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Leptin Causes the Early Inhibition of Glycolysis- and TCA Cycle-Related Genes in the Brain of Ob/Ob Mice to Restore Fertility

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Abstract

Introduction: Polycystic ovarian syndrome (PCOS) is undoubtedly the commonest androgen disorder in woman's fertile period and certainly one of the most prevalent causes of anovulation. The syndrome has an estimated prevalence of 4% - 10% among women of childbearing age. Previously, our group demonstrated the effect of gonadal white adipose tissue transplantation from wild-type lean and fertile female mice to isogenic obese anovulatory ob/ob mice. These complex metabolic interrelationships between obesity and PCOS have yet to be fully understood. The aim of this study was to evaluate the effect of gonadal white adipose tissue (WAT) transplantation from the wild-type lean and fertile female mice to isogenic obese, anovulatory mice (Lep ob/Lep ob) on the expression of glycolysis- and TCA cycle-related genes and obtain a general view of the glucose metabolism in the brain of these animals. Methods: Fifteen ob/ob mice ranging from 2 to 3 months of age were divided into 3 experimental groups: control normal weight (n = 5), obese control (n = 5) and obese 7 days leptin treated (n = 5). The whole brains of the mice were processed for RNA extraction. The samples from each group were used to perform PCR assays using an array plate containing 84 primers to study the glucose metabolism-related genes. Results: The glycolysis- and TCA cycle-related genes were significantly downregulated. The most significantly affected genes

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were as follows: for glycolysis (fold regulation with $p < 0.05$): *Pgm*1, *Bpgm*, *Aldob*, and *Eno*3 (119, 45, 18, and 28 times less, respectively); and for the TCA cycle (fold regulation with $p < 0.05$): *Cs*, *Idh*3*b*, and *Mdh*2 (84, 27, and 37 times less, respectively). Conclusion: The seven-day leptin treated mice show a decrease in the glucose metabolism. These results confirm the ability of the adipose tissue-derived hormone leptin to regulate early crucial genes that are related to glycolysis mechanisms and to the TCA cycle. This hormone seems to revert early the central physiological conditions that are associated with PCOS; however, the morphological alterations can only be observed within a 45-day treatment.

Keywords

PCOS, Obesity, Leptin, Glycolysis, TCA Cycle, Gene Expression

1. Introduction

Polycystic ovarian syndrome (PCOS) is undoubtedly the commonest androgen disorder during a woman's fertile period and certainly one of the most prevalent causes of anovulation [1]-[4]. PCOS is the classic example of the loss of functional cyclicity rhythm associated with anomalous feed-back or, as it is also known as inappropriate feed-back [5]. In this case, the excessive production of androgens and their subsequent extra glandular conversion into estrogens form the pathophysiological basis for chronic anovulation [6]-[9]. Additionally, the excessive androgen production can be explained by extra- and intra-ovarian factors [10]. This syndrome has an estimated prevalence of 4% - 10% among women of childbearing age [11]-[14] and is remarkable for the heterogeneity of its symptoms, reflected by the presence or by the absence of insulin resistance (IR) among these women, as well as, by individual differences regarding the magnitude of the symptoms that are observed in women that are affected by the syndrome [14]-[16].

The clinical manifestations of PCOS include classic menstrual disorders, obesity, adrenal enzyme deficiencies, hirsutism, metabolic syndrome, diabetes, infertility, and hyperandrogenism, which may also be associated with insulin resistance [13] [17]-[19]. A significant number of women with PCOS have an abnormally high IR when compared to those of matched controls. Moreover, PCOS can be considered the initial manifestation of metabolic syndrome [20].

In fact, PCOS is often associated with obesity in women. Obesity is a worldwide problem that threatens the lives of adults, adolescents and children. Obesity has many associated comorbidities, such as the well-known components of metabolic syndrome (MetS), which harms the health of men and women [21].

Obesity is associated with several metabolic disorders and has reproductive consequences that are complex and not well understood [22]. Adipose tissue produces leptin, which has dominated the literature on female fertility complications, but other adipokines, such as adiponectin and resistin, seem to be important as well, as our understanding of their biological functions improves. Leptin influences the developing embryo and the functioning of the ovary and of the endometrium and modulates the release and the activity of gonadotropins and of the hormones that control their synthesis (inhibin, GnRH and kisspeptins). The biological actions and the potential roles of the adipokines leptin, adiponectin and resistin are frequently studied in the context of female fertility and its interplay with the complexity of the obese metabolic state [23].

Until recently, PCOS has been recognized as a hyperandrogenic disorder originating from hypothalamic pituitary gonadotropin secretion or ovarian dysfunction [24]. Lately, however, at least some cases of PCOS have been regarded as disorders of metabolic origin that impair reproduction [24]. Because obesity, particularly in the abdominal region, is found in approximately 50% of women with PCOS and appears in mid-childhood and increases during puberty [25], excess adiposity has generated a great deal of attention. Furthermore, the clinical phenotype and the development of PCOS are thought to be reinforced by obesity. Additionally, PCOS also has a genetic component, and different genetic polymorphisms have also been linked to the syndrome or its associated disorders [26]. Altogether, these associations highlight the importance of disentangling the relationship between obesity and PCOS and in particular their common metabolic disorders.

Leptin, which is a product of the ob gene, is a major hormone that is secreted by white adipose tissue (WAT) and is involved in the regulation of body weight, glucose metabolism, and fertility. Leptin null mice (Lep ob/

Lep ob) are obese, hyperphagic, insulin resistant, and sterile. In addition, ob/ob females are invariably sterile, and few ob/ob males have been reported to occasionally reproduce. These ob/ob mice develop many pathological features that are common to human obesity, which is also marked by disturbances of reproductive function [27].

Therefore, the complex metabolic interrelationships between obesity and PCOS have yet to be fully understood. The action of leptin in the brains of ob/ob mice seems to regulate early two key metabolic biological processes, glycolysis and the TCA cycle, especially in those nervous cells that express leptin receptors. Curiously, these receptors are not expressed by GnRH-releasing neurons. Leptin controls GnRH release by acting in other hypothalamic nuclei. Leptin regulates kiss1 neurons, making of these neurons probable targets for the hormonal control of the interaction between nutrition and reproduction [28]. Aiming to better understand these complex neuronal networks, the aim of this study was to evaluate the effect of gonadal WAT transplantation from wild-type lean and fertile female mice to isogenic obese, anovulatory mice (ob/ob) on the expression of glycolysis- and TCA cycle-related genes and to obtain a general view of glucose metabolism in the brains of these animals.

2. Methodologies

2.1. Experimental Animals and Surgical Procedure

In this study, ten transgenic obese and anovulatory leptin-deficient mice (B6.V-Lep ob/J, designated as ob/ob mice) and five isogenic lean ovulatory littermates (wild-type) were obtained from the Center for the Development of Experimental Models, Federal University of São Paulo, Brazil (CEDEME). These animals were maintained in a temperature-controlled environment at approximately 24˚C under a 12/12-h light-dark cycle and were handled at least once a week. Five ob/ob mice received a white adipose tissue (WAT) transplant as described by Gavrilova and Marcus-Samuels *et al.* (2000) [29] and Pereira *et al.* (2011) [9]: the adipose gonad tissue samples that were obtained from the wild-type mice were placed in phosphate buffer solution (PBS) and fragmented into small pieces. The WAT grafts were implanted in the subcutaneous tissue through small, shaved skin incisions on the dorsal region of the animal, which was anesthetized with isoflurane. The brains of all of the animals were removed by the reported procedure and kept in liquid nitrogen until use.

2.2. Experimental Groups

The animals that were described in the previous section were divided into three experimental groups as follows:

2.2.1. Control Group (CG)

Five (5) normal-weight B6.V-Lep ob/J mice at two to three months of age and ovulatory cycles.

2.2.2. Obese Group (OG)

Five (5) ob/ob mice at two to three months of age and anovulatory cycles.

2.2.3. 7-Day Transplanted Mice Group (7dTM)

Five (5) ob/ob mice at two to three months of age and anovulatory cycles were implanted with adipose gonadal tissue from mice with ovulatory cycles. These animals were sacrificed seven (7) days after the surgical procedure.The template is used to format your paper and style the text. All margins, column widths, line spaces, and text fonts are prescribed; please do not alter them. You may note peculiarities. For example, the head margin in this template measures proportionately more than is customary. This measurement and others are deliberate, using specifications that anticipate your paper as one part of the entire journals, and not as an independent document. Please do not revise any of the current designations.

2.3. RNA Extraction

After using liquid nitrogen for cryogenic soaking, the tissues were homogenized in Trizol™ reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. After the complete dissociation of the nucleoprotein complexes, phase separation was achieved with chloroform and centrifugation. The precipitated RNA from the aqueous phase was washed with 75% ethanol. The RNA was dried and dissolved in RNase-free water. The total RNA was then purified with the Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA) and submitted to

DNase treatment. The amount and quality of the extracted RNA were assessed by spectrophotometry using NanoDrop v3.3.0 (NanoDrop Technologies Inc., Rockland, DE).

2.4. QPCR

The total RNA (1.0 μg) per plate/array from each experimental group pool was used to synthesize the cDNA. The samples were treated with buffers from the kit, and reverse transcription reactions were performed using the RT2 First Strand Kit from SA Biosciences (Qiagen Company) according to the manufacturer's protocol. The qPCR array was performed using the RT2 Profiler^{TM} PCR array of SA Biosciences (http://www.sabiosciences.com/ArrayList.php). For each experimental group, 51 genes (29 for the TCA cycle- and 22 for the glycolysis-related genes) were examined in triplicate (PAHS-006). The amplification, data acquisition and analysis curves were performed on an ABI Prism 7500 Fast Sequence Detection System (Applied Biosystems, Foster City, CA). In turn, each gene was checked for the efficiency and the minimum and maximum threshold curve pattern. To ensure accurate comparisons between the curves, the same threshold was established for every gene. Three genes were used for normalization (*Hsp*90*ab*1, *Gapdh* and *Actb*), and the average qC values were used to standardize the gene expression (2-CT change table) and to determine the difference between the groups. To consider a gene differentially expressed, we used a differential cut-off of two-fold (up or downregulated).

2.5. Histology

For the histological evaluation of all of the study groups, the uteri were obtained and processed immediately after euthanasia. The tissues were fixed in 4% formalin in saline, embedded in paraffin, and cut to a thickness of 5 mm. The sections were deparaffinized, rehydrated, stained with hematoxylin/eosin (H.E.), and evaluated under light microscopy.

2.6. Statistical Analysis

The p values were calculated based on Student's t-test of the three replicate $2^{(-\Delta ct)}$ values for each gene in the control and treatment groups, and p values less than 0.05 were considered significant. The qPCR reactions were processed through the online software RT2 Profiler^{TM} PCR Array Data Analysis (SA Biosciences).

2.7. Ethics

The procedures were performed in accordance with the ethical standards of the institution and national guidelines for the care and use of laboratory animals. The study protocol for the use of laboratory animals in research was approved by the local ethics committee (CEP/UNIFESP, number 0017/12).

3. Results

In general, we observed a marked downregulation of the glycolysis- and TCA cycle-related genes in the brain of 7dTM mice compared to those of the CG mice and a marked upregulation of these same groups of genes in the brains of the non-treated ob/ob mice (OG) compared to those of the CG mice. In the brains of the 7dTM mice, the most significantly downregulated genes were *Pgm*1, *Bpgm*, *Aldob* and *Eno*3, which were downregulated 119, 45, 18, and 28 times, respectively, for the glycolysis-related genes (fold regulation with $p < 0.05$), and *Cs*, *Idh*3*b*, and *Mdh*2, which were 84, 27, and 37 times, respectively, for the TCA cycle-related genes (fold regulation with $p < 0.05$). Curiously, *Pcx* was the only upregulated gene and was upregulated 27 times (fold regulation with $p < 0.05$). In contrast, the endometrial and ovarian morphologies could only be observed after a 45-day period.

To better describe the gene expression profiles that were obtained for each functional group of genes (glycolysis and TCA cycle) and in each experimental group comparison (7dTM with CG and OG with CG), the glycolysis- and TCA cycle-related genes are discussed separately in the following two distinct subsections.

3.1. 7dTM versus CG

Glycolysis—Seventeen out of the twenty-two (77%) glycolysis-related genes were differentially expressed in the 7dTM group, using the CG group as a calibrator. All of these seventeen genes were downregulated, and none

were upregulated. The downregulated genes included *aldolases* a, b and c; *Bpgm*; *Enolases* 1, 2 and 3; *Gpi*1; *Pfkl*; *Pgam*2; *Pgk*1; *Pgms*1, 2 and 3; *Pklr*; and *Tpi*1. Among the non-differentially expressed genes, *Gapdhs* and *Hk3* were detected in both of the groups (7dTM and CG), while *Gck* and *Hk*2 were only detected in the CG group, and *Pgk*2 could not be detected in any of the groups. The fold-regulation values of each assessed glycolysis-related gene can be observed in **Table 1**.

Table 1. Fold-regulation values that were obtained for the glycolysis- and TCA cycle-related genes in two different comparisons, 7dTM and OG, using the CG group as a calibrator. The data were processed with the online program Data Assist™ SA Biosciences (Qiagen Company). nde = non-differentially expressed.

	Gene Symbol	Gene Name	Refseq	7dTM × CG		OG × CG	
				Fold regulation	p value	Fold regulation	p value
GLYCOLYSIS	*Pgm*1	Phosphoglucomutase 1	NM_025700	−119.57	0.000312	nde	
	Bpgm	2,3-bisphosphoglycerate mutase	NM_007563	−44.68	0.000517	2.02	0.006556
	*Eno*3	Enolase 3, beta muscle	NM_007933	−27.95	0.000051	2.23	0.020764
	Aldob	Aldolase B, fructose-bisphosphate	NM_144903	−17.94	0.000004	2.04	0.000378
	*Tpi*1	Triosephosphate isomerase 1	NM_009415	−15.55	0.0000	2.26	0.000281
	*Eno*1	Enolase 1, alpha non-neuron	NM_023119	−12.89	0.000003	2.47	0.000001
	Aldoc	Aldolase C, fructose-bisphosphate	NM_009657	−12.51	0.000001	2.58	0.000007
	*Pgk*1	Phosphoglycerate kinase 1	NM_008828	−9.57	0.0000	2.08	0.000001
	*Eno*2	Enolase 2, gamma neuronal	NM_018870	−7.04	0.000151	2.52	0.002822
	Pfkl	Phosphofructokinase, liver, B-type	NM_028352	−6.36	0.019107	3.19	0.000051
	*Pgam*2	Phosphoglycerate mutase 2	NM_1177307	−6.08	0.000001	3.54	0.000187
	*Pgm*3	Phosphoglucomutase 3	NM_028352	−5.33	0.000191	2.88	0.000009
	Aldoa	Aldolase A, fructose-bisphosphate	NM_1177307	−5.31	0.000002	nde	
	*Gpi*1	Glucose phosphate isomerase 1	NM_008155	−5.18	0.000355	2.76	0.000129
	*Pgm*2	Phosphoglucomutase 2	NM_028132	−4.59	0.000001	2.04	0.000444
	Galm	Galactose mutarotase	NM_176963	−2.58	0.000261	nde	
	Pklr	Pyruvate kinase liver and red blood cell	NM_013631	−2.18	0.007846	nde	
	Gck	Glucokinase	NM_010292	nde		3.54	0.00246
	*Hk*2	Hexokinase 2	NM_013820	nde		2.36	0.00001
	*Hk*3	Hexokinase 3	NM_1033245	nde		2.45	0.001786
TCA CYCLE	*Cs*	Citrate synthase	NM_026444	−84.16	0.0000	2.43	0.000067
	Pcx	Pyruvate carboxylase	NM_008797	26.75	0.000456	nde	
	*Mdh*2	Malate dehydrogenase 2, NAD (mitochondrial)	NM_008617	−37.40	0.000244	2.03	0.001091
	*Idh*3*b*	Isocitrate dehydrogenase 3 (NAD+) beta	NM_130884	−27.32	0.000024	2.05	0.000005
	*Mdh*1*b*	Malate dehydrogenase 1B, NAD (soluble)	NM_029696	−23.73	0.0000	2.41	0.00011
	Dlat	pyruvate dehydrogenase complex)	NM_145614	−19.32	0.000096	nde	
	Dlst	oxo-glutarate complex)	NM_030225	−8.49	0.0000	3.05	0.000647
	*Sucla*2	Succinate-Coenzyme A ligase, ADP-forming, beta subunit	NM_011506	−6.85	0.0000	2.24	0.000012
	*Pck*1	Phosphoenolpyruvate carboxykinase 1, cytosolic	NM_011044	−6.77	0.000039	nde	

Continued

TCA CYCLE	*Dld*	Dihydrolipoamide dehydrogenase	NM_007861	−6.36	0.000001	nde	
	Sdha	Succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	NM_023281	−5.73	0.0000	2.85	0.000006
	*Suclg*1	Succinate-CoA ligase, GDP-forming, alpha subunit	NM_019879	−5.22	0.000342	nde	
	Sdhb	Succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	NM_023374	−4.94	0.000088	4.24	0.00075
	*Mdh*1	Malate dehydrogenase 1, NAD (soluble)	NM_008618	−4.81	0.000001	nde	
	Ogdh	Oxoglutarate dehydrogenase (lipoamide)	NM_010956	−4.26	0.0000	nde	
	Idh3g	Isocitrate dehydrogenase 3 (NAD+), gamma	NM_008323	−3.70	0.0000	nde	
	Sdhc	Succinate dehydrogenase complex, subunit C, integral membrane protein	NM_025321	−3.46	0.000001	nde	
	*Aco*1	Aconitase 1	NM_007386	−3.32	0.000006	2.80	0.000044
	Idh3a	Isocitrate dehydrogenase 3 (NAD+) alpha	NM_029573	−3.00	0.000091	3.21	0.00018
	Pdhb	Pyruvate dehydrogenase (lipoamide) beta	NM_024221	−2.30	0.000115	2.30	0.000196
	Acly	ATP citrate lyase	NM_134037	nde		2.68	0.000029
	*Aco*2	Aconitase 2, mitochondrial	NM_080633	nde		2.28	0.006074
	*Fh*1	Fumarate hydratase 1	NM_010209	nde		2.55	0.001199
	*Idh*1	Isocitrate dehydrogenase 1 (NADP+)	NM_010497	nde		2.31	0.000025
	*Pck*2	Phosphoenolpyruvate carboxykinase2	NM_028994	nde		2.17	0.036288
	*Pdha*1	Pyruvate dehydrogenase E1, alpha	NM_008810	nde		2.26	0.000247
	*Suclg*2	Succinate-Coenzyme A ligase, beta	NM_011507	nde		2.53	0.000292

TCA Cycle—Twenty out of the twenty-nine (67%) TCA cycle-related genes were differentially expressed in the 7dTM group, using the CG group as a calibrator. Nineteen of these genes were downregulated, and only one was upregulated. The downregulated genes included *Aco*1; *Cs*; *Dlat*; *Dld*; *Dlst*; *Idh*3*a*; *Idhg*; *Mdh*s 1, 1*b* and 2; *Suclg*1; *Ogdh*; *Pcks* 1 and 2; *Pdhb*; *Sdh*s *a*, *b* and *c*; *Sucla*2; and *Suclg*1. As mentioned earlier, *Pcx* was the only upregulated gene. Among the nine non-differentially expressed genes, *Acly*; *Aco*2; *Idh*s 1, 2 and 3*b*; *Pdha*1; *Sdhd*; and *Suclg*2 were detected in both of the groups (7dTM and CG), while *Fh1* could only be detected in the CG. The fold-regulation values of each assessed TCA cycle-related gene can be observed in **Table 1**.

3.2. OG versus CG

Glycolysis—Sixteen out of the twenty-two (72%) glycolysis-related genes were differentially expressed in the OG group, using the CG group as a calibrator. All of these sixteen genes were upregulated, and none were downregulated. The upregulated genes included aldolases b and c; *Bpgm*; Enolases 1, 2 and 3; *Gck*; *Gpi*1; hexokinases 2 and 3; *Pfkl*; *Pgam*2; *Pgk*1; *Pgms*2 and *3*; and *Tpi*1. Among the non-differentially expressed genes, *Aldoa*, *Galm*, *Gapdhs*, *Pgm*1 and *Pklr* were detected in both of the groups (OG and CG), while *Pgk2* could not be detected in any of these groups. The fold-regulation values of each assessed glycolysis-related gene can be observed in **Table 1**.

TCA Cycle—Eighteen out of the twenty-nine (62%) TCA cycle-related genes were differentially expressed in the OG group, using the CG group as a calibrator. All of these eighteen genes were upregulated, and none were downregulated. The upregulated genes included *Acly*; *Aco*1; *Aco*2; *Cs*; *Dlst*; *Fh*1; *Idh*1; *Idh*3*a*; *Idh*3*b*; *Mdh*s 1*b* and 2; *Pck*2; *Pdha*1; *Pdhb*; *Sdh*s *a* and *b*; *Sucla*2; and *Suclg2*. All of the eleven non-differentially expressed genes (*Dlat*; *Dld*; *Idh*s 2 and 3g; *Mdh*1; *Ogdh*; *Pck*1; *Pcx*; *Sdhc*; *Sdhd*; and *Suclg*1) were detected in both of the

groups (OG and CG). The fold-regulation values of each assessed TCA cycle-related gene can be observed in **Table 1**.

3.3. Histomorphology of the Uterus

With respect to the histology of the uteri, after a 7-day treatment period, the endometrium was similar to that of the OG group, with an absence of glandular tissue (**Figure 1**). However, Pereira *et al.*, 2011 demonstrated that after a 45-day treatment period, these mice presented an endometrium similar to that of the CG group, with the presence of numerous leukocytes in the endometrium, signaling the restoration of hormonal control and the surface epithelial renewal that are typical of the estrous phase (**Figure 1**).

4. Discussion

We believe that investigating the molecular changes underlying the fertility restoration of leptin-treated ob/ob mice is crucial to better understanding the intracellular pathways controlling the interplay between metabolism and the female sexual cycle. With this in mind, we observed a lack of studies on glucose-related gene alterations in the brain of leptin-induced fertile ob/ob mice and decided to assess the expression of glycolysis- and TCA cycle-related genes in these animals.

It is largely known that leptin plays a primary role in regulating homeostasis, obesity and fertility [23] [28]. Moreover, in a previous work, Pereira *et al.* (2009) clearly demonstrated that WAT transplantation decreases insulin resistance and restores fertility in female ob/ob mice [9]. Behind these physiological and morphological changes lay complex molecular interactions that deserve to be better understood.

As such, the aim of this work was to investigate the central effects of leptin on glucose metabolism accompanying neuropeptide Y inhibition and the restoration of fertility in the same animal model that was used by Pereira *et al.* [9]. Additionally, this work seems to be a pioneer in presenting an overall profile of glycollysis- and TCA cycle-related gene expression in the brains of leptin-treated ob/ob mice.

Indicating the weak points, the results shown herein are restricted to mRNA quantifications and whole brain extracts, instead of specific brain nuclei, and were used to assess early central actions of leptin. However, as mentioned earlier, there is a lack of studies of these genes in the brains of leptin-treated ob/ob mice. In addition, we believe that differential gene expression will reflect specific adipokine hormone actions in leptin receptor-expressing cells, such as kiss1 neurons.

Leptin deficiency, which was observed in the ob/ob mice, caused obesity and led these animals to develop metabolic disorders such as insulin resistance and type II diabetes, as well as hypertension and infertility [27]-[29]. We do not know exactly in which cells in the brains of these 7-day leptin-treated mice have altered metabolic processes, but our results demonstrate an important early downregulation of glycolysis- and TCA cy-

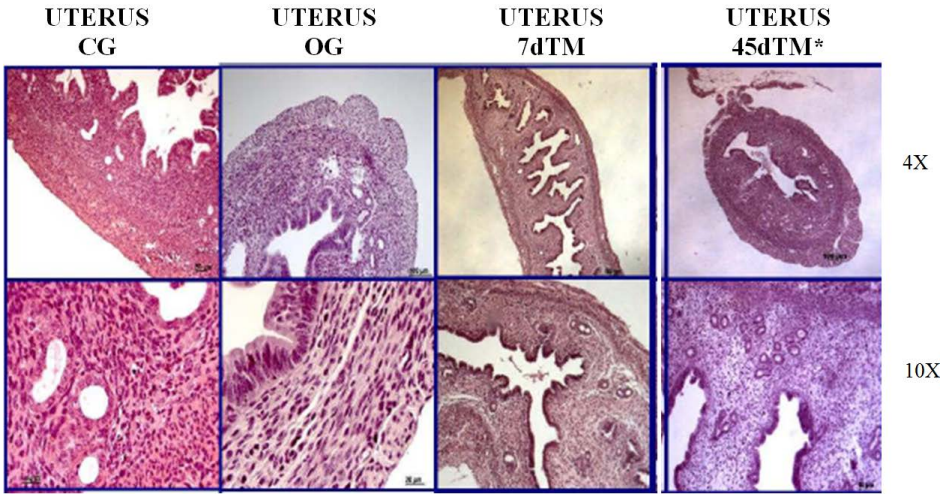

Figure 1. Histomorphological features of the uterus of animals from each experimental group at two different magnifications (4× and 10×). The 45dTM mice were obtained by Pereira *et al.* (2011) [9].

cle-related genesin the brains of ob/ob mice caused by this adipokine.

Khan *et al.*, 1998 have demonstrated through biochemical assays (glucose-6-phosphatase activity) that glucose cycling is increased in the brains of ob/ob mice [30]. Our gene expression results show the same, that is, except for *Pcx*, all of the other glycolysis-related genes assessed herein are upregulated in these mice, in contrast to the 7dTM and OG mice. Therefore, after 1 week of leptin treatment, there seems to be a marked reduction in the activity of these genes. We believe that among the early central neuronal circuitary changes caused by leptin and that will eventually lead to fertility restoration after a 45-day treatment, glucose cycling reduction, most likely in the hypothalamus, seems to be critical.

In general, similar to the profile that was observed for the glycolysis-related genes, the TCA cycle-related genes displayed a general downregulation in the 7dTM group compared to those of the CG group. However, these genes are upregulated in the brains of the OG mice compared to those in the CG mice. This profile matches previous reports, in which untreated ob/ob mice exhibited increased hepatic and central B-oxidation [31] [32], especially in the hypothalamic Arcuate and Ventromedial nuclei [33].

5. Conclusion

Overall, the data presented here indicate an early decrease in the central glucose metabolism after leptin treatment. These results confirm the ability of the adipose tissue-derived hormone leptin to regulate crucial genes that are related to glycolysis mechanisms and to the TCA cycle. In summary, this hormone seems to revert early the central physiological conditions that are associated with PCOS in the central nervous system; however, the morphological alterations that are associated with fertility in the peripheral tissues can only be observed within a 45-day treatment. The extrapolation of these results to patients with metabolic syndrome must await further investigation.

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