

Rapid in vivo PGC-1 mRNA upregulation in brown adipose tissue of Wistar rats by a β_3 -adrenergic agonist and lack of effect of leptin

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Abstract

Peroxisome proliferator-activated receptor- γ coactivator-1 (PGC-1) is highly expressed in brown adipose tissue (BAT) and plays an important role in adaptive thermogenesis. The aim of this study was to assess the acute effect of a β_3 -adrenergic agonist (Trecadrine) and leptin on the expression of PGC-1 and PPAR γ 2 mRNA in BAT. Trecadrine produced a marked increase (4.5-fold) in PGC-1 mRNA compared to controls ($P < 0.001$) without changes in PPAR γ 2 mRNA, whereas leptin administration did not alter either PGC-1 or PPAR γ 2 expression. These results show that selective stimulation of the β_3 -adrenoceptor rapidly upregulates the expression of PGC-1 in brown adipocytes without a concomitant increase in PPAR γ 2. Moreover, our results show that PGC-1 and PPAR γ 2 expression in BAT seems not to be acutely regulated by leptin. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: PGC-1; PPAR γ 2; Leptin; β_3 -adrenergic agonist; Uncoupling protein; Brown adipose tissue; Rat

1. Introduction

Adaptive thermogenesis is an important component of energy homeostasis and a physiological defence against obesity (Lowell and Spiegelman, 2000). Brown adipose tissue (BAT) is of special interest in the study of adaptive thermogenesis because is the only tissue that works exclusively as a thermogenic organ due to its abundance of mitochondria, with ability to uncouple the oxidative phosphorylation through uncoupling protein (UCP) (Ricquier and Bouillaud, 2000). In this context, it has been described that genetic ablation of BAT produces obesity in mice (Lowell et al., 1993). Peroxisome proliferator-activated receptor- γ (PPAR γ) are nuclear receptors strongly involved in adipogenesis and thermogenesis. Moreover, it has been described that these nuclear receptors are transcription factors controlling positively the expression of UCPs in BAT

(Kelly et al., 1998). The PPAR γ coactivator-1 (PGC-1) is a coactivator of PPAR γ , NRFs, TR β and RXR α highly expressed in BAT, but also in skeletal muscle, heart, kidney and brain. PGC-1 plays an important role in the expression of genes encoding proteins involved in adaptive thermogenesis through increases in the transcriptional activity of the above mentioned nuclear receptors (Puigserver et al., 1998; Wu et al., 1999).

Thermogenesis in BAT is primarily mediated by the sympathetic nervous system (SNS) through β -adrenergic receptors (β -AR) (Himms-Hagen, 1990). β_3 -AR is the major β -AR subtype expressed in rodent BAT (D'Allaire et al., 1995) and its selective activation induces the expression of UCP1 with no clear effect on UCP2 and UCP3 expression (Puigserver et al., 1996; Savontaus et al., 1998; Yoshitomi et al., 1998; Gómez-Ambrosi et al., 1999). It has been reported that β -AR activation by isoproterenol, a non-selective β agonist, increases the expression of PGC-1 mRNA in HIB 1B brown adipocytes differentiated in culture (Puigserver et al., 1998). It has also been published that in vivo selective β_3 -AR stimulation induces the expression of

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PGC-1 in skeletal muscle in mice (Boss et al., 1999). However, there is no evidence of *in vivo* regulation of PGC-1 mRNA expression in BAT by β -AR agonists or selective β_3 -AR agonists.

Leptin, the product of the *ob* gene, is an adipocyte-derived hormone which plays an important role in the energetic balance through regulation of food intake and energy expenditure (Ahima and Flier, 2000). The mechanism of increased energy expenditure by leptin appears to involve long-term increased expression of UCP1 in BAT (Scarpace et al., 1997). However, this increase does not take place acutely (Gómez-Ambrosi et al., 1999). The leptin-mediated stimulation of thermogenesis seems to be dependent on sympathetic innervation providing evidence of the existence of a loop involving SNS activity and leptin (Haynes et al., 1997) where PGC-1 could be involved.

In this study, we assessed the acute effect of intraperitoneal administration of either a β_3 -AR agonist or leptin on the expression of PGC-1 and PPAR γ 2 mRNA in BAT of fasted rats and their possible relation with some indicators of thermogenesis and lipolysis.

2. Materials and methods

2.1. Animals and procedures

Five-month-old male Wistar rats weighing 420–430 g were housed at $25 \pm 1^\circ\text{C}$ with 12-h light cycle (8:00–20:00 h) and fed *ad libitum*. Animals were fasted overnight before drug administration. Rectal temperature of the rats was measured prior to *i.p.* injections of: saline, leptin (Peprotech, London, UK; 0.25 mg/kg) or the β_3 -AR agonist Trecadrine (Wasserman-Chiesi, Barcelona/Milan; 1 mg/kg, see reference (Barrionuevo et al., 1996) for chemical structure and pharmacological properties). The doses tested were used based on previous experiments showing physiological effects (Pelley-mounter et al., 1995; Gómez-Ambrosi et al., 1999). One hour after administration, rectal temperature and oxygen consumption were measured. This time period has been shown to detect the maximal thermogenic effect after β_3 -AR agonist administration (Shih and Taberner, 1995). Animals were killed by decapitation and trunk blood was collected. Interscapular BAT was excised, immediately frozen in liquid nitrogen and stored at -80°C until analysis. All experimental procedures were performed according to the 'Ethical principles and guidelines for scientific experiments on animals' of the Swiss Academy of Medical Sciences.

2.2. Blood analyses

Serum non-esterified fatty acid (NEFA) concentrations were measured by an enzymatic method (NEFA-

C, ACS-ACOD Method, Wako Chemicals, Neuss, Germany). Glycerol levels were quantified by using an enzymatic colorimetric method (Randox Laboratories, Antrim, UK). Serum leptin concentrations were determined by means of a commercially available ELISA kit (ActiveTM Murine Leptin ELISA, Diagnostic System Laboratories, Webster, TX, USA).

2.3. Oxygen consumption and rectal temperature

Oxygen consumption was assessed by means of a Jacketed Oxygen Consumption Chamber (Harvard Apparatus, Edenbridge, UK). Rectal temperature was measured before and 1 h after drug administration by a rectal probe (Yellow Springs Instruments, Yellow Springs, OH, USA) connected to a Panlab thermometer pb 0331 (Panlab, Barcelona, Spain) as described previously (Gómez-Ambrosi et al., 1999).

2.4. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated by the ULTRASPECTM-II RNA ISOLATION SYSTEM (Biotech Laboratories, Houston, TX, USA) from 100 mg of BAT according to the manufacturer's instructions. The yield and quality of the RNA were assessed by measuring absorbance at 260 and 280 nm and by electrophoresis on 1.5% agarose gels. One microgram of RNA was used to synthesise first-strand cDNA after 30 min at 37°C treatment with 10 units of RNase free DNase I (Boehringer Mannheim GmbH, Mannheim, Germany). The RT reaction was carried out in a volume of 20 μl containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl_2 , 10 mM dithiothreitol, 100 ng of random hexamers (Boehringer Mannheim), 1 mM each dNTP (Bioline, London, UK), 20 units of RNase inhibitor (Promega, Madison, WI, USA), 200 units of M-MLV RT (GIBCO BRL, Life Technologies, Gaithersburg, MD, USA) and incubated at 37°C for 60 min. The enzyme was inactivated by heating at 95°C for 5 min. Four microlitres from the RT reaction were amplified in a 50 μl reaction mixture containing 40 ng of each primer, 16 mM $(\text{NH}_4)_2\text{SO}_4$, 67 mM Tris-HCl (pH 8.8), 2 mM MgCl_2 , 0.1% Tween-20, 0.2 mM each dNTP and 1 unit of BIOTAQTM polymerase (Bioline). Primers used to amplify PGC-1 cDNA (GenBank AB025784) were 5'-CCTTCT-GAACTTGATGTGA-3' (sense, 146–165) and 5'-AT-GCTCTTTGCTTTATTGCT-3' (antisense, 603–622), PPAR γ 2 (GenBank Y12882) 5'-TCTGATTATGGGT-GAACTC-3' (sense, 43–62) and 5'-TTTC-TACTCTTTTTGTGGATC-3' (antisense, 592–612) and β -actin (GenBank J00691) 5'-TCTACAAT-GAGCTGCGTGTG-3' (sense, 1599–1618) and 5'-GGTCAGGATCTTCATGAGGT-3' (antisense, 2357–2376). Primers for PGC-1 and PPAR γ 2 were

designed using the *Oligo*[®] 4.05 *Primer Analysis Software* (National Biosciences, Inc., Plymouth, MN, USA). cDNA was amplified for 34 (PGC-1), 43 (PPAR γ 2) and 25 cycles (β -actin), using the following parameters: 94°C for 30 s, 52°C (PGC-1), 51°C (PPAR γ 2) and 59°C (β -actin) for 30 s and 72°C for 30 s, with a final extension step at 72°C for 7 min. Although 43 cycles may seem to be a high number of cycles of amplification, it has been reported that during fasting, the otherwise elevated expression of PPAR γ 2 in fat is dramatically reduced (Vidal-Puig et al., 1996). Amplifications were linear under these conditions (Fig. 1) and were carried out in a GeneAmp[®] PCR System 2400 (Perkin Elmer, Norwalk, CT, USA). Primers and conditions used for UCP1 mRNA quantification were the same as described previously (Gómez-Ambrosi et al., 1999). All PCR reactions for each gene were performed at the same time and with the same batch of Taq polymerase in order to reduce variations in the efficiency of PCR. The amplified products were resolved in a 1.5% agarose gel with ethidium bromide. Levels of mRNA were expressed as the ratio of signal intensity for PGC-1 or PPAR γ 2 relative to that for β -actin. PCR bands intensity were determined by densitometric analysis with the *Gel Doc 1000 UV fluorescent gel documentation system* and *Molecular Analyst 1.4.1* software for quantitation of images (Bio-Rad, Hercules, CA, USA). The identity of PCR product amplifications was demonstrated after digestion with two restriction enzymes, yielding the predicted fragments in all the cases.

2.5. Statistical analysis

All results are expressed as mean \pm SE. Data were analysed using a one-way ANOVA followed by Fisher's LSD posthoc test. Pearson's correlation coefficient (r) was computed to explore the correlations between two variables. The calculations were performed using the SPSS/Windows version 7.5.2S statistical package (SPSS, Chicago, IL, USA). A P value lower than 0.05 was considered statistically significant.

3. Results

3.1. Effects on blood biochemistry and indicators of thermogenesis

The β_3 -AR agonist Trecadrine produced a marked lipolytic effect after acute administration as evidenced by the increase observed in circulating concentrations of NEFA and glycerol. Leptin did not significantly alter either NEFA or glycerol levels. Trecadrine-induced lipolysis was accompanied by a rise in rectal temperature and whole body oxygen consumption, as well as by an elevation of UCP1 mRNA expression in BAT. Leptin produced a rise in rectal temperature, but did not affect oxygen consumption or UCP1 mRNA expression despite a 20-fold increase in serum leptin concentrations (Table 1).

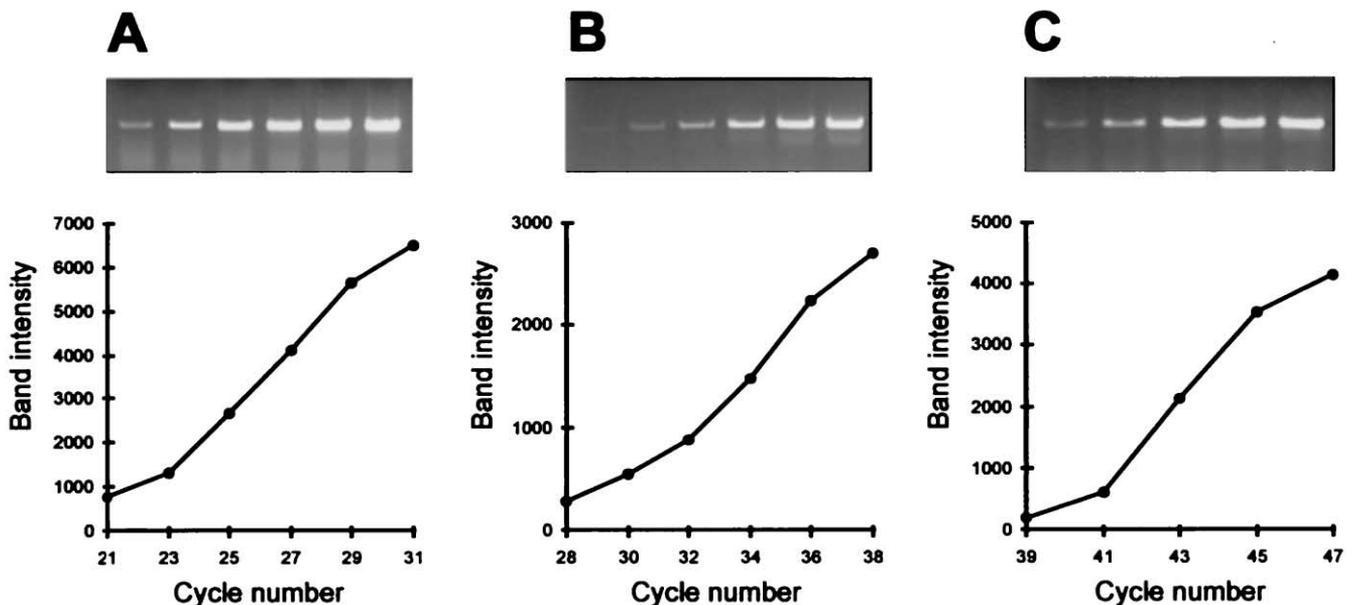


Fig. 1. Effects of the number of PCR cycles on the amplification of the cDNAs for β -actin (A), PGC-1 (B) and PPAR γ 2 (C) synthesised after RNA obtained from interscapular BAT. Top, representative photographs of PCR products at different numbers of amplification cycles; bottom, band intensities were quantified by using *Gel Doc 1000 UV fluorescent gel documentation system* and *Molecular Analyst 1.4.1* software (Bio-Rad) after ethidium bromide electrophoresis.

Table 1

Serum analyses and thermogenic indicators of animals treated with saline, the β_3 -AR agonist Trecadrine and leptin^a

	Saline	Trecadrine	Leptin
NEFA ^h (mmol/l)	0.93 ± 0.11	1.24 ± 0.07 ^b	1.00 ± 0.06
Glycerol (mmol/l)	0.33 ± 0.02	0.58 ± 0.04 ^d	0.28 ± 0.03 ^e
Leptin (ng/ml)	1.28 ± 0.51	0.81 ± 0.25	22.02 ± 1.12 ^{d,g}
Temperature change (°C)	0.12 ± 0.12	0.80 ± 0.30 ^b	0.74 ± 0.10 ^b
Oxygen consumption (ml/g per hour)	0.71 ± 0.05	1.09 ± 0.04 ^d	0.84 ± 0.08 ^e
BAT UCP1 mRNA (relative units)	1.00 ± 0.15	1.71 ± 0.11 ^c	0.83 ± 0.24 ^f

^a Data represent the mean ± SE of five rats per group.

^b $P < 0.05$.

^c $P < 0.01$.

^d $P < 0.001$.

^e $P < 0.05$.

^f $P < 0.01$.

^g $P < 0.001$ versus control and Trecadrine treated groups, respectively by one-way ANOVA and Fisher's LSD posthoc test.

^h Non-esterified fatty acids.

3.2. Effects on PGC-1 and PPAR γ 2 mRNA expression in BAT

Trecadrine induced a 4.5-fold increase in PGC-1 mRNA expression in BAT (Fig. 2) 1 h after i.p. administration ($P < 0.001$). However, PPAR γ 2 mRNA levels were not significantly modified. Levels of PGC-1 and PPAR γ 2 mRNA remained unaltered after acute leptin administration.

Pearson's correlation coefficient showed a statistically significant linear relationship between PGC-1 mRNA expression in BAT and UCP1 mRNA expression in BAT, circulating concentrations of NEFA and oxygen consumption (Fig. 3).

4. Discussion

PGC-1 is a coactivator deeply involved in adaptive thermogenesis regulating the transcriptional activity of PPAR γ and other nuclear receptors (Puigserver et al., 1998). In this sense, PGC-1 controls the expression of UCPs and turns on the biogenesis of mitochondria (Wu et al., 1999).

It has been shown previously that non-selective β -AR agonists induce the expression of PGC-1 in cultured HIB 1B brown adipocytes after 6 h of stimulation and that β -AR may mediate the effect of cold exposure on the induction of PGC-1 (Puigserver et al., 1998). Our results constitute the first evidence of the rapid (1 h) in vivo induction of PGC-1 mRNA expression in BAT by selective β_3 -AR stimulation. This finding seems to indicate that PGC-1 is one of the first molecules which are upregulated when it is necessary to activate the ther-

mogenic cellular pathways of BAT. This activation was confirmed by the marked stimulation of lipolysis and thermogenesis elicited by Trecadrine in this short period of time. An in vivo induction of PGC-1 mRNA in skeletal muscle after β_3 -AR treatment has also been described (Boss et al., 1999). However, this effect is likely to be indirect, given the fact that skeletal muscle does not seem to express physiologically significant amounts of β_3 -AR (Evans et al., 1996). Boss et al. (1999) have suggested that PGC-1 expression in muscle could be induced by NEFA. In this sense, we have found an association between NEFA and PGC-1 mRNA expression in BAT, suggesting that NEFA may induce the expression of PGC-1 in several tissues in addition to muscle.

It is known that β_3 -AR agonists produce hypertrophy of BAT (Ghorbani et al., 1997) and hypertrophy of adipose tissue is mediated by PPAR γ , at least in white adipose tissue (Kubota et al., 1999). Also, Gros et al. (1999) have recently reported that expression of the β_3 -AR in CHO cells increases the expression of PPAR γ . Moreover, exposure of rats to cold, which in turn

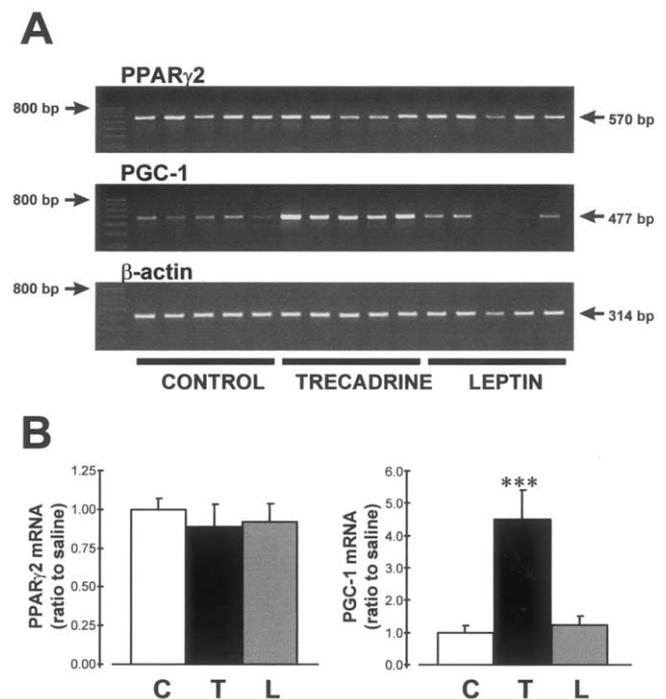


Fig. 2. Relative levels of PPAR γ 2 and PGC-1 mRNA in BAT of fasted rats treated acutely with the β_3 -AR agonist Trecadrine, leptin or saline: (A) photographs show sets of PCR products from BAT of individual rats ($n = 5$ per group). Size of PCR products are indicated. Left side lanes show a weight marker (100 Base-Pair Ladder, Pharmacia-Biotech, Uppsala, Sweden); (B) densitometric quantitation of PPAR γ 2 and PGC-1 mRNA expression in saline (C, white bar), Trecadrine (T, black bar) and leptin (L, grey bar) treated groups. Bars represent the mean ± SE of the ratio between PPAR γ 2 and PGC-1 to β -actin. The expression of PPAR γ 2 and PGC-1 in control rats was assumed to be 1. *** $P < 0.001$ versus saline- or leptin-treated animals by ANOVA and Fisher's LSD.

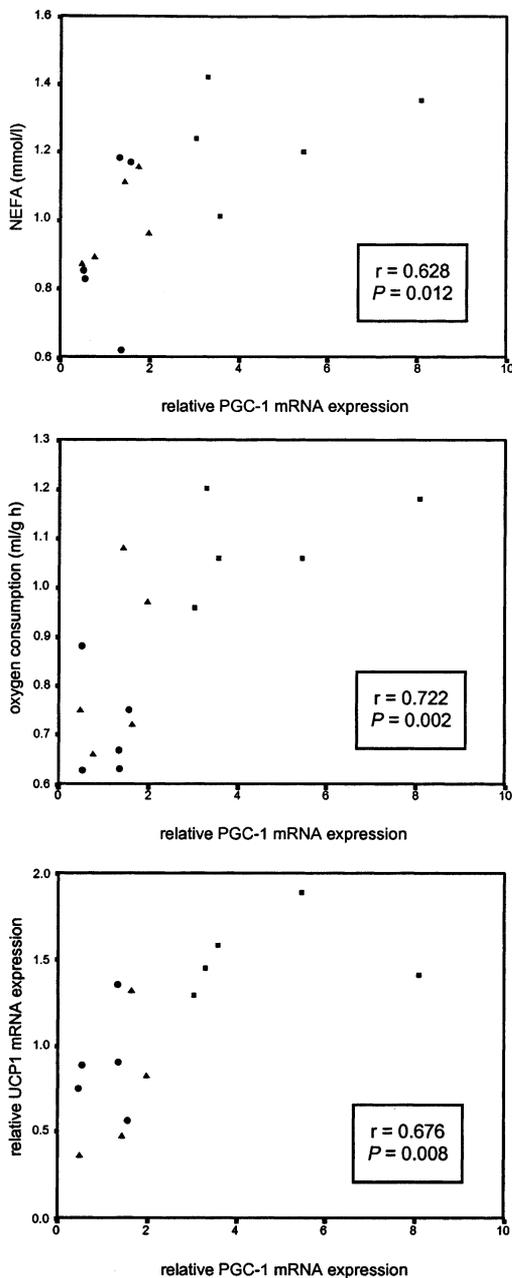


Fig. 3. Scatter diagrams showing the positive correlation found between PGC-1 mRNA expression in BAT and circulating concentrations of NEFA, oxygen consumption and UCP1 mRNA expression in BAT of fifteen male fasted rats, 1 h after i.p. administration of saline (●), Trecadrine (■) or leptin (▲). Pearson's correlation coefficient and P values are indicated.

stimulates β_3 -AR, has been shown to activate the PPAR pathway in BAT (Guardiola-Díaz et al., 1999). The fact that we have not detected any change in PPAR γ 2 may be due to the acute experimental conditions. Our results, taken together with published data, indicate that PPAR γ 2 receptors and their coactivation by PGC-1 are a key component of the early thermogenic response to β_3 -AR agonist in vivo in BAT, which is confirmed by the strong positive correlation

found between expression of PGC-1 and indicators of thermogenesis such as UCP1 expression in BAT and whole body oxygen consumption.

The PGC-1 is reduced in fasting and elevated upon refeeding (Wu et al., 1999). Therefore, we reasoned that leptin may be a candidate molecule to explain the observed changes. However, acute leptin treatment did not affect levels of PGC-1 in BAT. Leptin has been shown to increase lipolysis (Frühbeck et al., 1998) and thermogenesis (Scarpace et al., 1997). In this study, leptin did not stimulate lipolysis probably due to the low dose administered given the fact that higher doses are necessary to induce lipolysis in vivo (Frühbeck et al., 1998). Leptin-mediated long-term thermogenesis has been described to take place after several days by upregulation of UCP1 (Scarpace et al., 1997). However, this effect is not observed acutely and other mechanisms such as upregulation of UCP3 in skeletal muscle have been proposed to explain the short-term rise in temperature induced by leptin (Gómez-Ambrosi et al., 1999). These observations and the lack of effect of leptin in the expression of PGC-1 seems to indicate that the thermogenic activation of BAT by leptin is SNS-mediated and is only detected after long-term treatment.

There is no evidence of the effect of leptin on PPAR γ expression in BAT and the results concerning white adipose tissue are contradictory, since both upregulation (Qian et al., 1998) and downregulation have been found (Sanigorski et al., 2000).

In conclusion, the present study shows that administration of a selective β_3 -AR agonist induces a rapid upregulation of PGC-1 mRNA expression in BAT of fasted rats in parallel to stimulating thermogenesis and lipolysis, without a concomitant increase in PPAR γ 2 mRNA. Moreover, our results indicate that PGC-1 and PPAR γ 2 expression in BAT seems not to be acutely regulated by leptin.

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