THE EXPRESSION OF MITOCHONDRIAL BIOGENESIS SIGNALING FACTORS IN BROWN ADIPOCYTES IS INFLUENCED SPECIFICALLY BY 17β-ESTRADIOL, TESTOSTERONE AND PROGESTERONE

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Abbreviated title: Sex hormone effects on mitochondrial biogenesis factors.

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Abstract

The control of the mitochondrial biogenesis in brown adipose tissue (BAT), as part of the thermogenesis program, is a complex process that requires the integration of multiple transcription factors to orchestrate mitochondrial and nuclear gene expression. Despite the knowledge of the role of sex hormones on BAT physiology, little is known about the effect of these hormones on the mitochondrial biogenic program. The aim of this study was to determine the effect of testosterone, 17β-estradiol and progesterone on the expression of nuclear factors involved in the control of mitochondrial biogenesis and thermogenic function such as \( ppar \), \( pgc1a \), \( nrf1 \), \( gabpa \) and \( tfam \), and also an inhibitor of PI3K-Akt pathway, recently found to be involved in the control of mitochondrial recruitment (\( pten \)). For this purpose, an in vitro assay using cell cultured brown adipocytes was used to address the role of steroid hormones, progesterone, testosterone and 17β-estradiol on the mRNA expression of these factors by real time PCR. Thus, 17β-estradiol seemed to exert a dual effect, activating the PI3K-Akt pathway by inhibiting \( pten \) mRNA expression, and also inhibiting \( nrf1 \) and \( tfam \) mRNA expression. Progesterone seemed to positively stimulate mitochondrial biogenesis and BAT differentiation by increasing the mRNA expression of the \( gabpa-tfam \) axis and \( ppar \) respectively, but also exerted a negative output by increasing \( pten \) mRNA levels. Finally, testosterone inhibited the transcription of \( pgc1a \), the master factor involved in UCP1 expression and mitochondrial biogenesis. In conclusion, our results support the idea that sex hormones have direct effects on different mediators of the mitochondriogenesis program.

Keywords: brown adipose tissue, sex steroids, mitochondriogenesis program signaling, nuclear and mitochondrial transcription factors.
Introduction

Brown adipose tissue (BAT) is a specialised tissue, which, in small mammals and newborns, is responsible for non shivering thermogenesis (15, 39), the main mechanism for thermoregulatory heat production, with uncoupling protein 1 (UCP1) as the principal mediator (4, 5, 23). UCP1 is an inner-membrane mitochondrial protein whose function is to uncouple the respiratory chain from ATP synthesis by dissipating the proton gradient generated by the respiratory chain as heat (32).

The expression of uncoupling proteins and the regulation of mitochondrial biogenesis are critical points for the understanding of the transcriptional basis of adaptive thermogenesis to meet environmental and physiological stimuli such as cold exposure, diet, infection, exercise and oxidative stress (20).

The control of mitochondrial biogenesis is a complex biological process that requires the integration of multiple transcription factors to orchestrate the programs of mitochondrial and nuclear gene expression involved in energy production (12, 37).

The nuclear respiratory transcriptional factors NRF1 and GABPA (homologous to the human NRF2) are involved in the expression of many genes related to mitochondrial function and biogenesis, including those encoding subunits of the five respiratory complexes and the mitochondrial transcription factor A (TFAM), a nuclear encoded transcription factor that has been considered indicative of mitochondrial differentiation (9, 11, 19, 37).

Peroxisome proliferator activated receptor gamma (PPARγ) co-activator-1α (PGC1α) is a transcriptional co-activator that has the dual action of stimulating the expression and the transcriptional function of NRF1 and NRF2 (25, 47). Moreover, PGC1α participates in the induction of UCP1 expression by interacting with members of the nuclear
receptor superfamily such as PPARα (1, 46) and PPARγ (30). Thus, PGC1α co-
ordinates mitochondrial biogenesis and adaptive thermogenesis response in BAT (3)
through the co-activation of NRFs (47) and PPARs (38) and plays an essential role in
differentiation-induced mitochondrial biogenesis (43).

The PI3K-Akt (Phosphoinositide-3 kinase-v-akt murine thyoma viral oncogene
homolog 1) pathway, which is linked to radical oxygen species and insulin signaling,
converges with several other pathways in the regulation of NRF expression and function
(41), thus suggesting the involvement of this pathway and others in the control of
mitochondrial biogenesis in response to environmental and physiological stimuli (20)
and also in UCP1 expression (44). Thus, phosphatase and tensin homologue deleted on
chromosome 10 (PTEN), which inhibits the PI3K-Akt pathway, arises as an important
modulator involved in mitochondrial biogenesis and the thermogenesis program.

Sex differences in brown adipose tissue thermogenic capacity between males and
females have been described (31, 33, 35, 36). In particular, one of the most important
differences was related with mitochondrial recruitment, where female BAT showed
larger mitochondria and higher cristae density compared to males (36). To this extent,
17β-estradiol, testosterone and progesterone have been proposed as key factors that
could account in part for these differences. Thus, for example, previous data
demonstrated that these hormones modify β-adrenergic signaling and ucp1 expression
in brown adipocytes (24, 34) suggesting the importance of these hormones in the
thermogenic program.

To date, some studies have demonstrated effects of androgens and estrogens on gene
expression of some subunits of mitochondrial enzymes (10, 45). In the case of estrogens
it has also been demonstrated to increase some mitochondrial encoded genes directly
acting on mitochondrial DNA (6-8). Nevertheless, the information currently available as
to the direct effect of 17β-estradiol, testosterone and progesterone on brown adipose tissue mitochondrial recruitment is scarce.

The aim of this study was to determine the effect of 17β-estradiol, testosterone, and progesterone on the expression of some nuclear factors that are part of the circuits controlling mitochondrial biogenesis and thermogenic function (16) such as PPARγ, PGC1α, NRF1 and GABPA and TFAM, and also the inositol phosphatase PTEN. For this purpose, an in vitro assay using cell cultured brown adipocytes was used to address the role of the steroid hormones, progesterone, testosterone and 17β-estradiol on the mRNA expression of these factors “per se” but also in cultured adipocytes challenged with NA that modulates the expression of several mitochondrial transcription factors (47, 21) and is a key hormone involved in the recruitment of BAT. We hypothesize that sex hormones could exert a coordinate effect with NA in the mitochondrial recruitment.

In addition, we also investigated the expression of these factors in vivo in male and female BAT to analyze its expression as possible mechanism underlying the gender differences in the thermogenic and mitochondrial recruitment process.
Materials and Methods

Materials

Testosterone, 17-β-estradiol and progesterone were from Sigma (St Louis, MO, USA). Other cell culture reagents were supplied by Sigma (St Louis, MO, USA), Cultek (Madrid, Spain) and Gibco BRL (Gaithersburg, MD, USA); RNA isolation and PCR chemicals were from Roche Diagnostics (Basel, Switzerland) and routine chemicals were from Merck (Darmstadt, Germany) and Panreac (Barcelona, Spain). Microplate spectrophotometer was supplied by Bio-Tek Instruments, Inc (Vermont, USA); Gene Amp 9700 thermal cycler was supplied by Applied Biosystems (California, USA), and Lightcycler thermal cycler was supplied by Roche (Basel, Switzerland).

Animals and isolation of brown adipose tissue

Three-month male and female Wistar rats (n=14) were used for the in vivo assay (obtained from Charles River Laboratories, Wilmington, MA, USA). Animals were housed at 22 ºC, with a 12:12-h light-dark cycle, with free access to drinking water and standard chow pellets (Panlab, Barcelona, Spain). Animals were killed by decapitation at the start of the light cycle and interscapular brown adipose tissue rapidly removed, frozen in liquid N₂ and stored at -70 C until RNA isolation. Animal experiments were performed in accordance with general guidelines approved by our institutional ethics committee and EU regulations (86/609/EEC).

Primary cultures of brown adipocytes

Brown fat precursor cells were isolated from 4-week-old male NMRI mice (supplied by Charles River Laboratories, Wilmington, MA, USA) as previously described (26). The cervical, interscapular and axillar brown adipose tissue depots were dissected out from each mouse under sterile conditions. The tissue was pooled and incubated in HEPES...
buffer (pH 7.4, 2 ml/mouse), containing 0.2 % (w/v) crude collagenase type II. The tissue was digested for 30 min at 37 °C and vortexed every 5 min. The digest was poured through a 250 µm silk filter into 10 ml sterile tubes. The solution was then cooled at 4 °C for 15-30 min to allow the mature brown fat cells and lipid droplets to float. The infranatant was filtered through a 30 µm silk filter into 10 ml sterile tubes and precursor cells were collected by centrifugation for 10 min at 700xg, washed in DMEM, pelleted again, and resuspended in 0.5 ml of culture medium per mouse. 0.2 ml of pooled final precursor cell suspension was inoculated in 6 well plates each well containing 1.8 ml of culture medium. This was day 0. From that moment on, cells were incubated at 37 °C in 8 % CO₂.

For the first 6 days, the preadipocytes were grown in 2 ml of a medium consisting of DMEM supplemented with 10% newborn calf serum, 4 nM insulin, 4 mM glutamine, antibiotics (50 IU penicillin/ml and 50 µg streptomycin/ml), 10 mM HEPES and 25 µg sodium ascorbate/ml. This medium-containing serum was changed on day 1 (cells were previously washed with DMEM) and day 3. On day 6, the medium was discarded and a serum-free medium was added, consisting of DMEM-F12 (1:1), free fatty acid bovine serum albumin 0.5%, 4 nM insulin, 4 mM glutamine, antibiotics (50 IU penicillin/ml and 50 µg streptomycin/ml), 10 mM HEPES and 25 µg sodium ascorbate/ml.

The different treatments were carried out on day 6, when cells presented a differentiated morphology and lipid accumulation. Serum-free medium was used to avoid hormonal interference. Testosterone, 17-β-estradiol or progesterone (from 10⁻⁹ to 10⁻⁷ M) were dissolved in ethanol and added to the corresponding wells, with the final ethanol concentration never exceeding 0.01%. An equivalent volume of ethanol was added to untreated controls. On day 7, after 24h treatment, the cells were exposed to
noradrenaline (NE) $10^{-7}$M for 6 h. Then, the cells were harvested with TriPure™ for RNA isolation.

**RNA isolation**

Total RNA was isolated from BAT and cells using TriPure™ reagent, following the instructions of the manufacturer. RNA was determined in triplicate using a microplate spectrophotometer set at 260 nm.

**Reverse-transcription**

1 μg of total RNA was reverse transcribed to cDNA at 42 °C for 15 min with 25 U MuLV reverse transcriptase in a 10μl volume of RT reaction mixture containing 10mM Tris-HCl (pH 9.0 at 25°C), 50mM KCl, 0.1% Triton®X-100, 2.5 mM MgCl2, 2.5 μM random hexamers, 10 U RNAase Inhibitor and 500 μM of each dNTP in a DNA GeneAmp 9700 thermal cycler.

**Real time RT-PCR**

The primers used were designed using specific primer analysis software Primer3 (Whitehead Institute for Biomedical Research) and Oligo Analyzer 3.0 (Integrated DNA Technologies, Inc.) and the specificity of the sequences was analyzed by Fasta in the BLAST database (http://www.ncbi.nlm.nih.gov/BLAST/) (see Table 1). Real-time PCR was performed using a LightCycler™ rapid thermal cycler system (Roche Diagnostics, Basel, Switzerland). Reactions were performed per duplicate in a 10 μL volume containing 3 μL of cDNA sample (diluted 1/10) using 0.5 μM primers, 3 mM of MgCl2 as well as dNTPs, Taq DNA polymerase and reaction buffer provided in the LightCycler FastStart DNA Master SYBR Green I mix. All real-time conditions are summarized in Table 1. Product specificity was confirmed in initial experiments by agarose gel electrophoresis and routinely by melting curve analysis. For mathematical
analysis, the crossing points (CP) values were used for each transcript. CP is defined as the point at which fluorescence of the transcript rises appreciably above the background fluorescence. ‘Fit Point Method’ was performed in the LightCycler software 3.3, at which CP was measured at a constant fluorescence level. PCR efficiencies of each amplicon (between 1.7 and 1.9 in our study) were calculated using the following formula: \( E = \frac{F}{F_0} \left( \frac{1}{n-n_0} \right) \), where \( n \) and \( n_0 \) were the crossing point values of \( F \) and \( F_0 \), fluorescence signals of the linear phase of the logarithmic amplification curve.

Statistics

Statistical PCR data analysis was performed using the Relative Expression Software Tool (REST). The statistical model used was a Pair Wise Fixed Reallocation Randomisation Test© (28). Differences in expression between groups, expressed as Fold Change, were assessed using the means for statistical significance by randomisation tests, a proper model to avoid the normal distribution assumption of the data. The level of probability was set at \( p<0.05 \) as statistically significant. This method included the correction of data using 18S that was validated as housekeeping gene.
Results

Gender differences in the gene expression of mitochondrial biogenic factors in brown adipose tissue.

Female brown adipose tissue showed a lower expression of *nrf1* (0.5 fold change, \( p=0.03 \)) compared to males (Figure 1). Similarly *gabpa* was also lower in females compared to males, although it did not reach statistical significance (0.5 fold change, \( p=0.09 \)). *tfam*, a transcription factor regulated by NRfs, followed the same pattern (0.5 fold change in females) but failed to be significant (\( p=0.37 \)). There were no differences in the expression of *ppar\( \gamma \) (\( p=0.24 \)) or *pgc1\( \alpha \) (\( p=0.24 \)). *pten* expression was markedly lower in female brown adipose tissue compared to males (0.4 fold change, \( p<0.02 \)).

17\( \beta \)-estradiol, testosterone and progesterone have a direct and differential effect on mitochondrial biogenic factors gene expression in cultured brown adipocytes.

Progesterone treatment

Progesterone induced an upregulation of *tfam* mRNA levels at maximum dosage both in the absence and presence of NA (1.6 fold change, \( p=0.02 \) and \( p=0.04 \) respectively) (Figure 2a). This increase was accompanied with an increase in *gabpa*, although this only reached statistical significance in combination with NA (1.9 fold change, \( p=0.05 \)). No changes were observed for *nrf1*. This progesterone effect in the *gabpa*-tfam axis was not reflected either in its upstream link *pgc1\( \alpha \).*

Progesterone treatment increased the mRNA expression of *ppar\( \gamma \) at maximum concentration (2.4 fold change in the absence of NA, \( p=0.04 \)). Along the same lines, progesterone exerted an upregulation of *pten* mRNA in combination with NA treatment (1.4 fold change, \( p=0.04 \)).
 Estradiol treatment brought about a downregulation of nrf1 (Figure 2b), although this was only significant at the lower dosage tested in combination with NA (0.6 fold change, p=0.04). gabpa was also downregulated although differences were not statistically significant (p=0.19). Likewise tfam mRNA expression was also downregulated at the lower dosage tested in combination with NA (0.5 fold change, p=0.03). pgc1α, and pparγ mRNA levels were not modified under estradiol treatment, although pparγ showed a tendency to be downregulated.

pten mRNA expression was downregulated under estradiol treatment (0.8 fold change, p<0.02), both in the absence and presence of NA (10^{-7} M).

Testosterone treatment

Testosterone exerted a downregulation of pgc1α in the absence of NA (approx. 0.4 fold change, p<0.04 at all the concentrations tested except for 10^{-8}M which did not reach statistical significance) (Figure 2c). NA treatment in the presence of testosterone seemed to reverse the androgen effects. Nevertheless, this effect was only significant at maximum testosterone dosage (approx 2 fold with respect to non NA treated cells). Moreover, testosterone did not exert any effect on pgc1α downstream targets nrf1, gabpa or tfam mRNA expression.

No effect was observed after testosterone treatment on pparγ levels, although a tendency to be downregulated was observed. Testosterone treatment also induced a downregulation of pten levels at maximum concentration, but this was reversed in the presence of NA.
Discussion

Recent studies have provided insights into the pathways regulating mitochondrial biogenesis. The evidence supports a model where regulated coactivators communicate physiological signals to specific transcription factors. These events result in the activation of the genes needed for mitochondrial biogenesis and respiration function (16). In this paper we provide evidence that 17β-estradiol, progesterone and progesterone signaling might play an important role in the modulation of factors involved in the mitochondriogenesis program.

In vivo, female BAT showing lower nfr1 and tfam mRNA expression could apparently be in discrepancy with the higher mitochondrial recruitment and function and higher β-adrenergic signaling depicted in female BAT (36). Nevertheless, the higher female mitochondrial recruitment, could, at least in part, be explained by the lower pten phosphatase mRNA expression in female BAT compared to males, despite the low levels of nfr1-tfam mRNA, since the activation of PI3K-Akt pathway, which is inhibited by PTEN phosphatase (18), brings about the phosphorilation and nuclear translocation of NRF1 binding to TFAM and finally increase in mtDNA (29, 41), and UCP1 expression (44). It is relevant to consider the overall effect of these different signal transduction pathways to address the net signal input in the mitochondrial program.

Progesterone treatment exerted an upregulation of tfam mRNA in brown adipocytes in vitro. These results are supported in vivo by a strong correlation between serum progesterone and TFAM protein in BAT of female rats (Frontera M., unpublished data). The upregulation of tfam mRNA by progesterone could be due to the increased gabpa mRNA levels, and strongly suggest that progesterone by means of a co-ordinated rise in gabpa and tfam would lead to an enhanced replication and transcription of
mitochondrial DNA, at least in vitro, conducting to the stimulation of the
mitochondriogenesis program. Nevertheless, it must be considered that mRNA
expression does not necessary reflect activation of these factors in the frame of the
mitochondrial biogenesis.

Moreover, the increase in $p_p a_r_y$ expression found –that could be mainly $p_p a_r_y2$ (27)–
after progesterone treatment, could be responsible for the induction of UCP1 mRNA
expression (34) and for promoting the differentiation of brown adipocytes (42).  
Nevertheless, progesterone treatment in adipocytes challenged with NA treatment also
brought about an upregulation of $pt_e_n$ mRNA levels suggesting that progesterone may
also exert a negative input in the stimulation of mitochondrial program by inhibiting the
PI3K-Akt pathway.

$17\beta$-estradiol seemed to exert a dual effect on the mitochondrial biogenic program.
Firstly, inhibiting it, through the downregulation of $n_r_f_1$, $g_a_h_p_a$ and $t_f_a_m$ mRNA
expression showed in brown adipocytes stimulated with NA. Thus, $17\beta$-estradiol effect
may explain, at least partially, the lower $n_r_f_1$ and $t_f_a_m$ mRNA levels shown by female
BAT, which is more $\beta$-adrenergic activated than males (36). Secondly, $17\beta$-estradiol
also exerted a downregulation of $p_t_e_n$ mRNA levels in cell cultured brown adipocytes,
suggesting an activation of the PI3K-Akt pathway and therefore a mitochondrial
recruitment stimulatory signal. Recently, estradiol has been demonstrated to inactivate
PTEN activity by phosphorilation and hence influence PTEN activity, suggesting both a
chronic and acute control mechanism of PTEN by estrogens (14). To this extent, it is
tempting to speculate the estradiol signal could be responsible for the gender differences
in $p_t_e_n$ mRNA expression and hence contribute to the differences between genders in
mitochondrial biogenesis and thermogenic program. Nevertheless, it is worth to notice
that 17β-estradiol and testosterone may not necessary reflect a male or female profile in the mitochondrial biogenesis program framework (22, 40).

Testosterone treatment triggered a marked downregulation of pgc1α mRNA levels. Since PGC1α is also directly involved in the transcriptional control of UCP1 (1), this androgen induced downregulation in pgc1α mRNA levels could be related with the drop of ucp1 mRNA expression observed in a similar experimental model (34). No coordinate downregulation was observed either in nrf1 or gabpa mRNA, two PGC1 downstream targets, indicating a possible effect of sex hormones on its expression independently of PGC1 action. Thus, it could be hypothesized that testosterone could mainly exert its negative effect on thermogenesis by affecting a short term response such as UCP1 recruitment rather than a long term response such as the mitochondrial biogenesis process, although we can not discard that the hormonal exposure time is not enough to see this co-ordinated effect mediated on NRFs by PGC1α. To this extent, it has been recently discovered that this co-activator can interact with androgen and estrogen receptors (17), suggesting that sex hormones could exert a role in the PGC1α function in addition to transcriptional mechanisms. Curiously, although pgc1α mRNA is strongly induced by β-adrenergic agonists (13, 30) and also by physiological conditions known to increase ATP or heat demand, such as exercise and cold exposure, our study failed to show any modulation by NA at the concentration or time exposure used, although these conditions were enough to stimulate UCP1 transcription in a similar model (34). Nevertheless, NA reverted the downregulation of pgc1α under testosterone treatment.

Similarly to 17β-estradiol, a high dosage of testosterone induced a downregulation of pten mRNA expression. Since testosterone but not dihydrotestosterone were used in this experiment, we can not discard that the testosterone effect may in fact, have been due to
the conversion of testosterone into estradiol by means of aromatase (2). PTEN has been
demonstrated to be physiologically downregulated under cold exposure, a situation
where β-adrenergic signaling is activated. However, in our study, no regulation of pten
mRNA under NA treatment was reached, except in combination with testosterone,
where an upregulation of pten mRNA was observed. Further research is needed to
clarify this point.

In summary, we have demonstrated that 17 β-estradiol, testosterone and progesterone
play a role in the control of mitochondrial biogenesis by modifying the mRNA
expression of several mitochondrial transcription factors and other upstream links of
specific signaling pathways. To date, it is tempting to speculate that these changes
could, at least in part, be responsible for the gender differences in the mitochondrial
recruitment process. The characterization of these signaling pathways is a challenging
work that will fuel more research in the study of mitochondrion biology.
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References


46. Vega RB, Huss JM, and Kelly DP. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of

Figure legends

Figure 1. Gender differences in the mRNA expression of *pparγ, pgc1α, nrf1, gabpa, tfam* and phosphatase *pten*. mRNA expression of mitochondrial biogenesis related factors in male and female brown adipose tissue (n=7). The data represent fold changes of 6 samples per group and were established using each control as 1. Differences in expression between groups were assessed by a Pair Wise Fixed Reallocation Randomisation Test©. The level of probability was set at p<0.05 as statistically significant, were * denotes significant differences between males and females.

Figure 2. Effect of progesterone (a), 17β-estradiol (b) or testosterone (c) treatment on mRNA expression in primary culture of brown adipocytes. Differentiated brown adipocytes were either nontreated (open bars) or stimulated (closed bars) for 6 h with noradrenaline (10^{-7} M) after 24 h treatment with a) progesterone, b) 17β estradiol, or c) testosterone, (10^{-9}–10^{-7} M). The data represent fold changes of 5-6 samples per group and were established using each control as 1. Differences in expression between groups were assessed by a Pair Wise Fixed Reallocation Randomisation Test©. The level of probability was set at p<0.05 as statistically significant, were * denotes significant differences between hormone-treated versus control (nor sex hormone, nor NA treatment) and # denotes significant differences between hormone-treated versus hormone-treated plus NA.
# TABLES

**Table 1.** Oligonucleotide primer sequences and amplification conditions

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<th>Annealing Temp, °C (time, s)</th>
<th>Elongation Temp, °C (time, s)</th>
<th>Melting temp (°C)</th>
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