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Hyperplastic Obesity and Liver Steatosis as Long-Term Consequences of Suboptimal In Vitro Culture of Mouse Embryos[1]

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ABSTRACT

In the present study, we identify and describe an obese phenotype in mice as a long-term consequence of a suboptimal in vitro culture that resulted from the addition of fetal calf serum (FCS) into the culture medium. Mice produced with FCS displayed a high mortality rate (approximately 55% versus 15% in control mice within 20 mo) and increased sensitivity to the development of obesity in adulthood when fed either a standard or a high-fat diet. These mice developed hyperplastic obesity that was characterized by a significant expansion of the fat pads (approximately 25% and 32% higher body weight in male and female mice over controls, respectively) with unchanged adipocyte size. We observed a sexual dimorphism in the development of obesity in the mice produced with FCS. Whereas the female mice displayed hypertension, hyperleptinemia, and fatty liver, the male mice only displayed glucose intolerance. The mRNA expression of metabolically relevant genes in the adipose tissue was also affected. The males produced with FCS expressed higher mRNA levels of the genes that activate fatty acid oxidation (peroxisome proliferator-activated receptor alpha [*Ppara,* PPARalpha] and acyl-CoA oxidase 1 [*Acox1,* ACOX1]) and thermogenesis (uncoupling protein 1 [*Ucp1,* UCP1]), which may counteract the metabolic phenotype. Conversely, the females produced with FCS generally expressed lower levels of these metabolic genes. In the females, the obese phenotype was associated with inhibition of the lipogenic pathway (peroxisome proliferator-activated receptor gamma [*Pparg,* PPARgamma] and fatty acid synthase [*Fasn,* FAS]), indicating a saturation of the storage capacity of the adipose tissue. Overall, our data indicate that the exposure to suboptimal in vitro culture conditions can lead to the sexually dimorphic development of obesity in adulthood.

development, embryos, fatty liver, in vitro culture, mice, obesity

INTRODUCTION

Over the last few decades, the incidence of overweight and obesity in humans has grown at an accelerated rate to reach epidemic proportions [1]. However, the incidence of obesity has not paralleled food availability. For that reason, the roles of epigenetic effects on obesity risk have gained attention. Recently, suboptimal in vitro culture conditions have been proposed as a model for epigenetic modifications [2].

The use of serum, a common component of culture media [3–5], provides a rich but undefined environment for embryonic development [3]. In fact, it has been found that serum reduces the postimplantation viability of embryos [6–9]. Previous results from our group have revealed that mice derived from embryos cultured in suboptimal conditions due to the addition of fetal calf serum (FCS) can develop obesity and display abnormally large hearts and livers in adulthood [6]. These results are in agreement with previous reports describing malformations in some viscera of animals produced by nuclear transfer [10] or in vitro culture [11]. It has been suggested that the epigenetic modifications of sensitive imprinted genes during early embryogenesis may affect gene expression during later fetal development, influencing the phenotype of the offspring. In this regard, we have observed that supplementation of the culture medium with serum affects the mRNA expression of some growth-related imprinted genes in blastocysts, leading to aberrant fetal growth and development [6, 12]. Furthermore, we have also described that supplementation with FCS has effects on the behavior of adult mice, such as increased anxiety or deficiencies in their implicit memory systems [6].

In addition to the effects on the imprinted genes, other epigenetic alterations may affect gene expression later in life. For example, it has been reported that the composition of the culture medium used for embryonic development affects the methylation pattern at the two-cell stage, contributing to a high incidence of developmental failure [13]. Moreover, the adult mice derived from these procedures might have subtle genetic

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and epigenetic defects that are below the detectable threshold but that are manifested later in life and threaten viability [2, 14]. Interestingly, it has also been reported that the culture and transfer of mouse preimplantation embryos resulted in altered postnatal growth and organ sizing in the offspring that persisted into the second generation, which suggests heritable epigenetic modifications [15].

Recently, we have reported that suboptimal in vitro culture with FCS affects the fertility of male mice and that this effect is related to a modified gene expression pattern and apoptosis in the adult male testes [16]. Moreover, these mice can partially transmit the aberrant phenotypes produced by in vitro culture, such as glucose intolerance and hepatomegaly, to the next two generations [16]. Interestingly, these adverse effects are transmitted to the male offspring but not to the female offspring. In addition to sex hormone-dependent effects and the differences in the regulatory pathways underlying the sex-specific development of most organs, genetic and epigenetic differences between males and females have been reported during the preimplantation stage [17–19]. At this early stage of development, male and female blastocysts respond differently to the culture conditions, consequently affecting later development in a gender-specific manner. These gender-specific sensitivities could explain the differential ability of males and females to restore epigenetic marks between generations.

Following the general characterization of the impact of suboptimal in vitro culture conditions on mice, the present study focused on the development of obesity in the offspring. Several studies in mice have reported that obesity is a long-term consequence of suboptimal culture conditions [6, 20, 21]. Our results confirm that mice produced with FCS (a suboptimal culture condition) developed obesity and that this effect was more severe in females than in males. We have focused on metabolically relevant organs such as the liver and visceral white adipose tissue because these organs have a critical role in energy homeostasis, and obesity or overweight leads to the dysfunction of these organs due to excessive fat accumulation. We have evaluated whether the addition of FCS altered the mRNA expression of genes directly involved in lipolytic and lipogenic pathways in the adipocytes and the expression of adipose-derived hormone genes. In addition, we have also evaluated the expression of other genes involved in the regulation of energy intake and expenditure, including the cannabinoid receptor type 1 (CB_1), the growth hormone receptor (GHr), and the glucose transporter type 4 (GLUT4). The results indicate that mice produced with FCS display visceral and hyperplastic obesity that is associated with fatty liver and metabolic changes in these tissues (including changes in gene expression) mainly in female mice.

MATERIALS AND METHODS

Experimental Animals and In Vitro Embryo Production

All the experiments were performed on adult mice produced by in vitro culture of embryos. These mice were maintained under a 14L:10D photoperiod at 22°C to 24°C in a room with temperature and humidity control. Unless otherwise indicated, water and food were available ad libitum throughout the course of the studies.

One-cell embryos obtained from superovulated female B6CBAF1 mice (C57BL/6xCBA; Harlan Laboratories, Horst, The Netherlands) were cultured for 4 days in K^+-modified simplex optimized medium in the presence of 10% FCS (+FCS) or in the presence of 1 g/L bovine serum albumin (–FCS). The embryos that reached the blastocyst stage were transferred into the oviduct of 0.5 days postcoitum pseudopregnant CD1 females. All of the pregnant dams were allowed to deliver spontaneously. The day of delivery was designated as Day 1 of age of the neonates (the error in estimations of the time of birth was ± 6 h). Upon delivery, the litter size of each dam was recorded, and each pup was checked for gross abnormalities. The pups were nursed by their natural dams until weaning. To ensure standardized nutrition and maternal care, all the litters were redistributed on the day after birth to normalize the litter sizes at six to eight pups. Different groups of mice were used for each experiment to avoid interactions due to specific treatments or experimental conditions.

All the animal care and experimental procedures were approved by the INIA Ethical Committee on animal research in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Society for Study of Reproduction, European legislation (2010/63/UE), and Spanish legislation (RD 53/2013).

Glucose Tolerance Test

Glucose tolerance tests were performed in 4-mo-old mice (18 mice, 7 males and 11 females) produced with FCS (+FCS) and 16 control mice (7 males and 9 females) produced without FCS (–FCS). Food was withdrawn 12 h before the procedure. Awakened mice were injected with an intraperitoneal glucose load of 2 g/kg body weight. Tail blood samples were collected before (0 min) and after (15, 30, 45, 60, and 120 min) the glucose administration. The glucose level was determined using a standard glucose oxidase method as previously described [22]. In addition, the area under the glucose curves was calculated with a time interval of 0–60 min (starting at 0 min and ending at 60 min after the glucose administration) for each animal, and the baseline was established from the lowest glucose value at 0 min within each group.

A 15-Day Study of Feeding Behavior

The feeding studies were performed on adult mice at two different ages, 4 and 8 mo, using a specific cohort of mice for each age. We used 37 mice of each age (17 males and 20 females) produced with FCS and 37 control mice of each age (17 males and 20 females). These mice were fed a high-fat diet (HFD) (45% kcal fat diet, TD.08811; Harlan Ibérica) or a standard diet (SD) (2014; Harlan Ibérica) for 15 days. The food intake (kcal/kg of body weight) and the body weight (g) were measured daily for 15 days of diet exposure.

Survival Curves

Twenty mice (10 males and 10 females) produced with FCS and 20 control mice (10 males and 10 females) were monitored for 20 mo, and their mortality was recorded. The survival curves were drawn for each group of mice according to the in vitro culture conditions and gender using the Kaplan-Meier method [23, 24]. We fixed 20 mo as the endpoint because the biochemical determinations/assays were performed in 20-mo-old adult mice.

Blood Pressure

The systolic blood pressure was determined in conscious 20-mo-old mice (19 mice, 8 males and 11 females, produced with FCS and 20 control mice, 9 males and 11 females) after 1 h of acclimatization with an automated multichannel system by tail-cuff plethysmography using a NIPREM model 546 blood pressure monitor (Cibertec S.A.) and data acquisition software and hardware (PowerLab system; ADInstruments) following the manufacturer's instructions. The recordings were blind to +FCS or –FCS mice. The experimental room was kept at a constant temperature of 30°C.

Tissue Sample Collection

Adult mice (20 mice [9 males and 11 females] produced with FCS and 19 control mice [8 males and 11 females]) were sacrificed by decapitation. The blood plasma was obtained from the blood by centrifugation (2100 × *g* for 10 min at room temperature) and stored frozen. Samples from the liver, white adipose tissue, testicles, ovaries, and forepaws were collected and immediately frozen at −80°C until analysis.

Size of Adipocyte

Following dissection of the perigonadal white adipose tissue, a small piece of this fat tissue was immersed in Bouin solution overnight and then transferred to 70% ethanol and stored at 4°C. The tissue was fixed and embedded with a random orientation in paraffin, and 10-μm thick sections were stained with hematoxylin and counterstained with eosin. For each sample, the size of the adipocytes was determined for 80 ± 6 adipocytes by measuring the diameter of the cell obtained from four sections of tissues taken in each group of animals. The cell diameters were determined at 200× magnification using digitalized images obtained with a Nikon microscope (Nikon Instruments Inc.). Those adipocytes with irregular cell size were excluded from the measurement.

TABLE 1. Primer sequences used for qRT-PCR.

Gene symbol	Gene description		Oligo primer (5′–3′)	GenBank accession no.
Cnr1	Cannabinoid receptor 1 (CB_1)	Sense:	5′-GTCGCAATCTGTTGCTCAG-3′	NM_007726.3
		Antisense:	5′-TTGCCATCTTCTGAGGTGTG-3′	
Ppara	Peroxisome proliferator-activated receptor alpha (PPARα)	Sense:	5′-TGCTGTCCTCCTTGATGAAC-3′	NM_011144.6
		Antisense:	5′-GCTTAAGCACGTGCACAATC-3′	
Pparg	Peroxisome proliferator-activated receptor gamma (PPARγ)	Sense:	5′-GGAGATGTTGGAATGACAGG-3′	NM_001127330.1
		Antisense:	5′-CGAATGGGTACATTGGGAAC-3′	
Fasn	Fatty acid synthase (FAS)	Sense:	5′-TTCGTGATGGAGTCGTGAAG-3′	NM_007988.3
		Antisense:	5′-GGTCTTGGAGATGGCAGAAA-3′	
Adipoq	Adiponectin hormone	Sense:	5′-GGAGATGTTGGAATGACAGG-3′	NM_009605.4
		Antisense:	5′-CGAATGGGTACATTGGGAACA-3′	
Lep	Leptin hormone	Sense:	5′-AGGAAAATGTGCTGGAGACC-3′	NM_008493.3
		Antisense:	5′-ATACCGACTGCGTGTGTGAA-3′	
Acox1	Acyl-CoA oxidase 1 (ACOX1)	Sense:	5′-TCTCACGGATAGGGACAACA-3′	NM_015729.3
		Antisense:	5′-TATGACCCCAAGACCCAAGA-3′	
Ghr	Growth hormone receptor (GHR)	Sense:	5′-ACAAATGGTGATTTGCTGGA-3′	NM_010284.3
		Antisense:	5′-TCACTTGGATGTCTCCACGA-3′	
Ucp1	Uncoupling protein 1 (UCP1)	Sense:	5′-TGCCACACCTCCAGTCATTA-3′	NM_009463.3
		Antisense:	5′-CAGAAGGATTGCCGAAACTG-3′	
Slc2a4	Solute carrier family 2 (facilitated glucose transporter), member 4, GLUT4 or SLC2A4	Sense:	5′-GGATGAGAAACGGAAGTTGG-3′	NM_009204.2
		Antisense:	5′-AGACCGTATTGACCACACCA-3′	
Actb	Beta actin	Sense:	5′-TTTGCTCCAACCAACTGC-3′	NM_007393.3
		Antisense:	5′-GCTGCGTTTTACACCCTTTC-3′	

Total Fat Extraction from Tissues

To evaluate the expansion of the fat pads throughout the body, total lipids were extracted from frozen liver, testicle, ovary, and forepaw samples according to the Bligh and Dyer [25] method using chloroform:methanol (2:1, v/v) and butylated hydroxytoluene (0.025% w/v). The tissue samples were weighed and homogenized in saline solution (100 mg tissue/ml, 4°C). After two centrifugation sessions (2800 × *g* for 10 min at 4°C), the lower phase containing the lipids was extracted using Pasteur pipettes. The fat content was expressed as a percentage of the tissue weight.

Triglyceride Extraction and Fatty Acid Analyses in the Liver

The lipid extracts obtained previously from liver samples were separated by thin layer chromatography using hexane:diethylether:acetic acid (80:20:1, v/v/v) as the solvent system. After separation, the lipid spots corresponding to triglycerides were scraped from silica gel plates (Merck KGaA), and fatty acid methyl esters extracted with methanol:benzene (4:1, v/v) for 1 h at 90°C. Fatty acid methyl esters were then analyzed by gas-liquid chromatography, and individual fatty acids were identified with the following synthetic standards purchased from Supelco Analytical (Sigma Aldrich Co.): lauric (12:0); myristic (14:0); palmitic (16:0); stearic (18:0); arachidic (20:0); palmitoleic (16:1); oleic (18:1); vaccenic (18:1); eicosenoic (20:1); linoleic (18:2); γ-linolenic (18:3); α-linolenic (18:3); eicosadienoic (20:2); dihomo-γ-linolenic (20:3); arachidonic (20:4); eicosapentaenoic (20:5); adrenic (22:4); docosapentaenoic (22:5); and docosahexaenoic acid (22:6). Fatty acids were quantified by area normalization, and each fatty acid was expressed as a percentage of total fatty acids. They were finally grouped into saturated (SFA), monounsaturated (MUFA), or polyunsaturated (PUFA) fatty acids.

Oil Red O Staining in the Liver

Other liver samples were analyzed for lipids and fat depots by staining with a fat-specific dye, Oil Red O. Frozen liver samples from each mouse group were cut into 10 μm-thick sections using a sliding microtome (Leica SM200R) and fixed with 10% formol calcium. The sections were washed with distilled water and rinsed with 60% isopropanol. Then, the sections were stained with freshly prepared Oil Red O working solution (Sigma Aldrich Co.) for 20 min. The sections were rinsed with 60% isopropanol, lightly stained with Mayer hematoxylin, rinsed with tap water, and mounted in aqueous mountant.

Biochemical Parameters in the Plasma

The plasma levels of glycerol and triglycerides were measured by colorimetric methods using commercial kits (Randox laboratories Ltd.). The plasma leptin levels were determined with an enzyme-linked immunosorbent assay kit (Abcam Plc). A calibration curve and an internal control were included in each plate. The intraassay coefficient of variation (CV) was 5.3% for glycerol. The intra- and interassay CVs were 5.35% and 5.1% for triglycerides, respectively. The intra- and interassay CVs were <10% and <12% for leptin, respectively.

RNA Isolation and Quantitative Real-Time PCR Analysis

The RNA from adipose tissue was extracted using the Trizol method, according to the manufacturer's instruction (Life Technologies Co.). The adipose tissue samples were placed into 1–1.5 ml of Trizol reagent and homogenized. In order to ensure purity of the mRNA sequences excluding molecules smaller than 200 nucleotides and proteins, the RNA samples were isolated with RNeasy MinElute Cleanup Kit (Qiagen). Total mRNA concentration was quantified using a Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc.) to ensure A260/280 ratios of 1.8 to 2.0.

The reverse transcript reaction was performed from white adipose tissue mRNA using the Transcriptor Reverse Transcriptase kit and random hexamer primers (Transcriptor RT; Roche Diagnostic GmbH). Negative controls included reverse transcription reactions omitting reverse transcriptase. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) was performed using an ABI PRISM 7300 Real Time PCR System (Applied Biosystems; Life Technologies Co.) and the FAM dye label format for the TaqMan Gene Expression Assays (TaqMan; Life Technologies Co.). Cycling parameters were: 50°C for 2 min to deactivate single and double-strained DNA containing dUTPs, 95°C for 10 min to activate Taq DNA polymerase followed by 40 cycles at 95°C for 15 sec for cDNA melting and 60°C for 1 min to allow annealing and extension of the primers in which fluorescence was acquired. The melting curves analysis was performed to ensure only a single product was amplified. The qRT-PCR amplification was performed using the primers outlined in Table 1. The primers for qRT-PCR reaction were designed based on the National Center for Biotechnology Information (NCBI) database sequences of mouse reference mRNA and checked for specificity with the BLAST (Basic Local Alignment Search Tool) software from the NCBI Web site (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The quantification was performed with a standard curve run at the same time as the samples, with each reaction run in duplicate. Absolute values from each sample were normalized with regard to a housekeeping gene. We analyzed several housekeeping genes, including β-actin, cyclophilin, and the Sp1 transcription factor. Among them, β-actin was the most stable, and it was selected as housekeeping gene for normalization.

Statistical Analysis

The survival data over 20 mo for the mice produced in different culture conditions (+FCS and −FCS) were assessed by a Fisher exact test at the endpoint. All the data in the graphs and tables are expressed as means ± SEM. The intra- and interassay CVs were within the normal range of the enzymatic kits data sheets. The statistical analysis of the results was performed using GraphPad Prism version 5.04 (GraphPad Software Inc.). The significance of

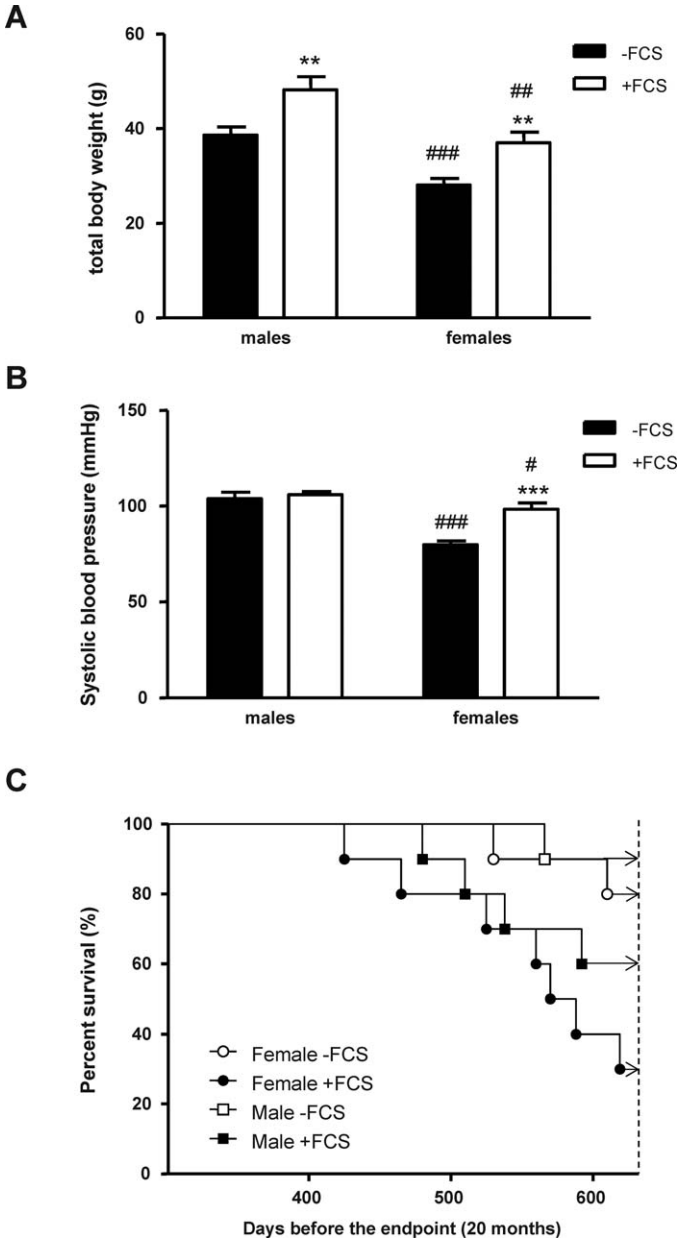

FIG. 1. Effects of FCS supplementation of the culture media on postnatal growth and body weight in adult mice. Total body weight (**A**), systolic blood pressure (**B**), and survival curves (**C**) of male and female mice (20-mo-old mice) produced in vitro in the presence or absence of FCS. The total body weight and systolic blood pressure were analyzed by two-way ANOVA (culture condition and gender) and a Bonferroni post hoc test. $^{**}P < 0.01$ and $^{***}P < 0.001$ denote significant differences compared with the −FCS mice. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ denote significant differences compared with the male mice (n = 8–11 animals per group).

differences within and between groups was primarily evaluated by two-way analysis of variance (ANOVA) (factors: the culture condition [+FCS/−FCS] and gender [male/female]) followed by a post hoc test for multiple comparisons (Bonferroni test). A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Effects of FCS Supplementation of Culture Media on Postnatal Growth in Adult Mice

We evaluated the effect of FCS supplementation on the body weight and blood pressure of 20-mo-old mice. In addition, survival curves of up to 20 mo of age were evaluated in +FCS and −FCS mice.

As shown in Figure 1A, a two-way ANOVA indicated that the total body weight of adult mice was significantly affected by the culture condition ($F_{1,35} = 20.46$; $P < 0.001$) and gender ($F_{1,35} = 27.95$; $P < 0.001$), but there was no interaction between these two variables. The post hoc tests showed that the +FCS mice had significantly increased body weight compared with their respective −FCS controls ($^{**}P < 0.01$ in male and female mice). Moreover, the female mice had significantly lower body weight than the male mice ($^{\#\#\#}P < 0.001$ in −FCS mice and $^{\#\#}P < 0.01$ in +FCS mice).

Both the culture condition ($F_{1,53} = 13.34$; $P < 0.001$) and gender ($F_{1,53} = 30.91$; $P < 0.001$) had a significant primary effect on the systolic blood pressure of the mice (Fig. 1B). An interaction between culture condition and gender was also detected ($F_{1,53} = 8.39$; $P < 0.01$). The female +FCS mice had significantly higher blood pressure than the female −FCS mice ($^{***}P < 0.001$), but no significant changes were observed in the male +FCS mice. However, the female mice had significantly lower systolic blood pressure than the male mice ($^{\#\#\#}P < 0.001$ in −FCS mice and $^{\#}P < 0.05$ in +FCS mice).

Figure 1C shows the survival curves up to 20 mo of age in the female and male mice produced with or without FCS. The percentage of mice that reached the 20-mo endpoint for each group was as follows: 90% male −FCS, 50% male +FCS, 80% female –FCS, and 30% female +FCS mice. We observed that the presence of FCS in the culture medium significantly decreased the lifespan of the +FCS mice compared with the control −FCS mice ($P < 0.01$).

Effects of FCS Supplementation of Culture Media on Body Weight Gain in Mice Fed a HFD

We evaluated the effect of a SD and a HFD on the weight gain in mice produced with FCS as indicated in Figure 2. The study was performed during a 15-day exposure to either diet in 4- and 8-mo-old mice. The initial body weight for each group of mice is indicated in Supplemental Table S1 (Supplemental Data are available online at www.biolreprod.org).

Figure 2A shows the body weight gain of 4-mo-old male mice fed the SD or HFD. The body weight gain was significantly affected by the culture condition ($F_{1,30} = 122.2$; $P < 0.001$) and the diet ($F_{1,30} = 7.383$; $P < 0.05$), with a significant interaction between culture condition and diet ($F_{1,30} = 31.23$; $P < 0.001$). The +FCS male mice gained significantly more weight than their respective male −FCS controls ($^{***}P < 0.001$ in mice fed SD and HFD). Moreover, the increase in body weight gain was higher in the male +FCS mice fed the HFD than in those fed the SD ($^{\#\#\#}P < 0.001$). Overall, the male mice produced with FCS exhibited increased body weight gain that was enhanced by the HFD.

In the 8-mo-old male mice (Fig. 2B), both the culture condition ($F_{1,30} = 282.9$; $P < 0.001$) and the diet ($F_{1,30} = 51.53$; $P < 0.001$) had significant primary effects on body weight gain. Again, there was also a significant interaction between the culture condition and diet ($F_{1,30} = 509.0$; $P < 0.001$). At this age, the +FCS mice gained significantly less weight on the SD ($^{***}P < 0.001$) than the male −FCS mice but significantly more weight on the HFD ($^{***}P < 0.001$).

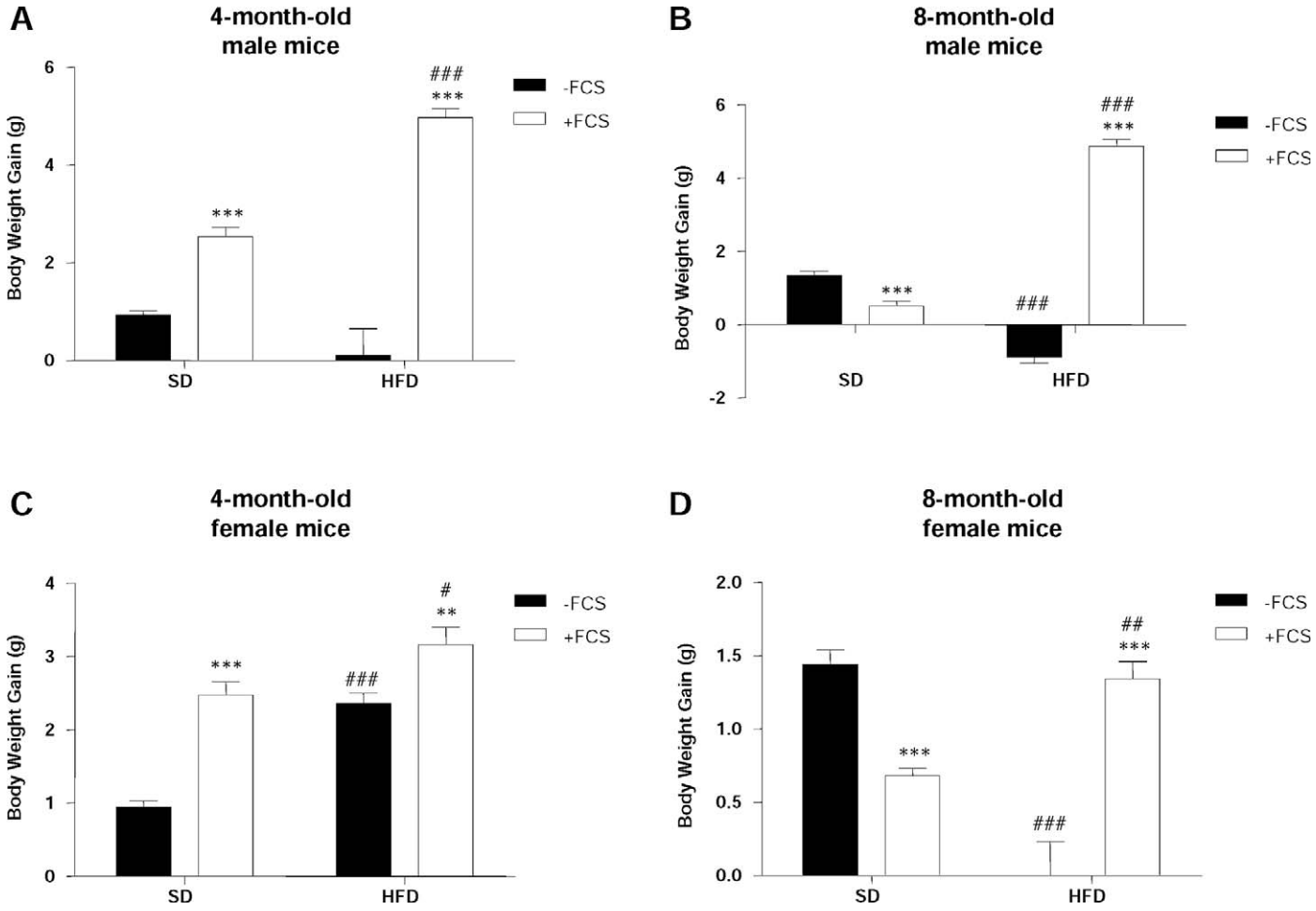

FIG. 2. Effects of FCS supplementation of the culture media on body weight gain in 4- and 8-mo old mice during a 15-day period under a standard diet (SD) (**A**, **B**) or a high-fat diet (HFD) (**C**, **D**). The bars represent means ± SEM (n = 8–11 animals per group). The data were analyzed by two-way ANOVA (culture condition and diet) and a Bonferroni post hoc test. $^{**}P < 0.01$ and $^{***}P < 0.001$ denote significant differences compared with the −FCS mice. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ denote significant differences compared with the mice fed SD.

Moreover, while the HFD significantly decreased the body weight gain of the male −FCS mice compared with the −FCS mice fed the SD ($^{\#\#\#}P < 0.001$), the +FCS mice fed the HFD displayed a significant increase compared with the +FCS mice fed SD ($^{\#\#\#}P < 0.001$). Therefore, the pattern of body weight gain observed in the male mice produced with FCS depended on the diet that the mice were fed; body weight increased with the HFD in the +FCS mice, while the control −FCS mice exhibited the opposite effect, a decrease with the HFD.

Regarding the female mice, Figure 2C shows the body weight gain of 4-mo-old mice fed a SD or a HFD. The statistical analysis indicated that both the culture condition ($F_{1,36} = 49.24$; $P < 0.001$) and the diet ($F_{1,36} = 40.34$; $P < 0.001$) had a significant primary effect on body weight gain, and there was also a significant interaction between these two factors ($F_{1,36} = 4.742$; $P < 0.05$). The female +FCS mice gained significantly more weight than their respective −FCS controls ($^{***}P < 0.001$ in mice fed the SD and $^{**}P < 0.01$ in mice fed the HFD). Furthermore, the female mice fed the HFD gained more weight than the females fed the SD ($^{\#\#\#}P < 0.001$ in −FCS mice and $^{\#}P < 0.05$ in +FCS mice). Thus, the female mice produced with FCS exhibited increased body weight gain that was enhanced with the HFD, while the control −FCS mice exhibited an increase only with the HFD.

In the 8-mo-old female mice (Fig. 2D), both the culture condition ($F_{1,36} = 5.364$; $P < 0.05$) and the diet ($F_{1,36} = 9.701$; $P < 0.01$) had a significant primary effect on body weight gain, and a significant interaction between these two variables ($F_{1,36} = 70.32$; $P < 0.001$) was also detected. At this age, the female +FCS mice fed the SD gained significantly less weight than the –FCS controls on the SD ($^{***}P < 0.001$), whereas the +FCS mice fed the HFD gained significantly more weight ($^{***}P < 0.001$) than the −FCS mice on the HFD. In addition, we observed the opposite effects of diet on the body weight gain in the −FCS mice. While the −FCS mice fed the HFD gained significantly less weight than the −FCS mice fed the SD ($^{\#\#\#}P < 0.001$), the +FCS mice fed the HFD gained significantly more weight than the female +FCS mice fed the SD ($^{\#\#}P < 0.01$). Overall, the female mice produced with FCS showed a different response to the diet that resulted in an opposite pattern of body weight gain than that observed in the control −FCS mice. We recorded the daily food intake of each group of animals during the 15 days of exposure to both diets, but we found no significant differences in the relative food intake (data not shown).

Effects of FCS Supplementation of Culture Media on the Adipocyte Size, Tissue Lipid Content, and Plasma Metabolites of Adult Mice

Adipocyte size. Changes in the total body weight were detected when the +FCS mice were compared with the −FCS

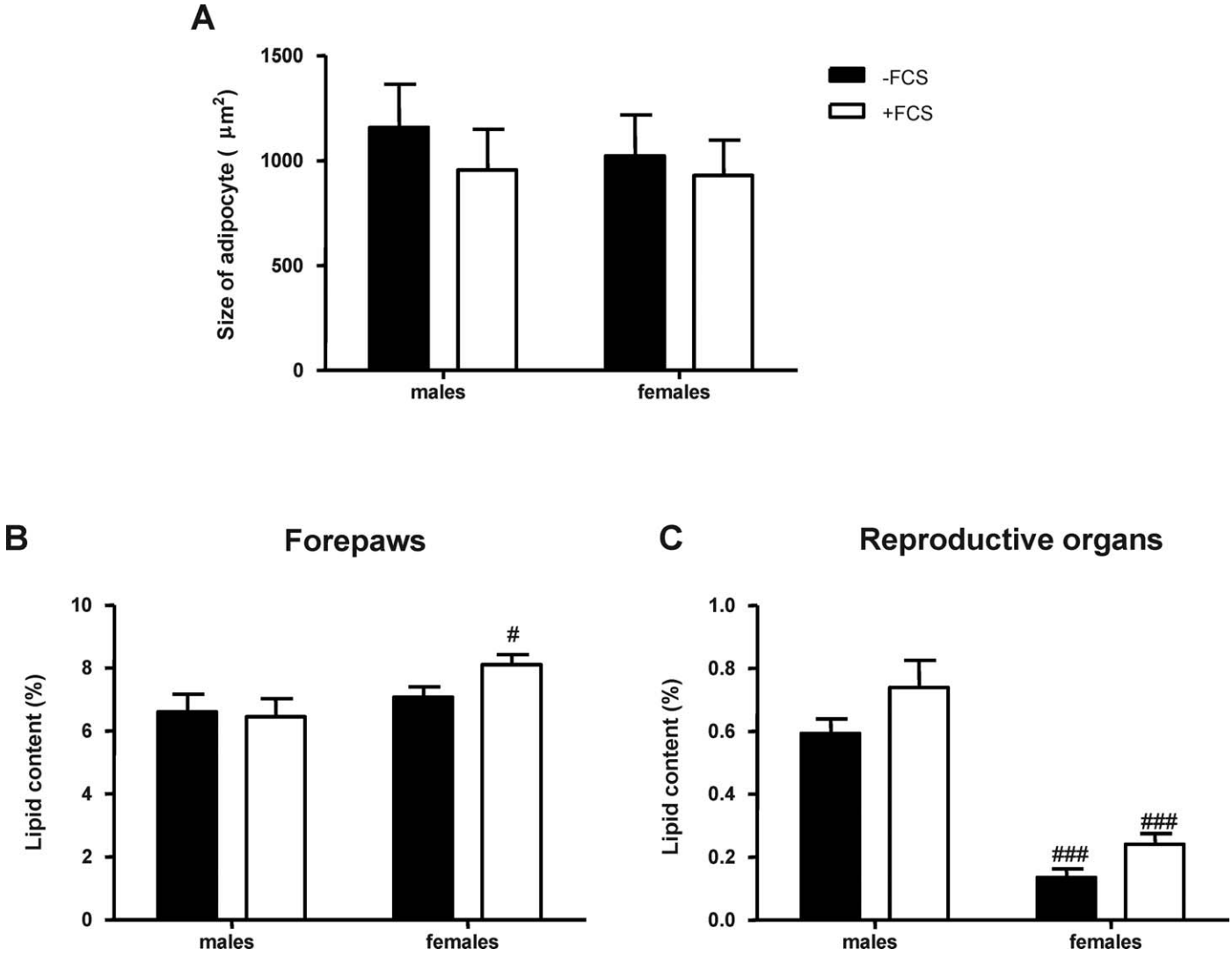

FIG. 3. Effects of FCS supplementation of the culture media on adipocyte size (**A**) and lipid content in forepaws (**B**) and reproductive organs (**C**) in adult mice. The bars represent means ± SEM (n = 8–11 animals per group). The data were analyzed by two-way ANOVA (culture condition and gender) and a Bonferroni post hoc test. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ denote significant differences compared with the male mice.

mice with a robust increase in the body weight of mice produced with FCS. However, we did not observe hypertrophic growth of the visceral adipose tissue in the +FCS mice. The adipocyte size from the perigonadal adipose tissue was found to be similar in all groups with no significant primary effects or interactions between the culture condition and gender as indicated in Figure 3A. Additionally, we also evaluated the effect of FCS supplementation on subcutaneous and visceral hyperplasia in 20-mo-old mice.

Lipid content in the forepaws. As shown in Figure 3B, the lipid content was determined in the forepaws. A two-way ANOVA indicated that only gender had a significant primary effect on forepaw lipid content ($F_{1,35} = 5.930$; $P < 0.05$). Indeed, the post hoc test showed that although the female mice displayed an overall increased lipid content in the forepaws, the increase was only significant in the female +FCS mice ($^{\#}P < 0.05$).

Lipid content in the reproductive organs. The lipid content of the reproductive organs was also evaluated in the adult mice (Fig. 3C). Both the culture condition ($F_{1,35} = 6.764$; $P < 0.05$) and gender ($F_{1,35} = 97.35$; $P < 0.001$) had a significant primary effect on the lipid content in the testes and ovaries. Although the +FCS mice exhibited a modest increase in fat content, this increase was not significant when the males and females were compared with their respective −FCS controls. However, the mice displayed less lipid content in the ovaries than in the testes ($^{\#\#\#}P < 0.001$ in −FCS and +FCS mice).

Lipid content and composition in the liver. We also analyzed the lipid content of the liver (total fat and triglycerides) and its fatty acid composition in the adult mice to identify any effects of the culture condition or gender and any interaction between these factors (Table 2). Gender had a significant primary effect ($F_{1,35} = 47.58$; $P < 0.001$) on the total liver fat content, and there was also a significant interaction between the culture condition and gender ($F_{1,35} = 7.336$; $P < 0.05$). While the two-way ANOVA showed no overall effect of the culture condition on the total liver fat considering all groups, the comparison between paired groups indicated that the female +FCS mice exhibited a significant increase in liver fat content compared with the female −FCS controls ($^{**}P < 0.01$). The most evident difference was the significantly increased liver fat content observed in the female

TABLE 2. Lipid parameters in the liver and plasma of adult mice produced from embryos cultured in vitro with (+) or without (–) FCS.

	Male		Female	
Parameter	−FCS	+FCS	−FCS	+FCS
Total fat content (% tissue)	7.38 ± 0.32	6.63 ± 0.92	9.70 ± 0.56$^{\#\#}$	11.95 ± 0.35$^{**,\#\#\#}$
Triglycerides (% tissue)	2.91 ± 0.52	1.28 ± 0.28*	3.49 ± 0.31	5.60 ± 0.36$^{***,\#\#\#}$
SFA (% total fatty acid)	63.32 ± 2.83	62.66 ± 8.49	65.14 ± 6.14	57.53 ± 4.17
MUFA (% total fatty acid)	17.10 ± 1.83	22.09 ± 6.88	23.49 ± 5.14	24.62 ± 3.64
PUFA (% total fatty acid)	19.58 ± 1.60	15.25 ± 0.61	11.48 ± 1.89$^{\#\#}$	20.19 ± 2.86*
Glycerol (mmol/L)	228.46 ± 16.66	183.59 ± 17.25	235.76 ± 17.97	226.69 ± 18.09
Triglycerides (mmol/L)	0.97 ± 0.09	0.94 ± 0.09	0.95 ± 0.12	0.66 ± 0.05
Leptin (ng/ml)	3.56 ± 0.90	3.53 ± 0.91	5.41 ± 1.48	8.04 ± 1.26$^{\#}$
Glucose (mg/dl)	76.7 ± 3.4	125.3 ± 15.3***	79.0 ± 3.1	64.8 ± 2.9$^{\#\#\#}$

* $P < 0.05$ and ***$P < 0.001$ denote significant differences compared with the −FCS mice.
$^{\#}$ $P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ denote significant differences compared with the male mice.

mice compared with the male mice ($^{\#\#\#}P < 0.001$ in +FCS mice and $^{\#\#}P < 0.01$ in −FCS mice).

The overall analysis also indicated that gender had a significant primary effect ($F_{1,35} = 41.06$; $P < 0.001$) on triglyceride content in the liver, and a significant interaction was observed between the culture condition and gender ($F_{1,35} = 24.00$; $P < 0.001$). The post hoc comparisons showed that whereas the male +FCS mice displayed decreased triglyceride levels (*$P < 0.05$), a clear increase was observed in the female +FCS mice (***$P < 0.001$) compared with the respective −FCS mice. Moreover, the female mice exhibited an overall increase in hepatic triglyceride content compared with the male mice that resulted from a significant effect in the +FCS mice ($^{\#\#\#}P < 0.001$). Overall, we observed the opposite response in triglyceride content in male and female mice produced with FCS, while the triglyceride content in control −FCS mice of both genders was unaltered.

Additionally, the triglyceride fatty acids in the livers were identified and quantified by chromatography. The data were grouped into SFA, MUFA, and PUFA and statistically analyzed using the culture condition and gender as factors. We did not detect primary effects on these groups of fatty acids by either factor; however, PUFA showed a significant interaction between the culture condition and gender ($F_{1,35} = 8.019$; $P < 0.01$). Focusing on the detected differences in the paired comparisons, the female +FCS mice exhibited a significant increase in the PUFA levels compared with the female −FCS mice (*$P < 0.05$). In addition, the PUFA content was significantly decreased in the female −FCS mice compared with the male −FCS mice ($^{\#\#}P < 0.01$). Again, we observed a different response in this parameter (PUFA) in the mice produced with FCS that was opposite to the response observed in the control −FCS mice.

The biochemical analysis of the liver fat content agreed with the results obtained with the specific lipid and fat depot stain Oil Red O in the liver (Fig. 4, A–D). The female +FCS mice exhibited increased fat content and triglycerides in the liver according to the results with Oil Red O as shown in Figure 4D. Consistent with these results, we further observed that the female mice produced with FCS developed hepatomegaly and liver steatosis (Fig. 4, E and F).

Plasma metabolites. We measured the plasma levels of glycerol, triglyceride, leptin, and glucose (Table 2). The glycerol and triglyceride levels were not affected by the culture condition or gender, and there was no interaction between these factors for glycerol or triglyceride levels. When the circulating levels of leptin were analyzed in these mice, only gender had a significant effect ($F_{1,35} = 6.511$; $P < 0.05$), and a significant increase in the leptin levels was observed in the female +FCS mice compared with the male +FCS mice ($^{\#}P < 0.05$).

Finally, the statistical analysis showed that both culture condition ($F_{1,30} = 6.099$; $P < 0.05$) and gender ($F_{1,30} = 17.46$; $P < 0.001$) had a significant primary effect on the glucose levels. Additionally, there was a significant interaction between the two factors ($F_{1,30} = 20.33$; $P < 0.001$). Thus, the male +FCS mice displayed higher plasma glucose levels compared with the male −FCS mice (***$P < 0.001$). However, the female +FCS mice were found to have significantly decreased glucose levels compared with the corresponding male mice ($^{\#\#\#}P < 0.001$). Additionally, Supplemental Figure S1 shows the effects of the culture condition and gender on the time course of glucose tolerance in adult mice. We observed a significant decrease in the glucose levels in the female −FCS mice compared with the male −FCS mice ($^{\#\#\#}P < 0.001$ at 30 min after glucose load and $^{\#}P < 0.05$ at 45 min), which was reflected in the area under the curves of the glucose levels during the first 60 min after the glucose injection (Supplemental Fig. S1A). The male +FCS mice had increased glucose levels compared with the male −FCS mice (*$P < 0.05$ at 15 min after glucose load and ***$P < 0.001$ at 30–60 min) (Supplemental Fig. S1B). However, the glucose levels in the female mice produced with FCS were unaltered compared to those produced with no FCS (Supplemental Fig. S1C).

Effects of FCS Supplementation of Culture Media on Metabolic Gene Expression in the White Adipose Tissue of Adult Mice

To further explore the metabolic control of these animals, we analyzed the gene expression of enzymes and regulatory factors involved in lipid metabolism and thermogenesis that have a putative role in energy metabolism. First, we analyzed the mRNA expression of genes encoding proteins implicated in lipolysis (peroxisome proliferator-activated receptor alpha [PPARα] and acyl-CoA oxidase 1 [ACOX]), lipogenesis (PPARγ and fatty acid synthase [FAS]), and thermogenesis (uncoupling protein 1 [UCP1]) (Fig. 5).

Lipolysis-related genes. Overall, the two-way ANOVA indicated that the relative expression of the lipolytic genes PPARα and ACOX was not affected by the culture condition or gender (Fig. 5, A and B). However, a significant interaction between both these factors was detected in these mice for expression of both genes ($F_{1,21} = 13.95$, $P < 0.01$ for PPARα; and $F_{1,21} = 8.300$, $P < 0.01$ for ACOX). The post hoc comparisons between the groups showed that the male +FCS mice exhibited a significant increase in the mRNA expression of PPARα and ACOX compared with their respective −FCS

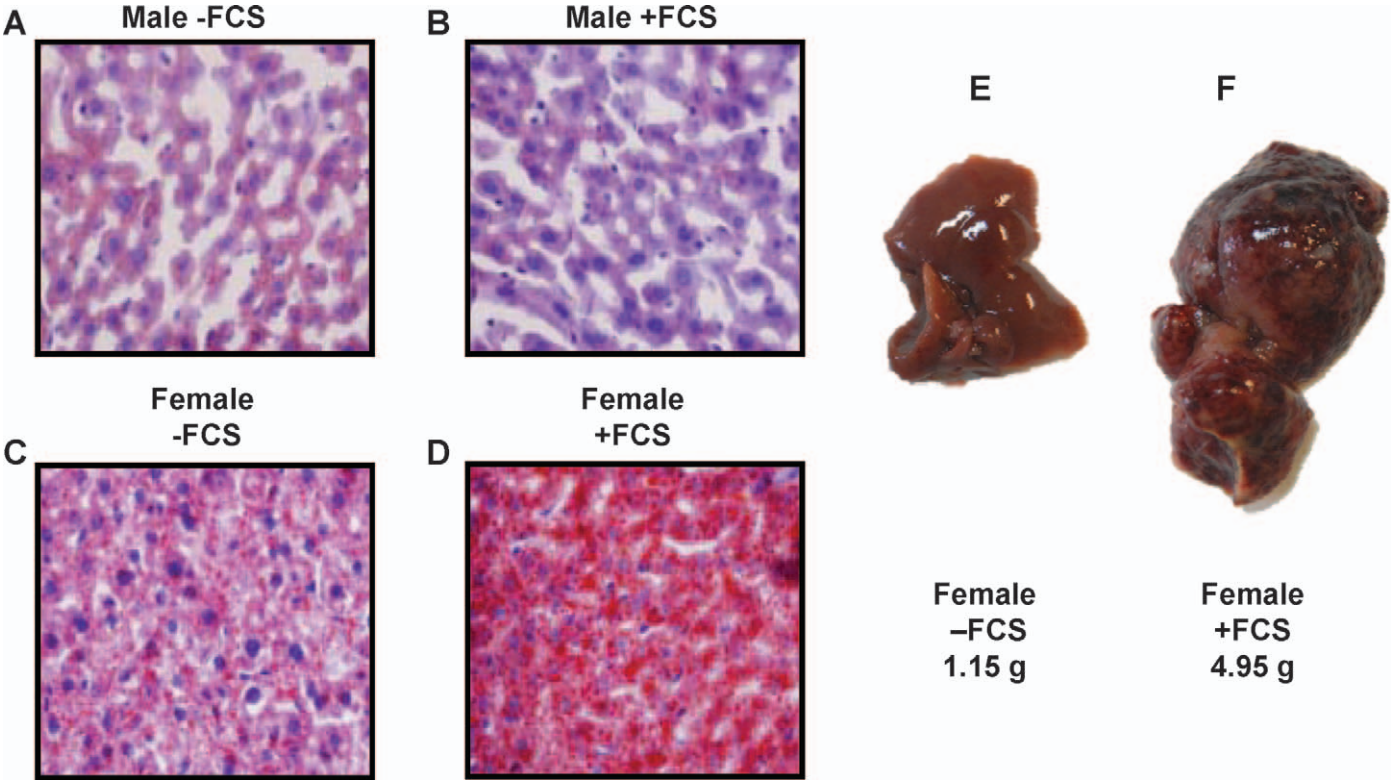

FIG. 4. Effects of FCS supplementation of the culture media on lipid content in the liver in adult mice. Liver sections stained with Oil Red O of male and female mice produced in vitro with or without FCS (magnification ×40; **A**–**D**). The images of liver of female mice produced in vitro with or without FCS (**E**, **F**).

mice ($^{*}P < 0.05$). By contrast, the female +FCS mice displayed a significant decrease in the mRNA expression of PPARα compared with the female −FCS mice ($^{*}P < 0.05$). Additionally, PPARα expression was significantly increased in female −FCS mice compared with male −FCS mice ($^{\#}P < 0.05$). No other significant changes were detected in ACOX expression. In summary, we observed that the mice produced with FCS exhibited differential responses in PPARα and ACOX expression based on gender with increased expression of these lipolytic genes in the +FCS males compared with control −FCS mice, which was opposite from the change observed in females.

Lipogenesis-related genes. For the lipogenic genes, the effects of the culture condition and gender on the relative expression of PPARγ and FAS are represented in Figures 5, C and D. The statistical analysis of the mRNA expression of PPARγ revealed a significant interaction between the culture condition and gender ($F_{1,20} = 8.699$, $P < 0.01$). The comparisons of paired groups showed that PPARγ expression was significantly decreased in the female +FCS mice compared with the female −FCS controls ($^{**}P < 0.01$). In contrast, the PPARγ expression was significantly increased in the female −FCS mice compared with the male −FCS mice ($^{\#}P < 0.05$).

The mRNA expression of FAS was significantly affected by the culture condition ($F_{1,23} = 5.237$, $P < 0.05$) and the gender ($F_{1,23} = 4.628$, $P < 0.05$). In addition, there was an interaction between the culture condition and gender ($F_{1,23} = 5.663$, $P < 0.05$). FAS expression in the adipose tissue of the female +FCS mice was significantly decreased compared with the female −FCS mice ($^{**}P < 0.01$). Again, as observed for expression of the PPAR genes, the relative expression of FAS was significantly increased in the female −FCS mice compared with the male −FCS mice ($^{\#}P < 0.05$). In this case, we observed that the mice produced with FCS exhibited no changes in the expression of PPARγ or FAS based on gender and, therefore, the increased expression of these lipogenic genes observed in female −FCS mice was absent in female +FCS mice.

Thermogenesis-related gene. Figure 5E indicates the relative gene expression of UCP1, a protein involved in the control of thermogenesis that was also analyzed in the white adipose tissue. A two-way ANOVA indicated that gender had a significant effect on UCP1 expression ($F_{1,20} = 5.834$, $P < 0.05$). A significant interaction between the culture condition and gender was also detected ($F_{1,20} = 5.251$, $P < 0.05$). After the post hoc test, only the male +FCS mice exhibited a significant increase in UCP1 expression compared with the male −FCS controls ($^{*}P < 0.05$).

Peptide hormone-related genes. Both adiponectin and leptin are peptide hormones that are released from the adipose tissue and play a key role in regulating energy homeostasis (Fig. 6). The mRNA expression of adiponectin was not affected by either the culture condition or gender (Fig. 6A). For leptin expression, we only observed a significant interaction between the two factors ($F_{1,24} = 9.293$, $P < 0.01$) (Fig. 6B). Focusing on leptin expression, the female +FCS mice showed a pronounced and significant decrease in leptin expression compared with the female −FCS mice ($^{*}P < 0.05$) and the male +FCS mice ($^{\#}P < 0.05$). Overall, we observed opposite responses in adiponectin and leptin mRNA expression between the mice produced with FCS and the control −FCS mice.

Other genes. As shown in Figure 7, the gene expression of GHr, GLUT4, and CB_1 in the adipose tissue were also analyzed by qRT-PCR. The statistical analysis of GHr expression revealed a significant interaction between the

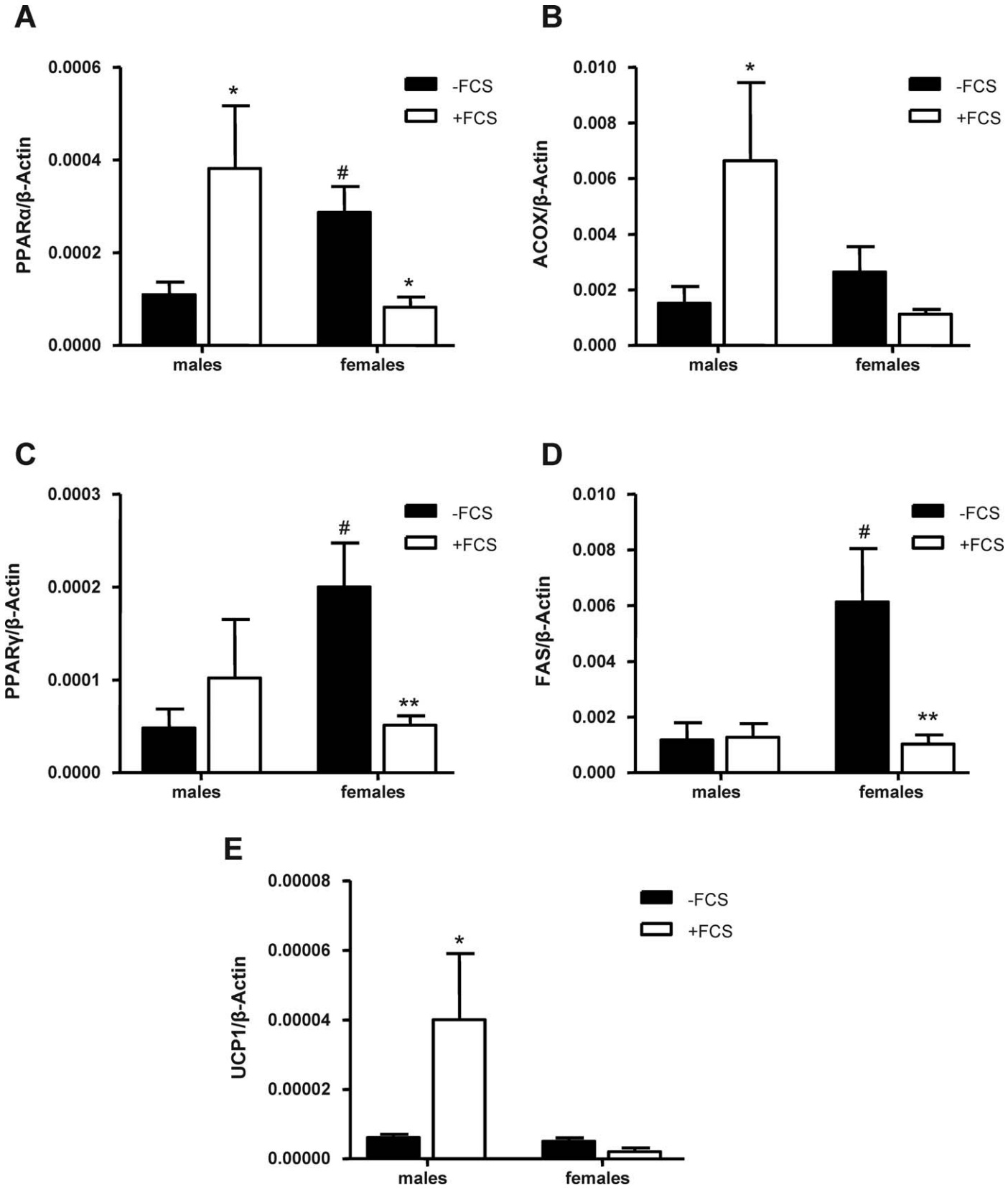

FIG. 5. Effects of FCS supplementation of the culture media on gene expression of lipid metabolism-related proteins in the white adipose tissue of adult mice. The gene expression of PPARα (**A**), ACOX (**B**), PPARγ (**C**), FAS (**D**), and UCP1 (**E**) were determined in male and female mice produced in vitro with or without FCS. The bars represent means ± SEM (n = 8–11 animals per group). The data were analyzed by two-way ANOVA (culture condition and gender) and a Bonferroni post hoc test. $^{*}P < 0.05$ and $^{**}P < 0.01$ denote significant differences compared with the –FCS mice. $^{\#}P < 0.05$ denotes significant differences compared with the male mice.

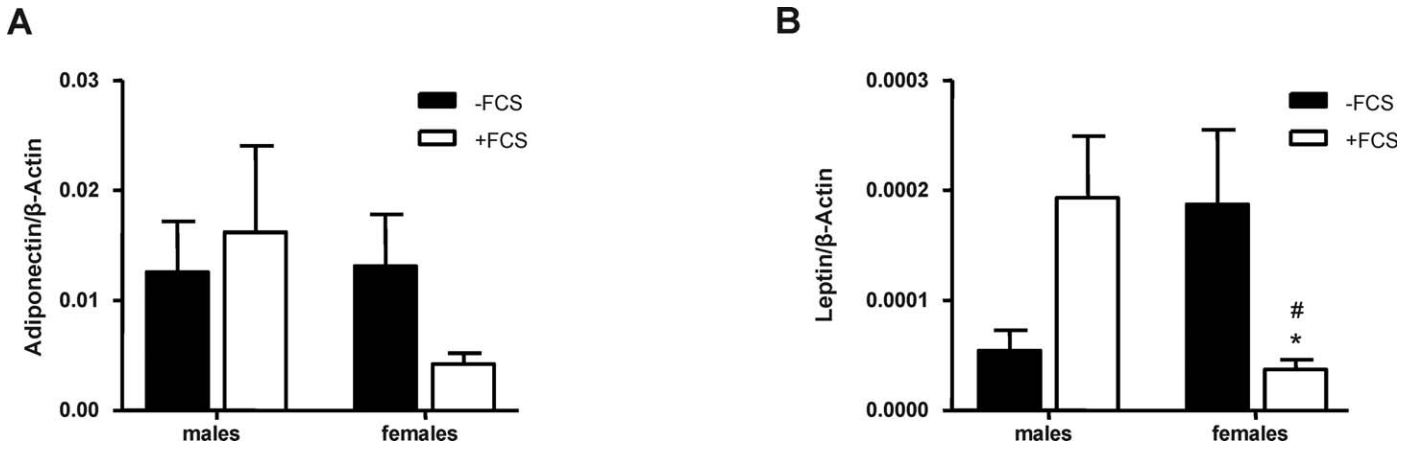

FIG. 6. Effects of FCS supplementation of the culture media on gene expression of adipocyte-derived hormones in the white adipose tissue of adult mice. The gene expression of adiponectin (**A**) and leptin (**B**) were determined in male and female mice produced in vitro with or without FCS. The bars represent means ± SEM (n = 8–11 animals per group). The data were analyzed by two-way ANOVA (culture condition and gender) and a Bonferroni post hoc test. $^{*}P < 0.05$ denotes significant differences compared with the −FCS mice. $^{\#}P < 0.05$ denotes significant differences compared with the male mice.

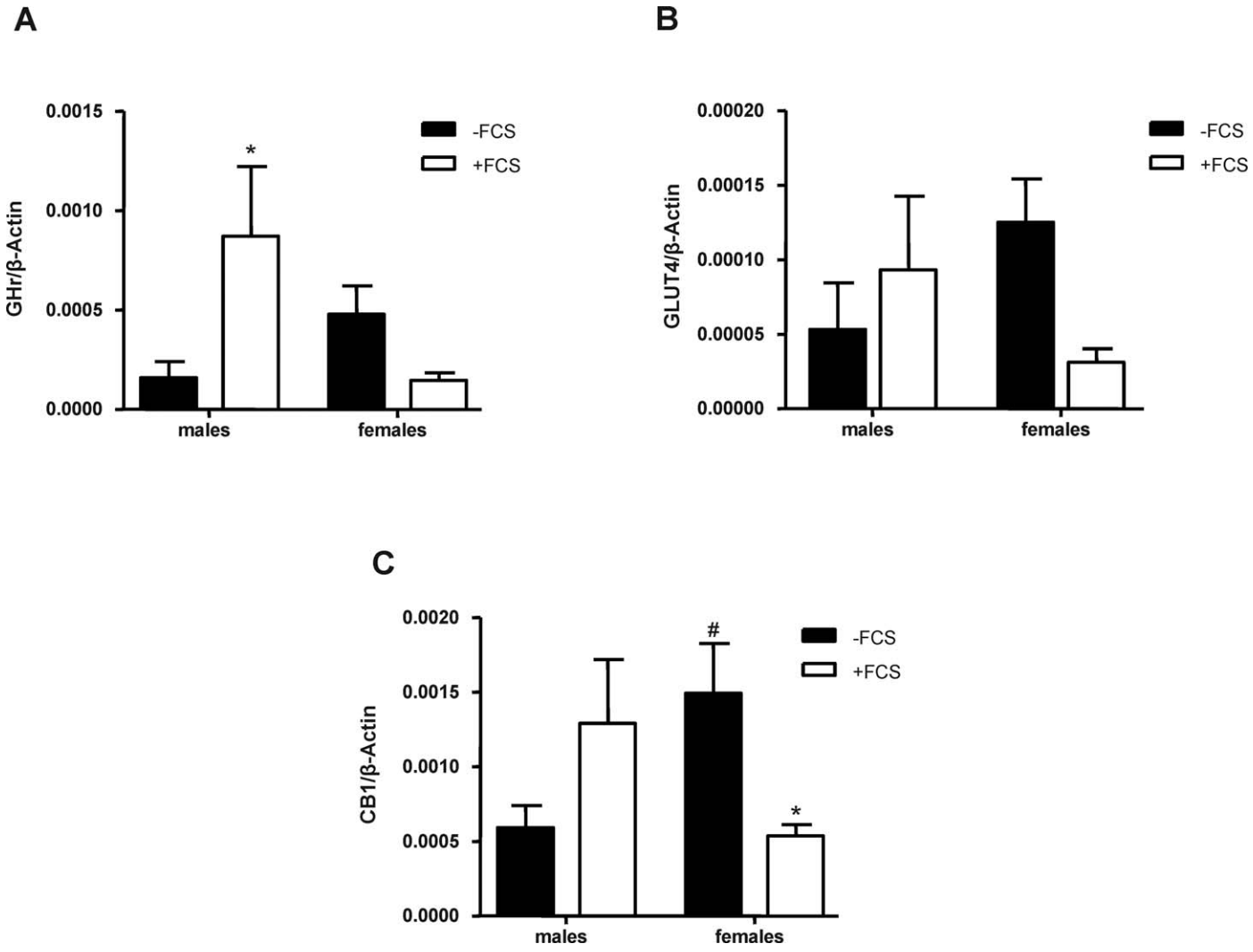

FIG. 7. Effects of FCS supplementation of the culture media on gene expression of GHr (**A**), GLUT4 (**B**), and cannabinoid CB_1 receptor (**C**) were determined in male and female mice produced in vitro with or without FCS. The bars represent means ± SEM (n = 8–11 animals per group). The data were analyzed by two-way ANOVA (culture condition and gender) and a Bonferroni post hoc test. $^{*}P < 0.05$ denotes significant differences compared with the −FCS mice. $^{\#}P < 0.05$ denotes significant differences compared with the male mice.

culture condition and gender ($F_{1,24} = 9.223$, $P < 0.01$) (Fig. 7A). The post hoc test showed that GHr mRNA expression was significantly increased in the male +FCS mice compared with the male −FCS mice (*$P < 0.05$), while GHr expression was unaltered in the female +FCS mice. An opposite response was observed for the −FCS mice.

GLUT4 mRNA levels were not affected by the culture condition or gender, but a significant interaction between these factors ($F_{1,21} = 5.232$, $P < 0.05$) was detected (Fig. 7B). However, the Bonferroni post hoc test showed no significant changes.

For CB_1 mRNA expression, we observed a clear interaction between the culture condition and gender ($F_{1,25} = 9.392$, $P < 0.01$) (Fig. 7C). In this case, the post hoc test showed significant differences. The female +FCS mice exhibited a significant decrease in the relative expression of the CB_1 compared with the female −FCS controls (*$P < 0.05$). By contrast, the female −FCS mice displayed higher levels of CB_1 mRNA than the male −FCS mice (#$P < 0.05$). In summary, a differential and opposite response to CB_1 expression was also observed in mice produced with FCS and compared with control −FCS mice based on gender.

DISCUSSION

In the present study, we evaluated the effects of embryo culture in the presence of FCS on the development of obesity in the offspring. Obesity caused by FCS supplementation was characterized by an expansion of the adipose tissue due to visceral hyperplasia. This obese phenotype was also associated with the induction of liver steatosis and additional complications in female mice.

In our mouse model, the survival rate of the control animals (−FCS) was similar for both genders (80%–90% at 20 mo). By contrast, 70% of female and 50% of male mice generated in the presence of serum (+FCS) died during the first 20 mo of life. Previous observations have suggested that long-term programming of postnatal development, growth, and physiology can be irreversibly affected during the preimplantation period of embryo development by suboptimal in vitro culture conditions. Several studies have reported a reduction in the postimplantation viability of embryos cultured in medium supplemented with serum [6–9]. It is thought that under these conditions the embryo does not receive the right cues, resulting in dysregulation of genes or aberrant epigenetic modifications in the genome [26]. In this regard, we have previously demonstrated that the use of serum affected not only the mRNA expression of several growth-related imprinted genes, leading to aberrant fetal growth and development [6], but also multiple genes involved in other biological processes and molecular functions [27].

The present results show an increase in body weight in mice produced with FCS. In males, the increase was approximately 24% more than that observed in the control group, while in females the increase in body weight was approximately 32% more than in controls. The development of obesity in these mice may be related to epigenetic alterations. This idea is consistent with previous reports that suggest that susceptibility to obesity in cloned mice derived by nuclear transfer is likely due to epigenetic errors [28, 29]. In accordance with these observations, a 15-day exposure to a standard laboratory chow caused a higher increase in body weight gain in 4-mo-old mice produced with serum than in the control mice, while the opposite effect was observed at 8 mo of age. We also tested the effect of serum in animals exposed to a HFD for 15 days. This diet caused a significant increase in body weight gain in the mice produced with serum compared with the control mice.

We also observed an increase of systolic blood pressure in females produced with serum. This effect was not evident in the males, although in general, systolic blood pressure was higher in males than in females. This increase may be due to the increase in body weight observed in these animals. These results are in agreement with previous studies that show that embryo culture produces an increase of systolic blood pressure in mice [30]. Similarly, studies using rats and sheep have demonstrated that maternal undernutrition during the periconceptional or preimplantation periods may result in an elevated systolic and arterial blood pressure in the offspring [31–33].

The increase in the size (hypertrophy) and/or number (hyperplasia) of adipocytes can be factors in the growth of adipose tissue, contributing to the development of obesity. As mentioned previously, mice produced with serum developed obesity; however, their obesity was not due to hypertrophy of adipose tissue because we observed normal-sized adipocytes in all the groups. On the other hand, obesity can lead to the accumulation of lipids in nonadipose tissues. Here, we showed that the use of serum in the culture induced a slight increase in the lipid content of the reproductive organs, while there was no effect on the subcutaneous fat distribution. Moreover, we observed serum-induced alterations in the level of lipids in the female mouse liver. Indeed, mice produced with serum displayed fatty liver, which was confirmed by a significant elevation in the total fat and triglyceride content of the livers compared with the livers of controls. It is known that excessive fat accumulation in the liver may lead to the development of nonalcoholic fatty liver, which is one of the problems associated with obesity [34]. We also observed significant gender differences in fat tissue accumulation and fatty acid composition of hepatic triglycerides. This is in agreement with previous studies in mammals that described differential distributions and amounts of fat in males and females [35–38]. These differences may be due to sex hormone influences [39, 40].

In addition to the liver, adipose tissue has a key role in peripheral energy homeostasis because obesity is also associated with dysfunctional adipose tissue. In our study, we observed important changes at the transcript level of several enzymes and hormones involved in lipid metabolism and energy homeostasis in adipocytes. We grouped the data into lipolytic or lipogenic genes to evaluate the net effect of the culture condition and/or gender. We found an important effect of both factors on the gene expression of receptors (PPARs) and enzymes involved in lipid metabolism. Serum supplementation increased the mRNA levels of PPARα and ACOX in male mice, suggesting a stimulation of lipolysis. Serum also caused a significant increase in UCP1 mRNA expression in the adipose tissue of these animals. These results are in agreement with a previous report that describes the induction of UCP1 gene expression by PPARα [41]. By contrast, serum supplementation inhibited the mRNA expression of PPARα and ACOX in female mice. It is important to note that ACOX expression is PPARα dependent. Similar inhibitory effects were observed for the prolipogenic PPARγ receptor and the lipogenic enzyme FAS. The suppressed transcription of these lipogenic genes may be related to the increase in PUFA observed in these animals [42]. Additionally, the inhibitory effect of serum on lipogenesis signaling may be due to compensatory actions that result from the suppression of lipolytic genes described above (PPARα and ACOX). Moreover, the inhibition of lipolytic/lipogenesis mechanisms in the adipose tissue may be related to the decrease in

circulating triglyceride levels observed in female mice produced with serum, suggesting a reduction of lipid mobilization in these animals.

Adipose tissue is also an endocrine organ. Adiponectin and leptin are the major adipocyte-derived hormones, and both are involved in the regulation of energy homeostasis, glucose metabolism, and lipid metabolism [43–45]. We observed no significant changes in adiponectin mRNA levels after serum supplementation, although a trend toward decreased adiponectin expression was observed in females. In agreement with these data, it has been reported that adiponectin mRNA levels in adipose tissue are lower in obese humans and rodents than in lean humans and rodents [46–48]. Serum supplementation reduced leptin transcription in female mice, while the circulating levels of leptin were elevated in these animals. The changes to this hormone may be related to the development of obesity displayed in these mice. In fact, it is known that obese rodents develop leptin resistance as a consequence of elevated circulating leptin levels. Moreover, we found a clear sex difference in leptin levels. Previous studies have described the existence of a sexual dimorphism in leptin production, reporting higher plasma and adipose leptin levels in females than in males [49, 50]. It has been suggested that these differences are not due to sex hormones but rather the different fat distributions of males and females may be involved [50].

We evaluated the expression of the CB_1 in adipose tissue, which controls adipogenesis and lipogenesis in adipocytes [51, 52]. Serum supplementation reduced CB_1 expression in female mice, preventing the increase observed in female controls. These results are in agreement with previous reports that described a reduction in CB_1 mRNA in the adipose tissue of obese women [53]. Another study described a decrease in CB_1 expression in adipose tissue, suggesting activation of the endocannabinoid system in obesity [54]. The gender-specific effect of the culture condition on CB_1 expression agrees with a recent study of healthy, normal-weight subjects that demonstrated gender differences in endocannabinoids [55].

GHr plays a key role in the regulation of postnatal growth and is essential for normal lipid metabolism and the maintenance of lipid homeostasis. The secretion of GH is subject to metabolic regulation; for example, obesity leads to reduced levels of the hormone [56]. We found that male mice produced with FCS displayed elevated mRNA levels of GHr; however, the GHr levels were reduced in females produced with FCS, which is consistent with the previously observed changes in GHr in mouse blastocysts produced with FCS [27]. The differential expression of GHr in males and females could also underlie the sexual dimorphism observed for FCS-induced obesity because mice with liver-specific deletion of the GHr gene have been shown to display severe hepatic steatosis, and we have detected hepatic steatosis only in females produced with FCS [57, 58].

Although the present results indicate that suboptimal culture conditions might affect the expression of metabolically relevant genes, further research is needed to understand how these alterations are reflected in both expression and functionality of these proteins. It is important to have in mind that changes in the expression of a particular gene are not always translated into the expression and function of the protein encoded [59].

We also evaluated glucose handling in our animals using a glucose tolerance test. We observed a clear gender effect. In fact, we found that the male control mice displayed higher glycemic levels 30 min after glucose injection than the female controls. This significant difference was sustained until 45 min after glucose handling, although the glycemic levels were higher in males than in females at all the time points. This improvement in glucose handling in females may be explained by the activation of PPARγ because this receptor is an important regulator of glucose metabolism and these animals expressed high levels PPARγ mRNA. PPARγ is expressed in mature adipocytes, playing a main role in the expression of glucose transporters, the insulin receptor, and insulin signaling factors [60–63]. Moreover, we found higher levels of GLUT4 mRNA in the female controls than in the male mice. Previous studies have reported that PPARγ plays a role in the activation of GLUT4 transcription [64]. In addition to the gender effect, we also observed a clear effect of the serum supplementation on glucose handling in male mice, but no effect was observed in females.

In summary, our results show that in vitro culture in the presence of serum generates obese offspring. It is most likely the early embryo exhibits an adaptive response to these suboptimal conditions that may predispose it to an obese phenotype in adulthood. This maladaptive response observed in mice most likely extends to most assisted reproductive technology procedures in other mammals. Understanding the mechanisms involved in the development of this altered phenotype in response to suboptimal culture conditions may help to predict and prevent the transmission of maladaptive acquired phenotypes.

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