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FSH and bFGF regulate the expression of genes involved in Sertoli cell energetic metabolism

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ABSTRACT

The purpose of this study was to investigate if FSH and bFGF regulate fatty acid (FA) metabolism and mitochondrial biogenesis in Sertoli cells (SC). SC cultures obtained from 20-day-old rats were incubated with 100 ng/ml FSH or 30 ng/ml bFGF for 6, 12, 24 and 48 h. The expression of genes involved in transport and metabolism of FA such as: fatty acid transporter CD36 (*FAT/CD36*), carnitine-palmitoyltransferase 1 (*CPT1*), long- and medium-chain 3-hydroxyacyl-CoA dehydrogenases (*LCAD*, *MCAD*), and of genes involved in mitochondrial biogenesis such as: nuclear respiratory factors 1 and 2 (NRF1, NRF2) and transcription factor A (*Tfam*), was analyzed. FSH stimulated *FAT/CD36*, *CPT1*, *MCAD*, *NRF1*, *NRF2* and *Tfam* mRNA levels while bFGF only stimulated *CPT1* expression. A possible participation of PPARβ/δ activation in the regulation of gene expression and lactate production was then evaluated. SC cultures were incubated with FSH or bFGF in the presence of the PPARβ/δ antagonist GSK3787 (GSK; 20 μM). bFGF stimulation of *CPT1* expression and lactate production were inhibited by GSK. On the other hand, FSH effects were not inhibited by GSK indicating that FSH regulates the expression of genes involved in FA transport and metabolism and in mitochondrial biogenesis, independently of PPARβ/δ activation. FA oxidation and mitochondrial biogenesis as well as lactate production are essential for the energetic metabolism of the seminiferous tubule. The fact that these processes are regulated by hormones in a different way reflects the multifarious regulation of molecular mechanisms involved in Sertoli cell function.

1. Introduction

The process of spermatogenesis and consequently male fertility is dependent upon the somatic cells that are present in the testis. Leydig cells are essential because of androgen production, and Sertoli cells are absolutely necessary in order to provide an adequate and protected environment within the seminiferous tubules. Sertoli cell carbohydrate metabolism presents some interesting characteristics. Glucose is metabolized to lactate since germ cells situated beyond the blood testis barrier rely on Sertoli cell production of this hydroxyacid to obtain energy (Boussouar and Benahmed, 2004). Hence, glucose is not an essential source of energy for Sertoli cells; in fact it has been shown that this cell type can survive in culture for at least 48 h in the absence of glucose (Riera et al., 2009). In this metabolic context, it has been shown that the oxidation of fatty acids (FA) can yield much of the energy required by Sertoli cells (Jutte et al., 1985).

Needless to say, FA must enter into the cell in order to be metabolized. One of the proteins involved in FA uptake is *FAT/CD36*, an integral membrane glycoprotein which has been found in a wide variety of cells (Abumrad et al., 1993; Coburn et al., 2000; Bonen et al., 2004). Once FA are incorporated into the cells, they are activated by covalently linking to coenzyme A forming an acyl-CoA derivative. Thereafter, the carnitine-palmitoyltransferase 1 (*CPT1*) is responsible for the entrance of acyl-CoA into the mitochondria where β-oxidation takes place (Rasmussen and Wolfe, 1999). β-oxidation involves the stepwise removal of acetyl-CoA molecules from the shrinking FA chain. The first step is the α-β-dehydrogenation of the acyl-CoA by a family of specific chain length acyl-CoA dehydrogenases (Ghisla and Thorpe, 2004). This family includes, among others, long chain (*LCAD*) and medium chain (*MCAD*) dehydrogenases. The molecular events necessary for FA oxidation are strictly controlled and their regulation varies within different tissues (McGarry and Foster, 1980; Lopaschuk et al., 1994; Rasmussen and Wolfe, 1999). Additionally, an association between FA oxidation and regulation of mitochondrial biogenesis in various tissues has been observed

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(Deepa et al., 2013; O'Neill et al., 2013; Santillo et al., 2013). To this respect, it has been shown that regulation of mitochondrial biogenesis is a crucial mechanism for cellular adaptation in response to hormonal environment and energy deprivation. For instance, induction of mitochondrial biogenesis can be observed in skeletal muscle in response to exercise (Joseph et al., 2006), in brown adipose tissue in adaptative thermogenesis (Butow and Bahassi, 1999) and in white adipose tissue during differentiation (Wilson-Fritch et al., 2003). In Sertoli cells, a cell type that utilizes FA as the main source of energy, it is reasonable to assume that the genes involved in mitochondrial biogenesis and those involved in FA oxidation may be somehow associated and regulated by hormones.

It is well known that Sertoli cells are under the control of follicle-stimulating hormone (FSH) and a plethora of locally produced factors (Gnessi et al., 1997). Basic fibroblast growth factor (bFGF), which belongs to the family of locally produced peptides, regulates several biological processes in a wide range of tissues and organs including the testis (Han et al., 1993). We have previously observed that FSH and bFGF regulate several mechanisms involved in lactate production in Sertoli cells (Meroni et al., 2002; Riera et al., 2002, 2003). However, the possible participation of these hormones in the regulation of FA metabolism and in mitochondrial biogenesis in Sertoli cells has not been analyzed yet.

Recently, we have observed that pharmacological PPARα and PPARβ/δ activation regulates the expression of genes involved in FA metabolism such as *FAT/CD36*, *CPT1*, *LCAD* and *MCAD* in Sertoli cells. We have also observed that PPARβ/δ activation can simultaneously regulate the expression of the above-mentioned genes and lactate production. These results were interpreted as a reflection of a coordinated mechanism which will ensure the concomitant provision of energy to Sertoli and germ cells (Regueira et al., 2014). The mechanisms involved in a possible activation of this nuclear receptor under physiological conditions, which may include hormonal regulation, have not been analyzed yet in Sertoli cells.

In the present study, we investigated whether FSH and bFGF are able to regulate molecular mechanisms involved in FA metabolism and in mitochondrial biogenesis. In addition, in the case that FA metabolism and mitochondrial biogenesis are regulated by FSH and bFGF, if PPARβ/δ activation has any role in this hormonal regulation of Sertoli cell energetic metabolism.

2. Materials and methods

2.1. Materials

Human recombinant bFGF were purchased from Invitrogen (Life Technologies, Rockville, MD). Ovine FSH (NIH-oFSH-S-16) was obtained from the National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD. GSK and all other drugs and reagents were purchased from Sigma–Aldrich (St Louis, MO, USA).

2.2. Sertoli cell isolation and culture

Sertoli cells from 20-day-old Sprague–Dawley rats were isolated as previously described (Meroni et al., 2002). Briefly, decapsulated testes were digested with 0.1% collagenase and 0.006% soybean trypsin inhibitor in Hanks' balanced salt solution for 5 min at room temperature. Seminiferous tubules were saved, cut and submitted to 1 M glycine–2 mM EDTA (pH 7.4) treatment to remove peritubular cells. The washed tubular pellet was then digested again with collagenase for 10 min at room temperature to remove germinal cells. The Sertoli cell suspension, collected by sedimentation, was resuspended in culture medium which consisted of a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's medium, supplemented with 20 mM HEPES, 100 IU/ml penicillin, 2.5 μg/ml amphotericin B, 1.2 mg/ml sodium bicarbonate, 10 μg/ml transferrin, 5 μg/ml insulin, 5 μg/ml vitamin E and 4 ng/ml hydrocortisone. Sertoli cells were cultured in 25 cm^2 flasks, 6- or 24-multiwell plates (5 μg DNA/cm^2) at 34 °C in a mixture of 5% CO_2:95% air.

No myoid cell contamination was revealed in the cultures when an immunoperoxidase technique was applied to Sertoli cell cultures using a specific antiserum to smooth muscle α actin. Remaining cell contaminants were of germ cell origin and this contamination was below 5% after 48 h in culture as examined by phase contrast microscopy.

2.3. Culture conditions

Sertoli cells were allowed to attach for 48 h in the presence of insulin and medium was replaced at this time with fresh medium without insulin. Stimulation with FSH (100 ng/ml) or bFGF (30 ng/ml) was performed on day 3. Cells incubated for 6, 12, 24 or 48 h with FSH or bFGF in the absence or presence of 20 μM GSK3787 (GSK) harvested on day 5 were used to evaluate *Tfam*, *NRF1*, *NRF2*, *FAT/CD36*, *CPT1*, *LCAD*, *MCAD* mRNA levels and CPT1 protein levels. Cells treated for 24 or 48 h with FSH or bFGF in the absence or presence of GSK were used to perform fatty acid oxidation assay. The 48-h conditioned media obtained in the above-mentioned incubations were used to determine lactate production.

2.4. Real-time PCR (RT-qPCR)

Total RNA was isolated from Sertoli cells cultured in 6-multiwell plates with TRI Reagent (Sigma–Aldrich). The amount of RNA was estimated by spectrophotometry at 260 nm. Reverse transcription was performed on 2 μg RNA at 42 °C for 50 min with a mixture containing 200 U SuperScript II reverse transcriptase enzyme, 125 ng random primers and 0.5 mM dNTP Mix (Invitrogen).

RT-qPCR was performed by a Step One Real Time PCR System (Applied Biosystems, Warrington, UK). The specific primers for real-time PCR were: 5′-ACCAGGCCACATAGAAAGCA-3′ and 5′-CACCAATAACGGCTCCAGTAA-3′ for *FAT/CD36*; 5′-AAAGGTCTGGGAGTGATTGG-3′ and 5′-CCATTCTCCACCAAAAAGAGG-3′ for *LCAD*; 5′-CGAGCACAACACACAAAACC-3′ and 5′-TTCCTCTCTGGCAAACTTCC-3′ for *MCAD*; 5′-GGCAGAAACGCCTAAAGAAG-3′ and 5′-CCGAGGTCTTTTTGGTTTTC-3′ for *Tfam*; 5′-GCTCATCCAGGTTGGTACTG-3′ and 5′-TTTGTTCCACCTCTCCATCAG-3′ for *NRF1*; 5′-GGCAGAGACATTCCCATTTG-3′ and 5′-GATCAGGGGTGGTGAAGACT-3′ for *NRF2*; 5′-AGTTCTTTGCTGACCTGCTG-3′ and 5′-TTTATGTCCCCCGTTGACTG-3′ for *HPRT1*. Amplification was carried out as recommended by the manufacturer: 25 μl reaction mixture containing 12.5 μl of SYBR Green PCR Master mix (Applied Biosystems), the appropriate primer concentration and 1 μl of cDNA. The relative cDNA concentrations were established by a standard curve using sequential dilutions of a cDNA sample. The data were normalized to *HPRT1*. The amplification program included the initial denaturation step at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 1 min. Fluorescence was measured at the end of each extension step. After amplification, melting curves were acquired and used to determine the specificity of PCR products. The comparative $\Delta\Delta$Ct method was used to calculate relative gene expression.

2.5. Northern blot analysis

Total RNA was isolated from Sertoli cells cultured in 25 cm^2 tissue culture flasks using the TRI Reagent (Sigma–Aldrich). The amount of RNA was estimated by spectrophotometry at 260 nm.

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For Northern blot analysis 10 μg total RNA was electrophoresed on a 1% agarose–10% formaldehyde gel. After migration, RNAs were transferred to Hybond-N nylon membrane (Amersham Pharmacia Biotech, Buenos Aires, Argentina) by capillary transfer with 10 × SSC (1.5 M NaCl and 0.15 M sodium citrate, pH 7.4) and fixed with U.V. Stratalinker (Stratagene Cloning Systems, La Jolla, CA, USA). cDNA probes were labeled with [α-^{32}P]deoxy-CTP (Amersham Pharmacia Biotech) using a random-primed labeling kit (Prime-a-Gene Labeling System, Promega Corporation, Madison, USA). The cDNA probes used were the following: *CPT1a*, a 785b probe previously obtained using a RT-PCR technique with specific primers (5′-CACGAAGCCCTCAAACAGAT-3′ and 5′-AATGTGCGACGA TACAGCAG-3′) and a *18S* oligonucleotide. Blots were prehybridized for 5 h at 42 °C in 50% formamide, 0.75 M NaCl, 20 mM sodium phosphate (pH 7.5) and 1 mM EDTA, 5 × Denhart's solution, 10% dextran sulfate, 0.5% SDS and 100 μg/ml herring sperm DNA. Hybridization was then performed overnight at 42 °C in the same hybridization buffer containing 1–4 × 10^6 cpm/ml ^{32}P-labeled probe. Membranes were washed utilizing different astringency conditions depending on the probe utilized. Membranes were exposed to Amersham Hyperfilm™ ECL (Ge Healthcare Limited, Buckinghamshire, UK) at −70 °C, for variable periods of time according to the probe utilized. The intensities of the autoradiographic bands were estimated by densitometric scanning using NIH Image software (Scion Corporation, Fredereck, MD, USA). The 18S signal was used to standardize mRNA contents.

2.6. Western blot analysis

Cells cultured in 6-multiwell plates were washed once with PBS at room temperature. Then, 200 ml of PBS containing 2 μl of protease inhibitor cocktail (P-8340; Sigma–Aldrich), 1 mM NaF, 1 mM EGTA, 1 mM EDTA, 50 nM okadaic acid and 2 mM PMSF was added to each well. Cells were then placed on ice and disrupted by ultrasonic irradiation. For Western blot analysis, 200 μl of 2× Laemmli buffer (4% w/v SDS, 20% v/v glycerol, 10% v/v 2-mercaptoethanol, 0.004% w/v bromophenol blue, and 0.125 M Tris–HCl, pH 6.8) was added and thoroughly mixed. Samples were immersed in boiling water bath for 5 min and then immediately settled on ice. Proteins (40 μg in each lane) were resolved in 10% SDS–PAGE (10% acrylamide/bisacrylamide for the resolving gel and 4.3% acrylamide/bisacrylamide for the stacking gel) in a Mini Protean 3 cell (Bio-Rad, Hercules, CA, USA). After SDS–PAGE, gels were electrotransferred at 100 V for 60 min onto PVDF membranes (Hybond-P, GE Healthcare Life Sciences, Piscataway, NJ, USA) using a Mini Trans-blot cell (Bio-Rad). Membranes were probed with specific antibodies that recognized carnitine-palmitoyltransferase 1 (CPT1) [rabbit polyclonal anti-sera against a specific oligopeptide for rat liver CPT I isoform (Lavarías et al., 2009)] or total Akt (T-Akt) (T-Akt antibody, Cell Signaling Technology, Inc., Danvers, MA, USA). A 1:4000 (CPT1) or 1:1000 (T-Akt) dilutions of primary antibodies were used. For chemiluminescent detection of the blots, a commercial kit from Cell Signaling Technology was used. The intensities of the autoradiographic bands were estimates by densitometry scanning using NIH Image Software (Scion Corporation, Frederick, MD, USA). Levels of T-Akt were used as loading control.

2.7. Fatty acid oxidation assay

Fatty acid oxidation was performed measuring the release of ^3H_2O to the incubation medium from [^{3}H]palmitate. Briefly, Sertoli cells cultured in 24-multiwell plates, were incubated for 4 h at 34 °C with culture medium (500 μl/well) containing 1% fatty acid free BSA, 2 μCi/ml of [9,10(*n*)-^{3}H] palmitic acid, 10 μM palmitic acid and 0.25 mM L-carnitine. Triplicate incubations were performed. After the incubation period, medium was collected and treated on ice for 30 min with an equal volume of 10% trichloroacetic acid. Thereafter a centrifugation at 13,000*g* for 5 min was performed and the supernatant recovered and treated with 5 volumes of methanol:chloroform (2:1) and 2 volumes of KCl:HCl 2 M. Phases were separated by centrifugation at 3000*g* for 5 min. An adequate aliquot of the aqueous phase was taken and counted in a liquid scintillation spectrophotometer.

2.8. Mitochondrial/nuclear DNA ratios

Mitochondrial biogenesis was determined by the ratio of mitochondrial DNA (mtDNA) to nuclear DNA (nDNA), quantified by RT-qPCR, assuming that nDNA levels remain constant. Nuclear and mitochondrial DNA was isolated based on the use of cetyltrimethylammonium bromide (CTAB) lysis buffer and isoamyl alcohol–chloroform extraction (Del Sal et al., 1989). DNA primers were designed to detect a specific mitochondrial DNA sequence (D-loop) and the nuclear encoded GLUT1. The specific primers were: 5′-TCTACCATCCTCCGTGAAATCA-3′ and 5′-TCACCCCCAGGAC GAATG-3′ for D-loop; 5′-CGAGCACAACACACAAAACC-3′ and 5′-TT CCTCTCTGGCAAACTTCC-3′ for GLUT1. Amplification was carried out as recommended by the manufacturer: 25 μl reaction mixture containing 12.5 μl of SYBR Green PCR Master mix (Applied Biosystems), the appropriate primer concentration and 1 μl of DNA. The relative DNA concentrations were established by a standard curve using sequential dilutions of a DNA sample. The amplification program included the initial denaturation step at 95 °C for 10 min, 40 cycles of denaturation at 95 °C for 15 s, and annealing and extension at 60 °C for 1 min. Fluorescence was measured at the end of each extension step. After amplification, melting curves were acquired and used to determine the specificity of PCR products. Relative values for D-loop and GLUT1 within samples were used to obtain a ratio of mtDNA to nDNA in each sample. Each sample was analyzed in triplicate. Values represent mean fold change ± S.D.

2.9. Lactate determination

Conditioned media obtained from cells cultured in 24-multiwell plates were used to determine lactate production. Lactate was measured by a standard method involving the conversion of NAD^+ to NADH. The amount of NADH was determined as the rate of increase of absorbance at 340 nm. A commercial kit from Sigma–Aldrich was used.

2.10. Other assays

DNA was determined by the method of Labarca and Paigen (1980).

2.11. Statistical analysis

All experiments were run in triplicates and repeated 3–4 times. One way ANOVA and post hoc analysis using Tukey–Krämer's multiple comparisons test were performed using GraphPad InSat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA, USA). *P* values <0.05 were considered statistically significant.

3. Results

3.1. Regulation of fatty acid metabolism by FSH and bFGF in Sertoli cells

In order to evaluate FSH and bFGF regulation of the expression of genes involved in FA metabolism, Sertoli cells were incubated

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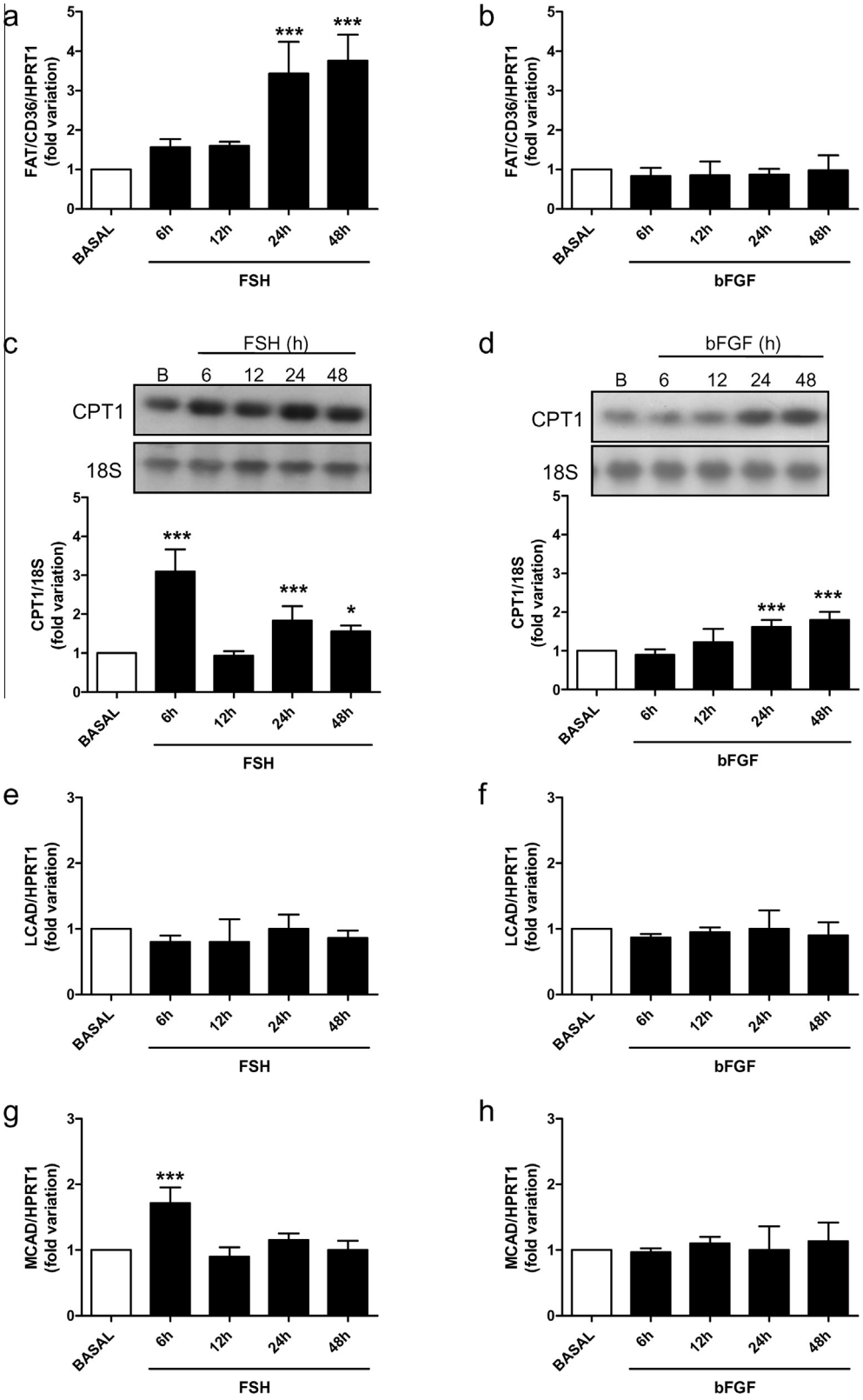

Fig. 1. Effect of FSH and bFGF on *FAT/CD36*, *CPT1*, *LCAD* and *MCAD* mRNA levels in Sertoli cells. Sertoli cells were maintained under BASAL conditions or stimulated with 100 ng/ml FSH (a, c, e and g) or 30 ng/ml bFGF (b, d, f and h) for variable periods of time (6, 12, 24 and 48 h). Total cellular RNA was then extracted. (a, b, e, f, g and h) RT-qPCR was performed. The comparative $\Delta\Delta$Ct method was used to calculate relative gene expression. (c and d) Northern blot analysis was performed. Membranes were hybridized with labeled cDNA probes for *CPT1*. The upper panels show a representative experiment out of three. The lower panels show pooled data of three independent experiments performed indicating the fold variation in mRNA levels (ratio of *CPT1* mRNA to *18S* in each sample) relative to BASAL. Results are expressed as means ± S.D. of three independent experiments performed, $^{***}P < 0.001$; $^{*}P < 0.05$ versus BASAL.

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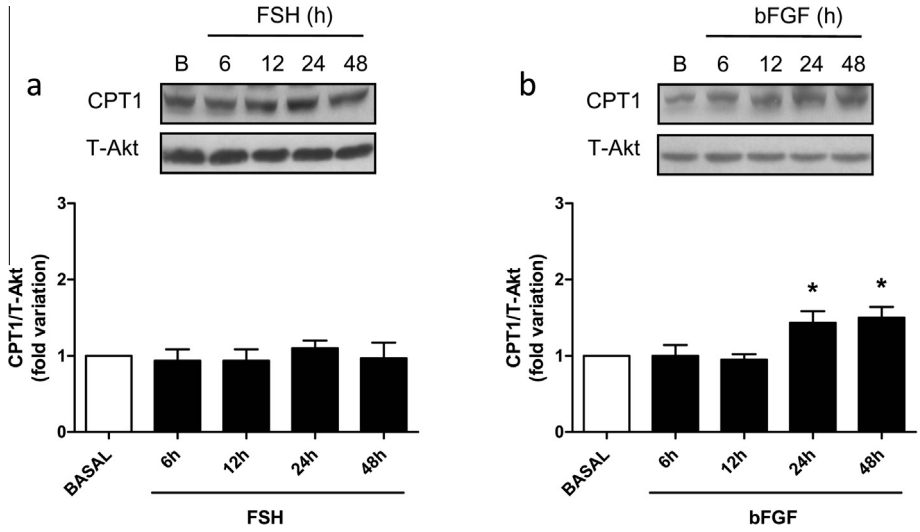

Fig. 2. Effect of FSH and bFGF on CPT1 protein levels in Sertoli cells. Sertoli cells were maintained under BASAL conditions or stimulated with 100 ng/ml FSH (a) or 30 ng/ml bFGF (b) for variable periods of time (6, 12, 24 and 48 h). Cell extracts were obtained and utilized for Western blot analysis using specific antibodies for CPT1 or Akt. The upper panels show a representative experiment out of three. The lower panels show pooled data of three independent experiments performed indicating the fold variation in the level of CPT1 (ratio of CPT1 to Akt in each sample) relative to BASAL. Results are expressed as means ± S.D. of three independent experiments performed, $^{*}P < 0.05$ versus BASAL.

Table 1
Effects of FSH and bFGF on fatty acid oxidation in Sertoli cell.

	pmol Pal/h/μg DNA
BASAL	3.3 ± 0.2
FSH 24 h	3.0 ± 0.1
FSH 48 h	2.3 ± 0.1***
bFGF 24 h	2.8 ± 0.2
bFGF 48 h	4.0 ± 0.1***

Sertoli cells were incubated in BASAL conditions or stimulated for 24 and 48 h with 100 ng/ml FSH or 30 ng/ml bFGF. The fatty acid oxidation was assessed by measuring $^{3}H_2O$ produced in the incubation medium. Results are expressed as pmol of palmitic acid/h/μg DNA ± S.D., $^{***}P < 0.001$ versus BASAL.

for different periods of time – 6, 12, 24 and 48 h – with 100 ng/ml FSH or 30 ng/ml bFGF, doses that have been shown to elicit maximal biological responses (Meroni et al., 2002; Riera et al., 2002). The levels of expression of *FAT/CD36*, *CPT1*, *LCAD* and *MCAD* were evaluated. Fig. 1 (left panels: a, c, e and g) shows that FSH stimulated *FAT/CD36*, *CPT1* and *MCAD* mRNA levels. *FAT/CD36* mRNA levels reached maximal values in 48-h incubations while *CPT1* and *MCAD* mRNA levels reached maximal values in 6-h incubations. On the other hand, Fig. 1 (right panels: b, d, f and h) shows that bFGF stimulated *CPT1* expression levels reaching maximal values in 24 and 48-h incubations.

Taking into account that both hormones stimulated *CPT1* mRNA levels and considering that CPT1 activity is the limiting step in fatty acid oxidation, we extended our study analyzing CPT1 protein levels. For this purpose, Sertoli cells were incubated for different periods of time – 6, 12, 24 and 48 h – with 100 ng/ml FSH or 30 ng/ml bFGF. Fig. 2 (left panel) shows that FSH did not modify CPT1 protein levels. On the other hand, Fig. 2 (right panel) shows that stimulation for 24 and 48 h with bFGF increased CPT1 protein levels.

The next experiment was designed to evaluate whether the effects observed in gene expression and in CPT1 protein levels were accompanied by a modification in fatty acid oxidation levels. To achieve this goal, fatty acid oxidation in Sertoli cells previously incubated for 48 h with 100 ng/ml FSH or 30 ng/ml bFGF was assessed. Table 1 shows that FSH decreased while bFGF increased fatty acid oxidation.

3.2. Regulation of the expression of genes involved in mitochondrial biogenesis by FSH and bFGF in Sertoli cells

In mammalian cells, a complex transcription network controls mitochondrial biogenesis (Scarpulla, 2008; Hock and Kralli, 2009). Nuclear respiratory factors 1 and 2 (*NRF1* and *NRF2*) and transcription factor A (*Tfam*) expression are considered as crucial regulators of mitochondrial biogenesis. In order to evaluate whether FSH and bFGF regulate the expression of above-mentioned genes, Sertoli cells were incubated for different periods of time – 6, 12, 24 and 48 h – with 100 ng/ml FSH or 30 ng/ml bFGF. Fig. 3 (left panels: a, c and e) shows that in a 6-h incubation period with FSH, the hormone stimulated *NRF1*, *NRF2* and *Tfam* expression. On the other hand, Fig. 3 (right panels: b, d and f) shows that bFGF did not modify the expression of the above-mentioned genes.

The next experiment was designed to evaluate whether the increases in the expression of *NRF1*, *NRF2* and *Tfam* were accompanied by an increase in mtDNA content. To achieve this goal, Sertoli cells were incubated for 24 and 48 h with 100 ng/ml FSH or 30 ng/ml bFGF. Fig. 4 shows that FSH increased mtDNA content while bFGF did not modify it.

3.3. Participation of PPARβ/δ in FSH regulation of Sertoli cells

As mentioned in the introduction, we have previously observed that PPARβ/δ activation can simultaneously regulate the expression of genes involved in FA metabolism and lactate production probably reflecting a coordinated mechanism which will ensure the concomitant provision of energy to Sertoli and germ cells. In order to analyze whether PPARβ/δ participates in FSH action, Sertoli cells were incubated with 100 ng/ml FSH in the absence or presence of the PPARβ/δ antagonist GSK3787 (Shearer et al., 2010; GSK, 20 μM) and the levels of gene expression and of lactate production were evaluated. Fig. 5 shows that the presence of GSK in the culture medium did not alter FSH action. Fig. 5 shows that FSH stimulation of lactate production was not inhibited by the antagonist either.

3.4. Participation of PPARβ/δ in bFGF regulation Sertoli cells

Finally, experiments were performed to evaluate the participation of PPARβ/δ in bFGF regulation of *CPT1* expression, FA oxidation

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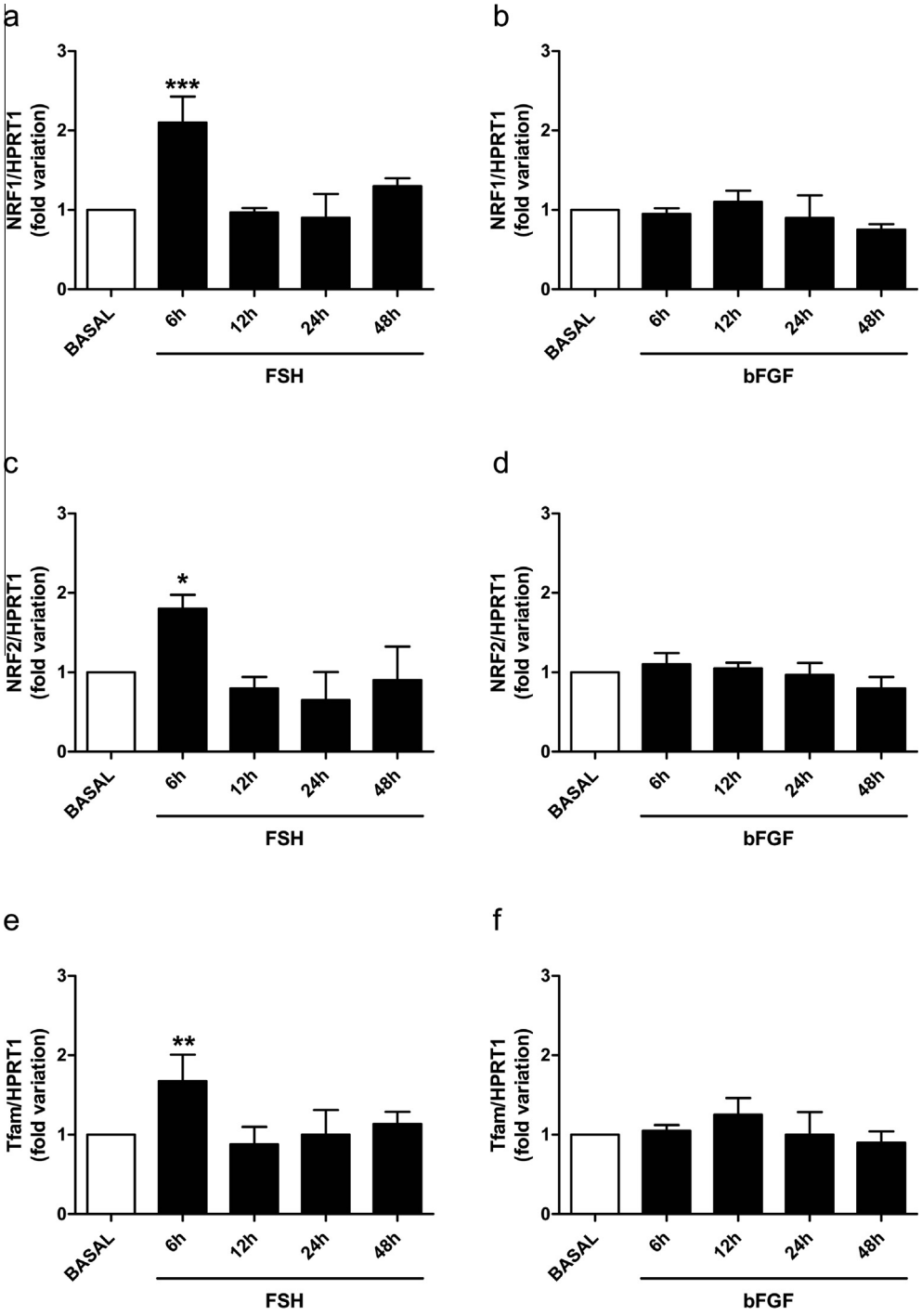

Fig. 3. Effect of FSH and bFGF on *NRF1*, *NRF2* and *Tfam* mRNA levels in Sertoli cells. Sertoli cells were maintained under BASAL conditions or stimulated with 100 ng/ml FSH (a, c and e) or 30 ng/ml bFGF (b, d and f) for variable periods of time (6, 12, 24 and 48 h). Total cellular RNA was then extracted and RT-qPCR was performed. The comparative $\Delta\Delta$Ct method was used to calculate relative gene expression. Results are expressed as means ± S.D. of three independent experiments performed, $^{***}P < 0.001$; $^{**}P < 0.01$; $^{*}P < 0.05$ versus BASAL.

and lactate production. Sertoli cells were incubated with 30 ng/ml bFGF in the absence or presence of 20 μM GSK. Figs. 6 and 7 show that GSK inhibited bFGF stimulation of *CPT1* expression, FA oxidation and lactate production.

4. Discussion

Spermatogenesis takes place in the seminiferous tubule, where Sertoli cells provide structural and nourishing support to the dividing and differentiating germ cells. This process requires a continuous cross talk between germ cells and Sertoli cells which exert multiple tasks critical for germ cell differentiation. In this context, regulation of Sertoli cell metabolism becomes essential to provide nutrients to germ cells and to fulfill its own energy requirements.

Energetic metabolism in the seminiferous tubule has been considered to have features of its own. As mentioned in the introduction, it has been shown that Sertoli cells mainly utilize FA as its energy source (Jutte et al., 1985). Few studies have dealt with FA

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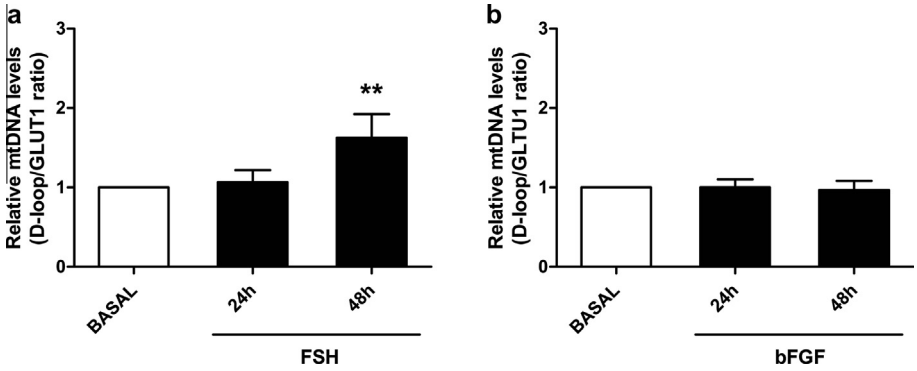

Fig. 4. Effect of FSH and bFGF on mtDNA content in Sertoli cells. Sertoli cells were maintained under BASAL conditions or stimulated with 100 ng/ml FSH (a) or 30 ng/ml bFGF (b) for variable periods of time (24 and 48 h). Total cellular DNA was then extracted and RT-qPCR was performed. The ratio of relative amount of mitochondrial DNA (D-Loop) and nuclear DNA (GLUT1) were determined. Results are expressed as means ± S.D. of three independent experiments performed, $^{*}P < 0.05$ versus BASAL.

metabolism in Sertoli cells since Jutte's early work. To this respect, a mitochondrial FA oxidation system (Fukasawa et al., 2010) and the presence of proteins which transport fatty acids – FAT/CD36 (Gillot et al., 2005) and CPT1 (Adams et al., 1998) – in Sertoli cells have been demonstrated. However, no studies have explored the hormonal regulation of these genes.

We have recently observed that activation of PPARα and PPARβ/δ in Sertoli cells regulates the expression of genes involved in fatty acid transport and metabolism (Regueira et al., 2014). Considering that FA oxidation is an important energy source in Sertoli cells, it may be assumed that this process must be strictly regulated. The last assumption is particularly pertinent to hormones such as FSH – the master hormone in the regulation of Sertoli cell physiology – and bFGF – a representative member of the locally produced testicular factors.

In Sertoli cells, FAT/CD36 has been found in the vicinity of residual bodies engulfed by these cells during the spermiation process (Gillot et al., 2005). It has been proposed that FAT/CD36 may not only be important in FA uptake but also in the phagocytosis of apoptotic germ cells and residual bodies, elements that probably represent an additional supply of FA available for ATP production in Sertoli cells (Xiong et al., 2009). Our results show that FSH, but not bFGF, increases *FAT/CD36* mRNA levels in Sertoli cells probably favoring the availability of FA as building blocks for complex lipids.

As for the genes involved in FA oxidation, we observed that FSH stimulates both the expression of *CPT1* and *MCAD*, while bFGF only stimulates *CPT1* mRNA levels. However, only bFGF stimulates CPT1 protein levels. In accordance with the positive regulation of CPT1 mRNA and protein levels, an increase in fatty acid oxidation under bFGF treatment was observed. This result pointed out that bFGF has a role in the ability of Sertoli cells to metabolize lipid matter in order to obtain energy. Simultaneously, bFGF stimulates lactate production which is exported and used as energy source by developing germ cells. On the other hand, FSH decreased fatty acid oxidation. This result was not unexpected as the anabolic effects of FSH are well recognized. To this respect, it has been demonstrated that FSH significantly stimulates acetate incorporation into triglycerides and phospholipids by regulating lipid esterification (Guma et al., 1997). In this way, FSH promotes on the one hand the synthesis of essential enzymes necessary to obtain energy and concomitantly the synthesis of lipids that may be essential to maintain spermatogenesis.

The importance of mitochondria as site of aerobic oxidation of metabolic fuels, such as FA, has been recognized for a long time. It has been observed that the number of mitochondria may vary in response to cellular metabolic requirements and hormonal environment (Gao et al., 2014). In order to sustain the changing energetic demands of spermatogenesis, a careful regulation of mitochondrial biogenesis may be predicted in Sertoli cells. A complex regulatory network, which includes nuclear respiratory factors (*NRF1* and *NRF2*) and mitochondrial transcription factor A (*Tfam*), coordinates mitochondrial biogenesis (Kelly and Scarpulla, 2004). *NRF1* stimulates transcription of the *Tfam* gene by binding to an *NRF1* response element in the promoter and subsequently, Tfam increases the transcription of mtDNA-encoded gene targets. Although the presence of *NRF1*, *NRF2* and *Tfam* in the testis had been demonstrated (Escrivá et al., 1999), no previous studies in Sertoli cells had analyzed whether hormones were involved in the transcriptional regulation of these genes and thus in mitochondrial biogenesis. Therefore, we decided to investigate whether FSH and bFGF, hormones that regulate carbohydrate and lipid metabolism in Sertoli cells, were able – concomitantly with the regulation of energetic metabolism – to regulate the expression of *NRF1*, *NRF2* and *Tfam* and consequently mtDNA content in this cell type. The present study shows that FSH, but not bFGF, increases the expression of *NRF1*, *NRF2* and *Tfam*. In accordance with this augment, FSH also stimulates mtDNA content. These results suggest that the trophic hormone participates in the control of mitochondrial biogenesis in Sertoli cells and reinforce the idea that it is essential to coordinate processes involved in energy homeostasis that may be necessary to maintain spermatogenesis.

Previous studies on the hormonal regulation of the expression of genes involved in mitochondrial biogenesis had been performed in various cell types. To this respect, it has been observed that testosterone increases mRNA levels of genes involved in mitochondrial biogenesis in skeletal muscle, which suggests a role in testosterone-induced increase in energy expenditure (Usui et al., 2014). Moreover, it has been observed that estradiol upregulates mitochondrial markers in skeletal muscle and L6 miotubes (Capllonch-Amer et al., 2014) and also in mammary gland and in uterus (Ivanova et al., 2013). Furthermore, it has been shown that thyroid hormone has a profound effect on mitochondrial biogenesis that is related with its effects on oxygen consumption and metabolic rate (Weitzel et al., 2003). The results presented herein suggest that in Sertoli cells, FSH leads to an increase in mitochondrial content in order to provide the machinery to utilize energetic substrates that will fulfill the high-energy demands of spermatogenesis.

As mentioned in the introduction, we have previously shown that only PPARβ/δ activation simultaneously regulates the expression of genes involved in FA metabolism and lactate production suggesting that this nuclear receptor may have a relevant physiological role in the seminiferous tubules. Hormones and growth factors, acting through their cognate receptors and regulating signal transduction, can play a role in determining the activity of PPARs

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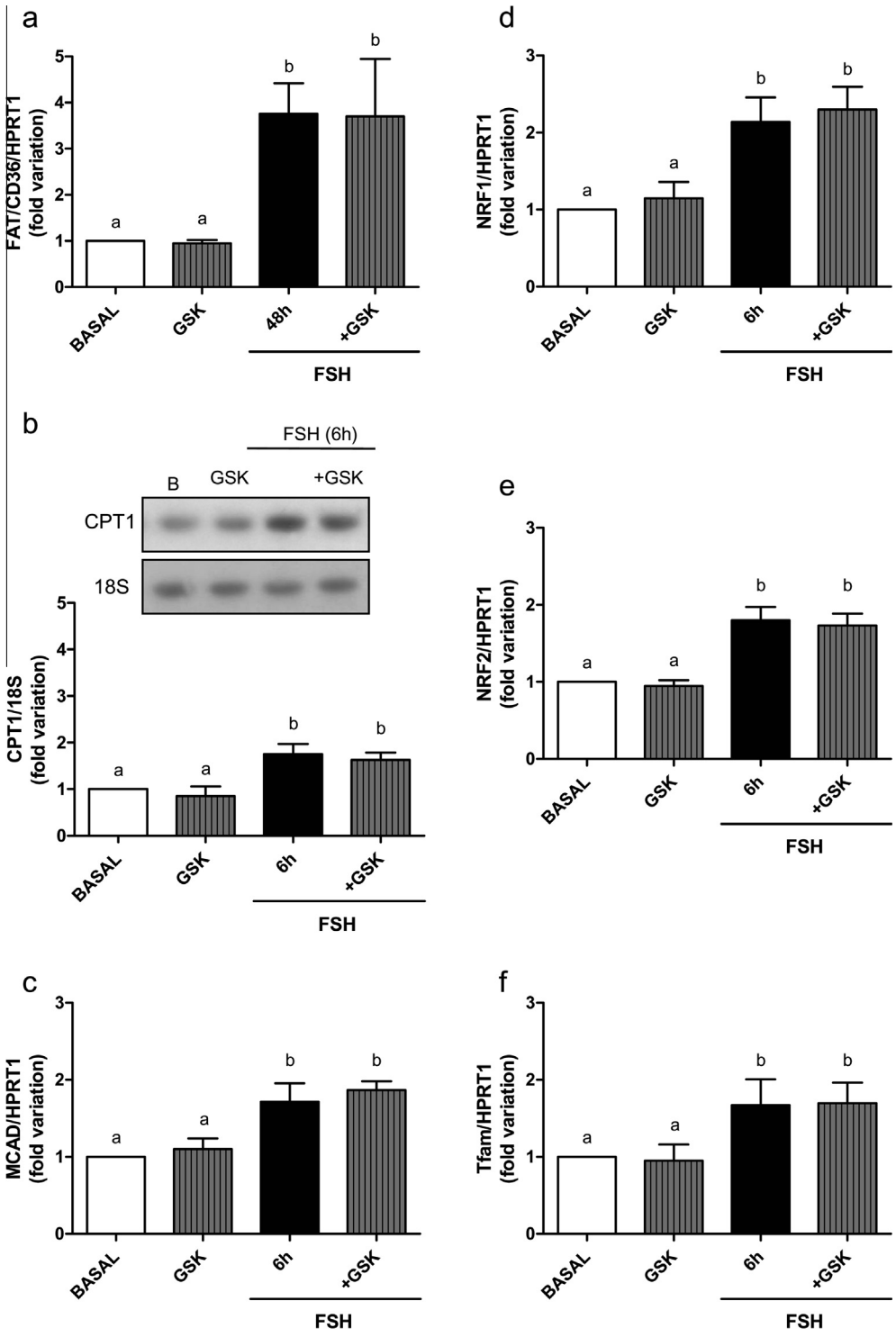

Fig. 5. Effect of GSK on FSH stimulation of *FAT/CD36*, *CPT1*, *MCAD*, *NRF1*, *NRF2* and *Tfam* mRNA levels in Sertoli cells. Sertoli cells were maintained under BASAL conditions or stimulated with 100 ng/ml FSH in the absence or presence of GSK (20 μM) for the times indicated in the figure. Total cellular RNA was then extracted. (a, c, d, e and f) RT-qPCR was performed. The comparative $\Delta\Delta$Ct method was used to calculate relative gene expression. (b) Northern blot analysis was performed. Membranes were hybridized with labeled cDNA probes for *CPT1*. The upper panels show a representative experiment out of three. The lower panels show pooled data of three independent experiments performed indicating the fold variation in mRNA levels (ratio of *CPT1* mRNA to *18S* in each sample) relative to BASAL. Results are expressed as means ± S.D. of three independent experiments performed. Different letters indicate statistically significant differences among treatment groups, $P < 0.05$.

and hence their effects on gene expression (Vanden Heuvel, 1999). Based on the latter observations, we hypothesized that PPARβ/δ activation might participate in the mechanism utilized by FSH and bFGF to regulate Sertoli cell energetic metabolism. Regarding FSH, no effects of GSK – a PPARβ/δ antagonist – were observed, indicating that this nuclear receptor is not involved in FSH action. As for the effects of bFGF on *CPT1* mRNA levels, fatty acid oxidation and lactate production, all of them were inhibited in the presence of GSK. The latter results suggest a prominent role of PPARβ/δ activation in bFGF regulation of Sertoli cell metabolism.

In summary, our findings indicate that bFGF stimulates *CPT1* expression, FA oxidation and lactate production with the participa-

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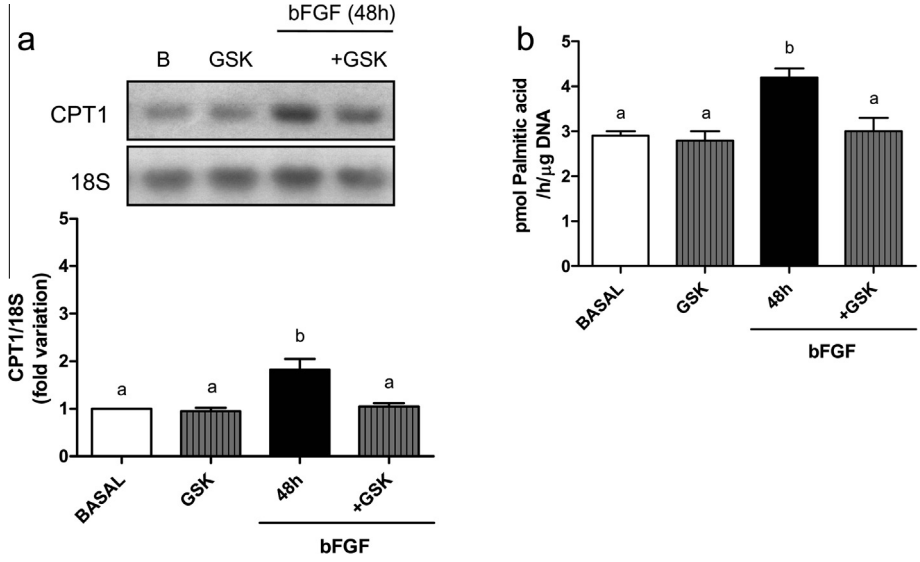

Fig. 6. Effect of GSK on bFGF stimulation of *CPT1* mRNA levels and fatty acid oxidation in Sertoli cells. Sertoli cells were maintained under BASAL conditions or stimulated with 30 ng/ml bFGF in the absence or presence of GSK (20 μM) for the times indicated in the figure. (a) Total cellular RNA was then extracted and Northern blot analysis was performed. Membranes were hybridized with labeled cDNA probes for *CPT1*. The upper panel show a representative experiment out of three. The lower panel show pooled data of three independent experiments performed indicating the fold variation in mRNA levels (ratio of *CPT1* mRNA to *18S* in each sample) relative to BASAL. Results are expressed as means ± S.D. of three independent experiments performed. (b) Fatty acid oxidation assay was performed as indicated in Section 2. Results are expressed as means ± S.D. of triplicate incubations performed in one representative experiment out of three. Different letters indicate statistically significant differences among treatment groups, $P < 0.05$.

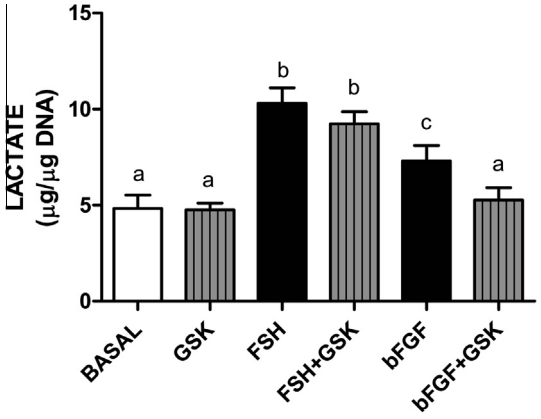

Fig. 7. Effect of GSK on FSH and bFGF stimulation of lactate production in Sertoli cells. Sertoli cells were maintained under BASAL conditions or stimulated with 100 ng/ml FSH or 30 ng/ml bFGF in the absence or presence of GSK (20 μM) for 48 h. Lactate was determined in the conditioned media. Values are expressed as means ± S.D. of triplicate incubations in one representative experiment out of three. Different letters indicate statistically significant differences among treatment groups, $P < 0.05$.

tion of PPARβ/δ activation. On the other hand, this investigation shows that FSH concomitantly regulates the expression of genes involved in FA metabolism and in mitochondrial biogenesis in Sertoli cells independently of PPARβ/δ activation. Metabolic processes such as FA oxidation, mitochondrial biogenesis and lactate production in Sertoli cells are essential for the energetic metabolism of the seminiferous tubule. The fact that these processes are regulated by hormones in a different way reflects the multifarious regulation of molecular mechanisms involved in Sertoli cell function.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

Abumrad, N.A., el-Maghrabi, M.R., Amri, E.Z., Lopez, E., Grimaldi, P.A., 1993. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. J. Biol. Chem. 268, 17665–17668.

Adams, S.H., Esser, V., Brown, N.F., Ing, N.H., Johnson, L., Foster, D.W., McGarry, J.D., 1998. Expression and possible role of muscle-type carnitine palmitoyltransferase I during sperm development in the rat. Biol. Reprod. 59, 1399–1405.

Bonen, A., Campbell, S.E., Benton, C.R., Chabowski, A., Coort, S.L., Han, X.X., Koonen, D.P., Glatz, J.F., Luiken, J.J., 2004. Regulation of fatty acid transport by fatty acid translocase/CD36. Proc. Nutr. Soc. 63, 245–249.

Boussouar, F., Benahmed, M., 2004. Lactate and energy metabolism in male germ cells. Trends Endocrinol. Metab. 15, 345–350.

Butow, R.A., Bahassi, E.M., 1999. Adaptive thermogenesis: orchestrating mitochondrial biogenesis. Cur. Biol. 21, R767–R769.

Capllonch-Amer, G., Sbert-Roig, M., Galmés-Pascual, B.M., Proenza, A.M., Lladó, I., Gianotti, M., García-Palmer, F.J., 2014. Estradiol stimulates mitochondrial biogenesis and adiponectin expression in skeletal muscle. J. Endocrinol. 221, 391–403.

Coburn, C.T., Knapp Jr., F.F., Febbraio, M., Beets, A.L., Silverstein, R.L., Abumrad, N.A., 2000. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. J. Biol. Chem. 275, 32523–32529.

Deepa, S.S., Walsh, M.E., Hamilton, R.T., Pulliam, D., Shi, Y., Hill, S., Li, Y., Van Remmen, H., 2013. Rapamycin modulates markers of mitochondrial biogenesis and fatty acid oxidation in the adipose tissue of db/db mice. J. Biochem. Pharmacol. Res. 1, 114–123.

Del Sal, G., Manfioletti, G., Schneider, C., 1989. The CTAB-DNA precipitation method: a common mini-scale preparation of template DNA from phagemids, phages or plasmids suitable for sequencing. Biotechniques 7 (5), 514–520.

Escrivá, H., Rodríguez-Peña, A., Vallejo, C.G., 1999. Expression of mitochondrial genes and of the transcription factors involved in the biogenesis of mitochondria Tfam, NRF-1 and NRF-2, in rat liver, testis and brain. Biochimie 81, 965–971.

Fukasawa, M., Atsuzawa, K., Mizutani, K., Nakazawa, A., Usuda, N., 2010. Immunohistochemicals localization of mitochondrial fatty acids β-oxidation enzymes in rat testis. J. Histochem. Cytochem. 58, 195–206.

Gao, A.W., Cantó, C., Houtkooper, R.H., 2014. Mitochondrial response to nutrient availability and its role in metabolic disease. EMBO Mol. Med. 6, 580–589.

Ghisla, S., Thorpe, C., 2004. Acyl-CoA dehydrogenase. A mechanistic overview. Eur. J. Biochem. 271, 494–508.

Gillot, I., Jehl-Pietri, C., Gounon, P., Luquet, S., Rassoulzadegan, M., Grimaldi, P., Vidal, F., 2005. Germ cells and fatty acids induce translocation of CD36 scavenger receptor to the plasma membrane of Sertoli cells. J. Cell Sci. 118, 3027–3035.

Gnessi, L., Fabbri, A., Spera, G., 1997. Gonadal peptides as mediators of development and functional control of the testis: an integrated system with hormones and local environment. Endocr. Rev. 18, 541–609.

Guma, F.C., Wagner, M., Martini, L.H., Bernard, E.A., 1997. Effect of FSH and insulin on lipogenesis in cultures of Sertoli cells from immature rats. Braz. J. Med. Biol. Res. 30, 591–597.

Han, I.S., Sylvester, S.R., Kim, K.H., Schelling, M.E., Venkateswaran, S., Blanckaert, V. D., McGuinness, M.P., Griswold, M.D., 1993. Basic fibroblast growth factor is a testicular germ cell product which may regulate Sertoli cell function. Mol. Endocrinol. 7, 889–897.

Hock, M.B., Kralli, A., 2009. Transcriptional control of mitochondrial biogenesis and function. Annu. Rev. Physiol. 71, 177–203.

Ivanova, M.M., Radde, B.N., Son, J., Mehta, F.F., Chung, S.H., Klinge, C.M., 2013. Estradiol and tamoxifen regulate NRF-1 and mitochondrial function in mouse mammary gland and uterus. J. Mol. Endocrinol. 51, 233–246.

Joseph, A.M., Pilegaard, H., Litvintsev, A., Leick, L., Hood, D.A., 2006. Control of gene expression and mitochondrial biogenesis in the muscular adaptation to endurance exercise. Essays Biochem. 42, 13–29.

Jutte, N.H., Eikvar, L., Levy, F.O., Hansson, V., 1985. Metabolism of palimitate in cultured rat Sertoli cells. J. Reprod. Fertil. 73, 497–503.

Kelly, D.P., Scarpulla, R.C., 2004. Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. Genes Dev. 18, 357–368.

Labarca, C., Paigen, K., 1980. A simple, rapid, and sensitive DNA assay procedure. Anal. Biochem. 102, 344–352.

Lavarías, S., Pasquevich, M.Y., Dreon, M.S., Heras, H., 2009. Partial characterization of a malonyl-CoA-sensitive carnitine O-palmitoyltransferase I from *Macrobrachium borellii* (Crustacea: Palaemodidae). Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 152 (4), 364–369.

Lopaschuk, G.D., Belke, D.D., Gamble, J., Itoi, T., Schönekess, B.O., 1994. Regulation of fatty acid oxidation in the mammalian heart in health and disease. Biochim. Biophys. Acta Lipids Lipid Metab. 1213, 263–276.

McGarry, J.D., Foster, D.W., 1980. Regulation of hepatic fatty acid oxidation and ketone body production. Annu. Rev. Biochem. 49, 395–420.

Meroni, S.B., Riera, M.F., Pellizzari, E.H., Cigorraga, S.B., 2002. Regulation of rat Sertoli cell function by FSH: possible role of phosphatidylinositol 3-kinase/protein kinase B pathway. J. Endocrinol. 174, 195–204.

O'Neill, H.M., Holloway, G.P., Steinberg, G.R., 2013. AMPK regulation of fatty acid metabolism and mitochondrial biogenesis: implications for obesity. Mol. Cell. Endocrinol. 366, 135–151.

Rasmussen, B.B., Wolfe, R.R., 1999. Regulation of fatty acid oxidation in skeletal muscle. Annu. Rev. Nutr. 19, 463–484.

Regueira, M., Riera, M.F., Galardo, M.N., Pellizzari, E.H., Cigorraga, S.B., Meroni, S.B., 2014. Activation of PPARA and PPARB/D regulates Sertoli cell metabolism. Mol. Cell. Endocrinol. 382, 271–281.

Riera, M.F., Meroni, S.B., Schteingart, H.F., Pellizzari, E.H., Cigorraga, S.B., 2002. Regulation of lactate production and glucose transport as well as of glucose transporter 1 and lactate dehydrogenase A mRNA levels by basic fibroblast growth factor in rat Sertoli cells. J. Endocrinol. 173, 335–343.

Riera, M.F., Meroni, S.B., Pellizzari, E.H., Cigorraga, S.B., 2003. Assessment of the roles of mitogen-activated protein kinase and phosphatidyl inositol 3-kinase/protein kinase B pathways in the basic fibroblast growth factor regulation of Sertoli cell function. J. Mol. Endocrinol. 31, 279–289.

Riera, M.F., Galardo, M.N., Pellizzari, E.H., Meroni, S.B., Cigorraga, S.B., 2009. Molecular mechanisms involved in Sertoli cell adaptation to glucose deprivation. Am. J. Physiol. Endocrinol. Metab. 297, E907–E914.

Santillo, A., Burrone, L., Falvo, S., Senese, R., Lanni, A., Chieffi Baccari, G., 2013. Triiodothyronine induces lipid oxidation and mitochondrial biogenesis in rat Harderian gland. J. Endocrinol. 219, 69–78.

Scarpulla, R.C., 2008. Transcriptional paradigms in mammalian mitochondrial biogenesis and function. Physiol. Rev. 88, 611–638.

Shearer, B.G., Wiethe, R.W., Ashe, A., Billin, A.N., Way, J.M., Stanley, T.B., Wagner, C. D., Xu, R.X., Leesnitzer, L.M., Merrihew, R.V., Shearer, T.W., Jeune, M.R., Ulrich, J. C., Willson, T.M., 2010. Identification and characterization of 4-chloro-N-(2-{[5-trifluoromethyl)-2-pyridyl]sulfonyl}ethyl)benzamide (GSK3787), a selective and irreversible peroxisome proliferator-activated receptor delta (PPARdelta) antagonist. J. Med. Chem. 53, 1857–1861.

Usui, T., Kajita, K., Kajita, T., Mori, I., Hanamoto, T., Ikeda, T., Okada, H., Taguchi, K., Kitada, Y., Morita, H., Sasaki, T., Kitamura, T., Sato, T., Kojima, I., Ishizuka, T., 2014. Elevated mitochondrial biogenesis in skeletal muscle is associated with testosterone-induced body weight loss in male mice. FEBS Lett. 588, 1935–1941.

Vanden Heuvel, J.P., 1999. Peroxisome proliferator-activated receptors: a critical link among fatty acids, gene expression and carcinogenesis. J. Nutr. 129, 575–580.

Weitzel, J.M., Iwen, K.A., Seitz, H.J., 2003. Regulation of mitochondrial biogenesis by thyroid hormone. Exp. Physiol. 88, 121–128.

Wilson-Fritch, L., Burkart, A., Bell, G., Mendelson, K., Leszyk, J., Nicoloro, S., Czech, M., Corvera, S., 2003. Mitochondrial biogenesis and remodeling during adipogenesis and in response to the insulin sensitizer rosiglitazone. Mol. Cell. Biol. 23, 1085–1094.

Xiong, W., Wang, H., Wu, H., Chen, Y., Han, D., 2009. Apoptotic spermatogenic cells can be energy sources for Sertoli cells. Reproduction 137, 469–479.

Please cite this article in press as: Regueira, M., et al. FSH and bFGF regulate the expression of genes involved in Sertoli cell energetic metabolism. Gen. Comp. Endocrinol. (2015), http://dx.doi.org/10.1016/j.ygcen.2015.08.011