]. BAT thermogenesis is under the control of its sympathetic innervation [3]. Recent findings have shown that the response of BAT to various stimuli can be modified by insulin [4–6]. In streptozotocin-diabetic rats, the interscapular BAT (IBAT) is atrophied and its basal and norepinephrine-stimulated total heat production rates, as well as its capacity to degrade fatty acids, are all markedly decreased [5]. This correlates with the observation that, in streptozotocin-diabetic rats, cold tolerance is decreased and the feeding of a palatable high energy diet (cafeteria diet) fails to increase the metabolic rate [4]. Conversely, in lactating cafeteria fed rats receiving insulin, hypertrophy of the IBAT occurs [6] and, as was shown recently, insulin injection increases the metabolic rate of fed, fasted or cafeteria fed rats [7].

The high BAT thermogenic capacity depends on both an adequate supply of substrate and on a unique mitochondrial pathway by which electron flow can be reversibly uncoupled from respiratory control. Fatty acids released from endogenous triglyceride stores or from circulating lipoproteins constitute the main substrate supply. The specialized mitochondrial mechanism involved is a regulated proton conductance pathway that can be blocked by purine nucleotides of which guanosine diphosphate (GDP) is the most potent, with consequent recoupling of the mitochondria [8]. The purine binding protein is a polypeptide with a molecular mass of 32000 (32000 $M_r$ protein) which is a major component of the mitochondria inner membrane [8–10].

The aim of this study was:

(i) to further characterize the defective thermogenic IBAT response in insulinopenia by measuring the mitochondrial proton conduc-

*Published by Elsevier Science Publishers B.V.*
00145793/84/$3.00 

tance pathway in streptozotocin diabetic rats;
(ii) to analyze the effects of a chronic increase in circulating insulin concentration on rat IBAT metabolism.

## 2. MATERIALS AND METHODS

Male Wistar rats weighing 250–330 g and fed laboratory chow containing 71% carbohydrate, 22% protein and 7% fat (by calories) were used. Rats were made diabetic with streptozotocin [5] or were infused with insulin through a chronically implanted jugular vein catheter [11]. In insulin-infused rats the amount of hormone required to achieve a plasma glucose of approximately 40 mg/dl was less than 5 ml/24 h (1.2 units/ml). At the onset of the infusion the plasma insulin concentration was about 17–20 ng/ml. The amount of insulin required to maintain the plasma glucose at 40 mg/dl decreased with time and the plasma insulin at the end of the 8-day infusion period was about 10 ng/ml [12]. Control animals received i.v. saline (plasma insulin 1.6 ng/ml).

The rats were killed by decapitation and both IBAT pads were excised. One pad, along with its nerve supply consisting of 5 intercostal nerves, was placed in a thermoregulated chamber (30°C) and perifused with a Krebs-Ringer bicarbonate buffer solution containing 5 mM glucose (pH 7.4) for spectrophotometric studies. The other pad was used for tissue analysis or enzymatic determinations.

On each neuroadipose preparation a non-destructive technique was used to record changes in redox state of the NAD(P) in response to various stimuli. The redox state was measured by surface-emitted fluorescence (>400 nm) obtained with an excitation light of 366 nm as in [13]. Electrical stimulation of the nerve supply was performed with pulse trains (60 V, 2 ms) of increasing frequency.

Mitochondria were isolated by differential centrifugation of IBAT homogenate as in [14]. The mitochondria recovery was quantified by comparing the activity of succinate dehydrogenase (EC 1.3.99.1) in the total homogenate with that in the mitochondria. The succinate dehydrogenase recovery in control rat mitochondria pellet was 49 ± 3% ($n$ = 12) and was not significantly different from that of treated rats. The recovery value being measured in each preparation, the corrected value for the total mitochondria of the IBAT of one rat could be calculated. The succinate dehydrogenase activity was measured spectrophotometrically as in [15]. Proteins were determined as in [16]. The GDP-binding to isolated mitochondria was measured using equilibrium dialysis with [8-$^3$H]GDP (spec. act. 11.3 Ci/mmol) as in [17]. The amount of the 32000 $M_r$ protein in isolated mitochondria was calculated from the densitometric traces of sodium dodecyl sulfate-polyacrylamide gel electrophoresis as in [9].

The fatty acid $\beta$-oxidative activity of the 1300 × $g$ homogenate, measured as the production of [1-$^{14}$C]acetyl CoA from [1-$^{14}$C]palmitoyl CoA, and tissue composition were obtained as in [5].

Student's unpaired $t$-test was used to determine the statistical significance. All organic and inorganic chemicals were of analytical grade and were purchased from E. Merck (Darmstadt). Streptozotocin was a gift from Dr W.J. Dulin, the Upjohn Company (Kalamazoo, MI), Actrapid insulin was supplied by Novo Industri (Copenhagen) and L-norepinephrine (arterenol) by Hoechst AG (Frankfurt). [8-$^3$H]GDP was obtained from the Radiochemical Center (Amersham), and [1-$^{14}$C]palmitoyl CoA from New England Nuclear (Boston, MA).

## 3. RESULTS

### 3.1. *GDP-binding and percentage of 32000* $M_r$ *protein in IBAT mitochondria*

The upper part of table 1 shows that, in IBAT mitochondria of diabetic rats, the total GDP-binding was decreased by 84.6% and the 32000 $M_r$ protein by 34.5% as compared to controls. The lower part of table 1 shows that chronic hyperinsulinemia produced opposite effects: the total GDP-binding was increased 4.4-fold and the % of 32000 $M_r$ protein 1.4-fold as compared to the controls.

### 3.2. *IBAT wet weight and composition*

As can be seen in table 2, the wet weight of IBAT of hyperinsulinemic rats as compared to the controls was increased 2-fold, the lipid concentration was increased 1.9-fold, the protein and DNA concentrations were decreased by 35.3 and 37.5%, respectively, and succinate dehydrogenase activity

Table 1

IBAT mitochondria: GDP-binding and 32000 $M_r$ protein

| | GDP-binding (nmol/IBAT) | 32000 $M_r$ protein as % of total protein |
|---|---|---|
| Controls (6) | 2.08 ± 0.20 | 8.4 ± 0.3 |
| Streptozotocin-diabetic (6) | 0.32 ± 0.05[a] | 5.5 ± 0.2[a] |
| Controls (6) | 1.06 ± 0.10 | 6.2 ± 0.3 |
| Insulin-infused (5) | 4.70 ± 0.64[a] | 8.8 ± 0.2[a] |

[a] $p < 0.001$ as compared to appropriate controls

The results are the means ± SE of the number of experiments indicated in parentheses and are expressed in nmol of GDP bound in the total mitochondria of the IBAT of one rat obtained by multiplying the specific value by the total amount of mitochondria (see section 2). The 32000 $M_r$ protein values are expressed as % of the total mitochondrial proteins

Table 2

Wet weight, lipid, protein, DNA concentrations and succinate dehydrogenase activity in IBAT of control and hyperinsulinemic rats

| | Control rats ($n$ = 5) | Insulin-infused rats ($n$ = 6) |
|---|---|---|
| Tissue wet wt (mg) | 212 ± 16 | 432 ± 27[a] |
| Lipids concentration ($mg \cdot g^{-1}$) | 229 ± 18 | 427 ± 25[a] |
| Protein concentration ($mg \cdot g^{-1}$) | 170 ± 10 | 110 ± 6[a] |
| DNA concentration ($mg \cdot g^{-1}$) | 3.60 ± 0.15 | 2.25 ± 0.14[a] |
| Succinate dehydrogenase ($\mu mol \cdot g^{-1} \cdot min^{-1}$) | 55 ± 6 | 88 ± 11[a] |

[a] $p < 0.001$

Values are means ± SE. Statistical analysis: hyperinsulinemic *vs* control rats

was increased 1.6-fold. Fig.1 shows that the tissue weight, the lipid and protein content and the total succinate dehydrogenase activity per IBAT expressed as % of control values changed in opposite direction in diabetic or hyperinsulinemic rats. The DNA content was not affected by either condition.

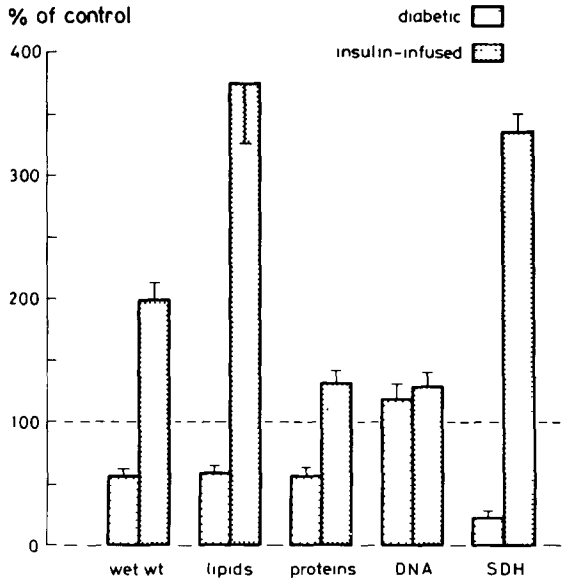


Fig.1. Wet wt, lipid, protein, DNA contents and succinate dehydrogenase (SDH) activity in IBAT of diabetic or insulin-infused rats expressed as % ± SE of appropriate controls. $n$ = 5–6 experiments.

### 3.3. *IBAT NAD(P) redox state level under electrical nerve stimulation, norepinephrine or octanoate additions*

In the IBAT of both control and hyperinsulinemic rats, electrical stimulation of the nerve supply caused a similar increase of the emitted fluorescence; i.e., of the NAD(P) reduction as a function of the stimulation frequencies. Norepinephrine (100–1000 nM) or octanoate (5 mM) additions produced a similar NAD(P) reduction in IBAT of both control and hyperinsulinemic rats (fig.2).

### 3.4. *IBAT fatty acid β-oxidation*

β-Oxidative specific activities were similar in the IBAT 1300 × $g$ homogenates of both control and hyperinsulinemic rats (690 ± 60 and 610 ± 100 pmol of reaction product formed $\cdot min^{-1} \cdot$ mg protein$^{-1}$, respectively; mean of 5–6 experiments ± SE). Total β-oxidative activity was increased in the IBAT homogenate of hyperinsulinemic rats compared to that of controls: 15.3 ± 2.0 and 7.8 ± 1.2 nmol of reaction product formed $\cdot min^{-1}$ per tissue, respectively ($p < 0.001$).

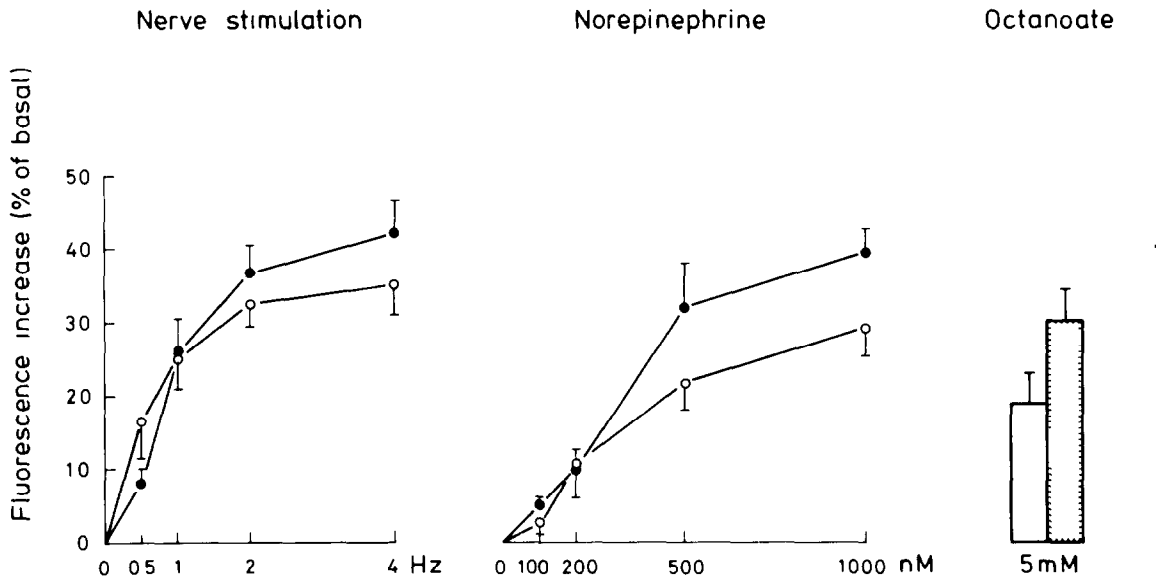


Fig.2. Pyridine nucleotide redox level at steady state in IBAT as measured by surface-emitted fluorescence in response to nerve stimulation, to norepinephrine or octanoate additions. Controls (○ and open columns), hyperinsulinemic rats (● and dotted columns). The results are the means ± SE of 8 (control) and 7 (hyperinsulinemic rats) experiments.

## 4. DISCUSSION

The results show that in the IBAT of the diabetic rat previously found to be atrophied and in which the $\beta$-oxidative capacity was markedly decreased [5], there is also a defect at the level of the mitochondrial proton conductance pathway. In fact, in the IBAT mitochondria of diabetic rats, as compared to the controls, the total GDP-binding as well as the percentage of 32000 $M_r$ protein were decreased. Thus the reduced IBAT thermogenic capacity and the consequent decrease of cold tolerance and norepinephrine-stimulated metabolic rate observed in streptozotocin-diabetic rats [4] would not only result from the described decrease in the $\beta$-oxidative capacity [5] but also from a decrease in the proton conductance pathway. In contrast to what was observed in the chronic insulinopenic state, in insulin-infused rats, the total IBAT $\beta$-oxidative activity, the total GDP-binding, and the percentage of 32000 $M_r$ protein of the mitochondria were increased as compared to the controls. The responses to nerve stimulation, norepinephrine or octanoate addition on NAD(P) redox state were only slightly greater in IBAT of insulin-infused rat than in those of the controls. This is in contrast to what was observed in the IBAT of diabetic rats where the responses were markedly different from those of the controls; i.e., the same stimuli induced a NAD(P) oxidation instead of a reduction [5].

It is generally accepted that the GDP-binding and to a lesser extent the 32000 $M_r$ protein content of the mitochondria are indices of the thermogenic capacity of the BAT. The GDP binding sites are increased in high thermogenic states like cold acclimation [18] or spontaneous overfeeding [19] and decreased in low thermogenic states like warm-adaptation [20] or genetic or experimentally induced obesity [21,22]. The 32000 $M_r$ protein is increased in cold acclimation [9,10,18] and decreased in warm adaptation [9,23] and in experimentally induced obesity associated with overfeeding [22]. In diabetes, a state where BAT thermogenesis is reduced [5], both GDP-binding and 32000 $M_r$ protein are significantly decreased as compared to the control which is another indication that these two parameters are indices of the thermogenic capacity of BAT.

The modifications observed in IBAT of hyperinsulinemic rats indicate that this tissue is in a high thermogenic state. These results raise the question

whether the observed effects of hyperinsulinemia are due to a direct action of insulin on BAT metabolic activity or are secondary to the increased sympathetic activity induced by either insulin or hypoglycemia. It was reported that insulin exerts a direct stimulatory action on the sympathetic nervous system independent of hypoglycemia in the rat [7], in the dog [24] and in man [25]. In insulin-infused rats, the plasma levels of catecholamines were slightly increased [12] but this was probably insufficient in itself to account for the observed effects on IBAT since the highest levels measured in the plasma are still about two orders of magnitude lower than those required to stimulate IBAT metabolism [3]. It cannot be excluded, however, that in hyperinsulinemic rat norepinephrine turnover in the IBAT would be enhanced. If this were the case, part of the effects of hyperinsulinemia could be attributed to some interactions between insulin and the sympathetic nervous system, even though the hyperplasia of IBAT observed under conditions of increased norepinephrine secretion like in cold acclimation or cafeteria feeding, was not observed here. Indeed, in IBAT of hyperinsulinemic rats, the DNA content was not modified, the main alterations consisted in lipid accumulation and in an increase of the mitochondrial content of this tissue.

The results obtained indicate that in hypo- and hyperinsulinemic states both the $\beta$-oxidative capacity and the proton conductance pathway are modified in opposite directions.

## ACKNOWLEDGEMENTS

We greatly appreciated the excellent technical help of Mr A. Gjinovci, Mrs C. Blanchard, A. Jousson, Miss C.A. Breitmeyer and C. Colomb, and we thank Mrs J. Steiner for typing the manuscript. This work was supported by grants 3.951.0.80, 3.071.181 and 3.246.0.82 from the Swiss National Science Foundation and by a grant-in-aid of Sandoz Stiftung zur Förderung der Medizinisch-Biologischen Wissenschaften (Basel).

## REFERENCES

[1] Foster, D.O. and Frydman, M.L. (1978) Can. J. Physiol. Pharmacol. 56, 110–122.
[2] Rothwell, N.J. and Stock, M.J. (1980) Can. J. Physiol. 58, 842–848.
[3] Seydoux, J. and Girardier, L. (1978) Experientia (suppl.) 32, 153–167.
[4] Rothwell, N.J. and Stock, M.J. (1981) Metabolism 30, 673–678.
[5] Seydoux, J., Chinet, A., Schneider-Picard, G., Bas, S., Imesch, E., Assimacopoulos-Jeannet, F., Giacobino, J.P. and Girardier, L. (1983) Endocrinology 113, 604–610.
[6] Agius, L., Rolls, B.J., Rowe, E.A. and Williamson, D.H. (1981) FEBS Lett. 123, 45–48.
[7] Rothwell, N.J., Saville, M.E. and Stock, M.J. (1983) Am. J. Physiol. 245, E160–E165.
[8] Nicholls, D.G. (1979) Biochim. Biophys. Acta 549, 1–22.
[9] Ricquier, D. and Kader, J.C. (1976) Biochem. Biophys. Res. Commun. 73, 577–583.
[10] Ricquier, D., Mory, G. and Hémon, P. (1979) Can. J. Biochem. 57, 1262–1266.
[11] Steffens, A.B. (1969) Physiol. Behav. 4, 833–836.
[12] Trimble, E.R., Weir, G.C., Gjinovci, A., Assimacopoulos-Jeannet, F., Benzi, R. and Renold, A.E. (1984) Diabetes, in press.
[13] Seydoux, J., Constantinidis, J., Tsacopoulos, M. and Girardier, L. (1977) J. Physiol. (Paris) 73, 985–996.
[14] Ricquier, D., Mory, G. and Hémon, P. (1975) FEBS Lett. 53, 342–346.
[15] Bachmann, E., Allmann, D.W. and Green, D.E. (1966) Arch. Biochem. Biophys. 115, 153–164.
[16] Lowry, D.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[17] Lin, C.S. and Klingenberg, M. (1980) FEBS Lett. 113, 299–303.
[18] Desautels, M., Zaror-Behrens, G. and Himms-Hagen, J. (1978) Can. J. Biochem. 56, 378–383.
[19] Brooks, S.L., Rothwell, N.J., Stock, M.J., Goodbody, A.E. and Trayhurn, P. (1980) Nature 286, 274–276.
[20] Rafael, J. and Heldt, H.W. (1976) FEBS Lett. 63, 304–308.
[21] Hogan, S. and Himms-Hagen, J. (1980) Am. J. Physiol. 239, E301–E309.
[22] Seydoux, J., Ricquier, D., Rohner-Jeanrenaud, F., Assimacopoulos-Jeannet, F., Giacobino, J.P., Jeanrenaud, B. and Girardier, L. (1982) FEBS Lett. 146, 161–164.
[23] Heaton, G.M., Wagenvoord, R.J., Kemp, A. and Nicholls, D.G. (1978) Eur. J. Biochem. 82, 515–521.
[24] Liang, C.S., Doherty, J.M., Faillace, R., Mackawa, K., Arnold, S., Gavras, H. and Hood, W.B. (1982) J. Clin. Invest. 69, 1321–1336.
[25] Rowe, J.N., Young, J.B., Minaher, K.L., Stevens, A.L., Pallotta, J. and Landsberg, L. (1981) Diabetes 30, 219–225.