PATRICIA CHIMIN

CARACTERÍSTICAS METABÓLICAS DIFERENCIAIS DE DISTINTOS TERRITÓRIOS ADIPOSOS EM RATOS SUBMETIDOS A TRATAMENTO PROLONGADO, CONTÍNUO E EXCESSIVO COM GLICOCORTICOIDES: UM MODELO DE SÍNDROME DE CUSHING IATROGÊNICA

Tese apresentada ao Programa de Pós-Graduação em Fisiologia Humana do Instituto de Ciências Biomédicas da Universidade de São Paulo, para obtenção do Título de Doutor em Ciências.

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RESUMO

Chimin P. Carcterísticas metabólicas diferenciais de distintos territórios adiposos em ratos submetidos a tratamento prolongado, contínuo e excessivo com glicocorticoides: um modelo de síndrome de Cushing iatrogênica. [tese (Doutorado em Fisiologia Humana)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2013.

O objetivo desse trabalho foi caracterizar um quadro experimental que apresenta excesso de glicocorticoides e verificar o efeito desse excesso de glicocorticoides sobre o metabolismo dos carboidratos no tecido adiposo branco. Vinte ratos Wistar foram usados, e após a aclimatação ao biotério, foram divididos aleatoriamente em dois grupos: controle (CON – receberam salina 0,9%) e dexametasona (DEX – receberam 0,25 mg/kg/d de dexametasona). Ao completarem 10 semanas, microbomba osmótica foi inserida entre as escápulas dos animais, e o tratamento foi liberado ao longo de 4 semanas. Ao final do período experimental, os animais foram sacrificados, e sangue do tronco, glândulas adrenais, músculo sóleo, além das gorduras subcutânea (SC), periepididimal (PE), retroperitoneal (RP) e mesentérica (MS) foram retirados e armazenados para análise. Adipócitos foram isolados e submetidos a ensaios biológicos (oxidação de D-[U-^{14}C]-glicose até $^{14}CO_2$, incorporação de D-[U-^{14}C]-glicose em triacilgliceróis, em ácidos graxos de triacilgliceróis e em glicerol de triacilgliceróis). Os resultados mostraram que o tratamento foi capaz de bloquear o eixo hipotálamo-hipófise-adrenal, como verificado pela menor concentração de corticosterona plasmática (CON = 337,4 ± 15,2 ng/ml; DEX = 140,5 ± 10,1 ng/ml, $P < 0,05$) e atrofia das glândulas adrenais, tanto direita (CON = 0,0264 ± 0,0015 g; DEX = 0,0213 ± 0,0011 g, $P < 0,05$), quanto esquerda (CON = 0,0284 ± 0,0011 g; DEX = 0,0229 ± 0,0010 g, $P < 0,05$) no grupo DEX. Além disso, os animais tratados com dexametasona apresentaram resistência à insulina, intolerância à glicose, dislipidemia e aumento nas concentrações de leptina e adiponectina. Esses animais também apresentaram adipócitos maiores (SC - CON = 42,9 ± 2,1 µm; DEX = 45,5 ± 2,0 µm; RP - CON = 49,4 ± 2,3 µm; DEX = 54,4 ± 3,9 µm; e MS - CON = 36,2 ± 3,3 µm; CS = 40,8 ± 2,6 µm; $P < 0,05$) que, juntamente com a análise de celularidade, demonstraram estar hipertrofiados. Em relação ao acúmulo de gordura, o grupo DEX apresentou maior lipogênese nos coxins RP e MS, sendo que houve maior incorporação de glicose na porção ácido graxo dos triacilgliceróis. Corroborando essa maior síntese *de novo* de ácidos graxos, a atividade máxima das enzimas envolvidas nessa via, como ácido graxo sintase; e das enzimas responsáveis pela formação de cofatores reduzidos, glicose-6-fosfato desidrogenase e enzima málica, também foram maiores no grupo DEX, principalmente no coxim MS. Essa maior resposta também foi refletida na expressão gênica dessas enzimas. Esses resultados mostram que diversos aspectos da indução iatrogênica de síndrome de Cushing em ratos foram obtidos, e que uma possível explicação para esse maior acúmulo na região visceral pode ser a maior atividade e expressão de enzimas envolvidas na formação de NADPH, ao invés da atividade de enzimas envolvidas mais diretamente na via de síntese propriamente dita.

Palavras-chave: Lipogenese. Glicocorticoides. Tecido adiposo.

ABSTRACT

Chimin P. Differential metabolic characteristics of distinct fat territories in rats submitted to a prolonged, continuous and excessive glucocorticoid treatment: a model for iatrogenic Cushing's syndrome. [Ph. D. thesis (Human Physiology)]. São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2013.

The aim of the present study was to characterize an experimental condition of glucocorticoid excess and to verify the effect of this glucocorticoid excess in carbohydrate metabolism in white adipose tissue. Twenty male Wistar rats were used, and after acclimatization to vivarium conditions, they were divided at random into two groups: control (CON – received saline 0.9%) and dexamethasone (DEX – received 0.25 mg/kg/d dexamethasone). After complete 10 weeks old, an osmotic micro pump was inserted in the subcutaneous dorsal interescapular region, and the treatment was derived for 4 weeks. At the end of experimental period, animals were sacrificed, and trunk blood, adrenal glands, soleus muscle, and also subcutaneous (SC), periepididymal (PE), retroperitoneal (RP) and mesenteric (MS) fat pads were excised and stored for further analysis. Also, adipocytes were isolated and submitted to *in vitro* tests (oxidation of D-[U-^{14}C]-glucose into $^{14}CO_2$, incorporation of D-[U-^{14}C]-glucose into triacylglycerol, incorporation of D-[U-^{14}C]-glucose into fatty acids of triacylglycerol and incorporation of D-[U-^{14}C]-glucose into glycerol of triacylglycerol). The results showed that the treatment was able to block hypothalamic-pituitary-adrenal axis, as verified by lower corticosterone plasma levels (CON = 337.4 ± 15.2 ng/ml; DEX = 140.5 ± 10.1 ng/ml, $P < 0.05$) and adrenal gland atrophy, in both right (CON = 0.0264 ± 0.0015 g; DEX = 0.0213 ± 0.0011 g, $P < 0,05$), and left glands (CON = 0.0284 ± 0.0011 g; DEX = 0.0229 ± 0.0010 g, $P < 0.05$) in DEX group. Furthermore, animals treated with dexamethasone presented insulin resistance, glucose intolerance, dyslipidemia and increased leptin and adiponectin levels. These animals also presented increased adipocytes diameters (SC - CON = 42.9 ± 2.1 µm; DEX = 45.5 ± 2.0 µm; RP - CON = 49.4 ± 2.3 µm; DEX = 54.4 ± 3.9 µm; and MS - CON = 36.2 ± 3.3 µm; CS = 40.8 ± 2.6 µm; $P < 0.05$), that together with cellularity analysis, demonstrated that these adipocytes were hypertrophied. In relation to lipid accumulation, DEX group presented increased lipogenesis in RP and MS fat pads, with increased incorporation into fatty acids of triacylglycerol. According to this increased fatty acids *de novo* synthesis, the maximal activity of enzymes involved in this pathway, like fatty acid synthase; and of enzymes that supply NAPDH for lipogenesis, glucose-6-phosphate dehydrogenase and malic enzyme, were also increased in DEX group, especially in MS fat pad. This increased response was also reflected in the gene expression of these enzymes. These data show that certain aspects of the induction of iatrogenic Cushing's syndrome were obtained, and that a possible explanation for this increased lipid accumulation in visceral depots can be due to increased activity and gene expression of enzymes that supply NADPH, required for lipogenesis, instead of the increase in enzymes that participate in lipogenesis itself.

Keywords: Lipogenesis. Glucocortioids. Adipose tissue.

1 INTRODUÇÃO

Nas últimas décadas, o tecido adiposo passou de um simples tecido “coadjuvante” para um órgão endócrino, com papel importante em várias funções do metabolismo. Sua função no controle da homeostase corporal é creditada, entre outras coisas, pela ampla capacidade de aumentar seu volume, dessa forma, aumentando a quantidade de triacilgliceróis (TAG) que podem ser armazenados sem que haja prejuízos para o tecido.

Por outro lado, essa mesma característica que o torna ímpar entre os órgãos corporais, também causa efeitos sistêmicos deletérios, dependendo do local onde esse acúmulo ocorre. Indivíduos que apresentam acúmulo de gordura na região mais central do corpo, chamado de gordura visceral, apresentam maior risco de desenvolverem complicações cardiovasculares, além de outras doenças associadas; enquanto que o acúmulo de gordura na região mais periférica, chamada de subcutânea, apresentam um risco menor de desenvolver essas doenças.

Além da genética, fatores hormonais também podem levar a um maior acúmulo de gordura corporal. Dentre os vários hormônios que atuam sobre o tecido adiposo, podemos citar os glicocorticoides. Os glicocorticoides são hormônios esteroides secretados pelo córtex da adrenal, e em condições de estresse, promovem, entre outros efeitos, “quebra” dos TAG liberando ácidos graxos (AG) e glicerol na circulação.

Apesar desse papel lipolítico já estabelecido, quando esse hormônio está presente em excesso, como no caso da síndrome de Cushing, ele promove acúmulo de gordura, principalmente na região central do corpo (gorduras viscerais), enquanto diminui a quantidade de gordura nas extremidades (gordura subcutânea). Além do acúmulo de gordura, na síndrome de Cushing os glicocorticoides promovem efeitos deletérios agindo sobre outros órgãos e tecidos corporais, como ossos, tecido conjuntivo, músculo, fígado, entre outros.

Ainda não se sabe ao certo como e por que os glicocorticoides causam esse efeito tão particular sobre o tecido adiposo. O que já está estabelecido é que os glicocorticoides, de alguma forma, alteram a atividade de enzimas que atuam na via lipogênica, mas até agora não há resultados conclusivos demonstrando por que e

como esse acúmulo diferenciado de gordura ocorre quando esse hormônio está presente em excesso.

8 CONCLUSÕES

A partir da análise dos dados podemos concluir que a administração de 0,25 mg/kg/d de dexametasona durante 4 semanas para ratos Wistar:

- foi capaz de reduzir a resposta normal do eixo hipotálamo-hipófise-adrenal, como verificado pela atrofia das glândulas adrenais e valores de corticosterona plasmática;

- promove características relacionadas com o quadro de excesso de glicocorticoides, como por exemplo, resistência à insulina, intolerância à glicose, alterações hormonais e dislipidemia;

- promove certo grau de acúmulo de gordura nos depósitos viscerais (especificamente no coxim MS), além de promover hipertrofia desses adipócitos;

- promove maior incorporação de glicose na porção AG dos TAG nos coxins viscerais;

- promove alteração na atividade de enzimas lipogênicas nos coxins RP e MS [principalmente daquelas envolvidas na geração de cofatores reduzidos (a saber, G6PDH e EM)];

- promove alteração na expressão gênica de enzimas lipogênicas (ACC, FAS, ACL, G6PDH e EM) tanto no coxim SC quanto no MS (porém com maior magnitude de aumento na expressão gênica da enzima G6PDH no coxim MS);

- não altera a expressão gênica da enzima 11βHSD1, que é responsável pela conversão da forma inativa da corticosterona para sua forma ativa;

Contudo, esses resultados mostram que certos aspectos da indução iatrogênica de síndrome de Cushing em ratos foram obtidos, e que uma possível explicação para o maior acúmulo de gordura na região visceral pode ser devido a maior atividade e expressão de enzimas envolvidas na formação de cofatores

reduzidos, ao invés da atividade de enzimas envolvidas na via de síntese propriamente dita.

REFERÊNCIAS*

Aarsland A, Chiinkes D, Wolfe RR. Hepatic and whole body fat synthesis in human during carbohydrate overfeeding. Am J Clin Nutr. 1997;65(6):1774-82.

Agarwal AK. Cortisol metabolism and visceral obesity: role of 11β-hydroxysteroid dehydrogenase type I enzyme and reduced co-factor NADPH. Endocr Res. 2003;29(4):411-8.

Ahima RS, Flier JS. Adipose tissue as an endocrine organ. Trends Endocrinol Metab. 2000;11(8):327-32.

Ahtikoski AM, Riso EM, Koskinen SO, Risteli J, Takala TE. Regulation of type IV collagen gene expression and degradation in fast and slow muscles during dexamethasone treatment and exercise. Pflugers Arch. 2004;448(1):123-30.

Ailhaud G, Grimaldi P, Négrl R. Cellular and molecular aspects of adipose tissue development. Annu Rev Nutr. 1992;12:207-33.

Arner P. Differences in lipolysis between human subcutaneous and omental adipose tissue. Ann Med. 1995;27(4):435-8.

Angelini N, Rafacho A, Boschero AC, Bosqueiro JR. Involvement of the cholinergic pathway in glucocorticoid-induced hyperinsulinemia in rats. Diabetes Res Clin Pract. 2010;87(2):184-91.

Asensio C, Muzzin P, Rohner-Jeanrenaud F. Role of glucocorticoids in the physiopathology of excessive fat deposition and insulin resistance. Int J Obes. 2004;28 Suppl 4: S45-S52.

Assimacopoulos-Jeannet F, Brichard S, Rencurel F, Cusin I, Jeanrenaud B. In vivo effects of hyperinsulinemia on lipogenic enzymes and glucose transporter expression in rat liver and adipose tissue. Metabolism. 1995;44(2):228-33.

Bagdade JD, Yee E, Alberts J, Pykalisto OJ. Glucocorticoids and triglyceride transport: effects on triglyceride secretion rats, lipoprotein lipase, and plasma lipoproteins in the rat. Metabolism. 1976;25(5):533-42.

Balachandran A, Guan H, Sellan M, van Uum S, Yang K. Insulin and dexamethasone dynamically regulate adipocyte 11β-hydroxysteroid dehydrogenase type 1. Endocrinology. 2008;149(8):4069-79.

Ballard FJ, Hanson RW. The citrate cleavage pathway and lipogenesis in rat adipose tissue: repleshiment of oxaloacetate. J Lipid Res. 1967;8(2):73-9.

*De acordo com:
International Committee of Medical Journal Editors. Uniform requeriments for manuscripts submitted to Biomedical Journal:sample references. Available from: http://www.icmje.org [2011 Jul 15].

Barel M, Perez OAB, Giozzet VA, Rafachi R, Bosqueiro JR, Amaral SL. Exercise training prevents hyperinsulinemia, muscular glycogen loss and muscle atrophy induced by dexamethasone treatment. Eur J Appl Physiol. 2010;108(5):999-1007.

Bazin R, Ferré P. Assays of lipogenic enzymes. Methods Mol Biol. 2001;155:121-7.
Bergmeyer HU, Bernt E, Schmidt F, Stork H. Methods of enzymatic analysis. Orlando, FL: Academic Press; 1974.

Bergmeyer HU, Bernt E, Schmidt F, Stork H. Methods of enzymatic analysis. Orlando, FL: Academic Press; 1974.

Bjorntorp P. Metabolic implications of body fat distribution. Diabetes Care. 1991;14(12):1132-43.

Bouillon D, Berdanier CD. Role of glucocorticoid in adaptive hyperlipogenesis in the rat. J Nutr. 1980;110(2):286-97.

Bradley RL, Cheatham B. Regulation of ob gene expression and leptin secretion by insulin and dexamethasone in rat adipocytes. Diabetes. 1999;48(2):272-8.

Bujalska IJ, Kumar S, Hewison M, Stewart PM. A switch in the dehydrogenase to reductase activity of 11β – hidroxyesteroid dehydrogenase type 1 upon differential of human omental adipose stromal cells. J Clin Endocrinol Metab. 2002;87(3):1205-10.

Bujalska IJ, Quinkler M, Tomlinson JW, Montague CT, Smith DM, Stewart PM. Expression profiling of 11β-hydroxysteroid dehydrogenase type-1 and glucocorticoid-target genes in subcutaneous and omental human preadipocytes. J Mol Endocrinol. 2006;37(2):327-40.

Cai Y, Song Z, Wang X, Jiao H, Lin H. Dexamethasone-induced hepatic lipogenesis is insulin dependent in chickens (*Gallus gallus domesticus*). Stress. 2011;14(3):273-81.

Campbell JE, Peckett AJ, D'souza AM, Hawke TJ, Riddell MC. Adipogenic and lipolytic effects of chronic glucocorticoid exposure. Am J Physiol Cell Physiol. 2011;300(1):C198-C209.

Chanson P, Salenave S. Metabolic syndrome in Cushing's syndrome. Neuroendocrinology. 2010;92 Suppl 1: S96-S101.

Christ-Crain M, Kola B, Lolli F, Fekete C, Seboek D, Wittman G, Feltrin D, Igreja SC, Ajodha S, Harvey-White J, Kunos G, Müler B, Pralong F, Aubert G, Arnaldi G, Giacchetti G, Boscaro M, Grossman AB, Korbonits M. AMP-activated protein kinase mediates glucocorticoid-induced metabolic changes: a novel mechanism in Cushing's syndrome. FASEB J. 2008;22(6):1672-83.

Cole MA, Kim PJ, Kalman BA, Spencer RL. Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. Psychoneuroendocrinology. 2000;25(2):151-67.

Coleman RA, Lee DP. Enzymes of triacylglycerol synthesis and their regulation. Prog Lipid Res. 2004;43(2):134-76.

Cushing's Support & Research Foundation [Internet]. Cushing's fact sheet. [cited 2012 Nov 26]. Available from: http://csrf.net/page/fact_sheet.php.

Del Prato S, Tiengo A. The importance of first-phase insulin secretion: implications for the therapy of type 2 diabetes mellitus. Diabetes Metab Res Rev. 2001;17(3):164-74.

Deveaud C, Beauvoit B, Salin B, Schaeffer J, Rigoulet M. Regional differences in oxidative capacity of rat white adipose tissue are linked to the mitochondrial content of mature adipocytes. Mol Cell Biochem. 2004;267(1-2):157-66.

Diamant S, Shafrir E. Modulation of the activity of insulin-dependent enzymes of lipogenesis by glucocorticoids. Eur J Biochem. 1975;53(2):541-6.

Dimitriadis G, Leighton B, Parry-Billings M, Sasson S, Young M, Krause U, Bevan S, Piva T, Wegener G, Newsholme EA. Effects of glucocorticoids excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle. Biochem J. 1997;321(Pt 3):707-12.

Diraison F, Dusserre E, Vidal H, Sothier M, Beylot M. Increased hepatic lipogenesis but decreased expression of lipogenic genes in adipose tissue in human obesity. Am J Physiol. 2002;282(1):E46-E51.

Djurhuus CB, Gravholt CH, Nielsen S, Mengel A, Christiansen JS, Schmitz OE, Møller N. Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. Am J Physiol Endocrinol Metab. 2002;283(1):E172-E7.

Dole VP, Meirnetz H. Microdetermination of long chain fatty acids in plasma and tissues. J Biol Chem. 1960;235:2595-9.

Drake AJ, Raubenheimer PJ, Kerrigan D, McInnes KJ, Seckl JR, Walker BR. Prenatal dexamethasone programs expression of genes in liver and adipose tissue and increased hepatic lipid accumulation but not obesity on a high-fat diet. Endocrinology. 2010;151(4):1581-7.

Drolet R, Richard C, Sniderman AD, Mailloux J, Fortier M, Huot C, Rhéaume C, Tchernof A. Hypertrophy and hyperplasia of abdominal adipose tissue in women. Int J Obes (Lond). 2008;32(2):283-91.

Einstein FH, Atzmon G, Yang XM, Ma XH, Rincon M, Rudin E, Muzumdar R, Barzilai N. Differential responses of visceral and subcutaneous fat depots to nutrients. Diabetes. 2005;54(3):672-8.

Elshourbagy NA, Boguski MS, Liao WS, Jefferson LS, Gordon JI, Taylor JM. Expression of rat apolipoprotein A-IV and A-I genes: mRNA induction during development and in response to glucocorticoids and insulin. Proc Natl Acad Sci U S A. 1895;82(23):8242-6.

Espínola-Antunes D, Kater CE. Adipose tissue expression of 11β – hydroxysteroid dehydrogenase type 1 in Cushing's Syndrome and in obesity. Arq Bras Endocrinol Metab. 2007;51(8):1397-1403.

Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol. 2005;115(5):911-9.

Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. Biochem Biophys Res Comun. 2002;290(3):1084-9.

Fernandez-Rodriguez E, Srewart PM. The pituitary-adrenal axis and body composition. Pituitary. 2009;12(2):105-15.

Fine JB, Di Girolamo M. A simple method to predict cellular density in adipocyte metabolic incubations. Int J Obes Relat Metab Disord. 1997;21(9):764-8.

Fonseca-Alaniz MH, Takada J, Alonso-Vale MIC, Lima FB. Adipose tissue as an endocrine organ: from theory to practice. J Pediatr. 2007;83 Suppl 5: S192-S203.

Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

Galton DJ, Wilson JPD. Lipogenesis in adipose tissue of patients with obesity and Cushing's disease. Clin Sci. 1972;43(6):17.

Gao Z, Zhang X, Zuberi A, Hwang D, Quon MJ, Lefevre M, Ye J. Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3-L1 adipocytes. Mol Endocrinol. 2004;18(8):2024-34.

Gathercole LL, Morgan SA, Bujalska IJ, Hauton D, Stewart PM, Tomlinson JW. Regulation of lipogenesis by glucocorticoids and insulin in human adipose tissue. PLoS ONE. 2011;6(11):e26223. doi: 10.1371/journal.pone.0026223. Epub 2011 Oct 14.

Girard J, Perdereau D, Foufelle F, Prip-Buus C, Ferré P. Regulation of lipogenic enzyme gene expression by nutrient and hormones. FASEB J. 1994;8(1):36-42.

Gounarides JS, Korach-André M, Killary K, Argentieri G, Turner O, Laurent D. Effect of dexamethasone on glucose tolerance and fat metabolism in a diet-induced obesity mouse model. Endocrinology. 2008;149(2):758-66.

Grasa MM, Cabot C, Balada F, Virgili J, Sanchis D, Monserrat C, Fernández-López JA, Remesar X, Alemany M. Corticosterone binding to tissues of adrenalectomized lean and obese Zucker rats. Horm Metab Res. 1998;30(12):699-704.

Haugen F, Drevon CA. The interplay between nutrients and the adipose tissue. Proc Nutr Soc. 2007;66(2):171-82.

Hellerstein MK. De novo lipogenesis in humans: metabolic and regulatory aspects. Eur J Clin Nutr. 1999;53 Suppl 1: S53-S65.

Heitzer MD, Wolf IM, Sanchez ER, Witchel SF, DeFranco DB. Glucocorticoid receptor physiology. Rev Endocr Metab Disord. 2007;8(4):321-30.

Hillgartner FB, Salati LM, Goodridge AG. Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. Physiol Rev. 1995;75(1):47-76.

Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. Obes Rev. 2010;11(1):11-8.

Jahng JW, Kim NY, Ryu V, Yoo SB, Kim BT, Kang DW, Lee JH. Dexamethasone reduces food intake, weight gain and the hypothalamic 5-HT concentration and increases leptin in rats. Eur J Pharmacol. 2008;581(1-2):64-70.

Karastergious K, Smith SR, Greenberg AS, Fried SK. Sex differences in human adipose tissue – the biology of pear shape. Biol Sex Differ. 2012;3(1):3-13.

Kola B, Christ-Crain M, Lolli F, Arnaldi G, Giacchetti MB, Grossman AB, Korbonits M. Changes in adenosine 5'-monophosphate-activated protein kinase as a mechanism of visceral obesity in Cushing's syndrome. J Clin Endocrinol Metab. 2008;93(12):4969-73.

Korach-André M, Gao J, Gounarides JS, Deacon R, Islam A, Laurent D. Relationship between visceral adiposity and intramyocellular lipid content in two rat models of insulin resistance. Am J Physiol Endocrinol Metab. 2005;288(1):E106-E16.

Kreier F, Fliers E, Voshol PJ, Eden CG, Havekes LM, Kalsbeek A, Van Heijningen CL, Sluiter AA, Mettenleiter TC, Romijn JA, Sauerwein HP, Bujis RM. Selective parasympathetic innervation of subcutaneous and intra-abdominal fat – functional implications. J Clin Invest. 2002;110(9):1243-50.

Lafontan M, Berlan M. Do regional differences in adipocyte biology provide new pathophysiological insights? Trends Pharmacol Sci. 2003;24(6): 276-83.

Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S. Adipokines: molecular links between obesity and atherosclerosis. Am J Physiol Heart Circ Physiol. 2005;288(5):H2031-H41.

Lee MJ, Gong DW, Burkey BF, Fried SK. Pathway regulated by glucocorticoids in omental and subcutaneous human adipose tissue: a microarray study. Am J Physiol Endocrinol Metab. 2011;300(3):E571-E80.

Lee MJ, Wu Y, Fried SK. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Mol Aspects Med. 2012; Oct 13. pii: S0098-2997(12)00115-X. doi: 10.1016/j.mam.2012.10.001. [Epub ahead of print].

Letexier D, Pinteur C, Large V, Fréring V, Beylot M. Comparison of the expression and activity of the lipogenic pathway in human and rat adipose tissue. J Lipid Res. 2003;44(11):2127-34.

Life Technologies [Internet]. Gene Expression Analysis using TaqMan® Assays. [cited 2012 Dez 21]. Available from: https://pt-br.invitrogen.com/site/br/pt/home/Products-and-Services/Applications/PCR/real-time-pcr/real-time-pcr-assays/taqman-gene-expression.html

Lima FB, Matsushita DH, Hell NS, Dolnikoff MS, Okamoto MM, Cippola-Neto J. The regulation of insulin action in isolated adipocytes. Role of periodicity of food intake, time of the day and melatonin. Braz J Med Res. 1994;27(3):995-1000.

Lott JA, Turner K. Evaluation of Trinde's glucose oxidase method for measuring glucose in serum and urine. Clin Chem. 1975;21(12):1754-60.

Macfarlane DP, Forbes S, Walker BR. Glucocorticoids and fatty acid metabolism in humans: fueling fat redistribution in the metabolic syndrome. J Endocrinol. 2009;197(2):189-204.

Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS. A transgenic model of visceral obesity and the metabolic syndrome. Science. 2001;294(5549):2166-70.

Mitsugi N, Kimura F. Simultaneous determination of blood levels of corticosterona and growth hormone in the male rat: relation to sleep-wake-fulness cycle. Neuroendocrinology. 1985;41(2):125-30.

Murakami T, Iida M, Shima K. Dexamethasone regulates obese expression in isolated rat adipocytes. Biochem Biophys Res Commun. 1995;214(3):1260-67.

National Adrenal Diseases Foundation [Internet]. Adrenal Diseases – Cushing's Syndrome. The facts you need to know. [cited 2012 Nov 26]. Available from: http://www.nadf.us/diseases/cushings.htm.

Novelli M, Pocai A, Chiellini C, Maffei M, Masiello P. Free fatty acids as mediators of adaptive compensatory responses to insulin resistance in dexamethasone-treated rats. Diabetes Metab Res Rev. 2008;24(2):155-64.

Nye CK, Hanson RW, Kalhan SC. Glyceroneogenesis is the dominant pathway for triglyceride glycerol synthesis in vivo in the rat. J Biol Chem. 2008;283(41):27565-74.

Ogias D. O jejum regula diferencialmente a *cortisone biding globulin* plasmática, o receptor de glicocorticoide e proteínas que controlam a progressão do ciclo celular na mucosa gástrica de filhotes e ratos adultos [Tese]. São Paulo: Instituto de Ciências Biomédicas da Universidade de São Paulo; 2009.

Ortega FJ, Mayas D, Moreno-Navarrete JM, Catalán V, Gómez-Ambrosi J, Esteve E, Rodriguez-Hermosa JI, Ruiz B, Ricart W, Peral B, Fruhbeck G, Tinahones FJ, Fernández-Real JM. The gene expression of the main lipogenic enzymes is downregulated in visceral adipose tissue of obese subjects. Obesity (Silver Spring). 2009;18(1):13-20.

Palou M, Priego T, Sánchez J, Rodríguez AM, Palou A, Picó C. Gene expression patterns in visceral and subcutaneous adipose depots in rats are linked to their morphologic features. Cell Physiol Biochem. 2009;24(5-6):547-56.

Palou M, Sánchez J, Priego T, Rodríguez AM, Picó C, Palou A. Regional differences in the expression of genes involved in lipid metabolism in adipose tissue in response to short- and medium-term fasting and reefeding. J Nutr Biochem. 2010;21(1):23-33.

Paulsen SK, Pedersen SB, Fisker S, Richelsen B. 11β-HSD type 1 expression in human adipose tissue: impact of gender, obesity, and fat localization. Obesity (Silver Spring). 2007;15(8):1954-60.

Pénicaud L, Cousin B, Leloup C, Lorsignol A, Casteilla L. The autonomic nervous system, adipose tissue plasticity, and energy balance. Nutrition. 2000;16(10):903-8.

Postic C, Girard J. The role of the lipogenic pathway in the development of hepatic steatosis. Diabetes Metab. 2008;34(6 Pt 2):643-8.

Rafacho A, Giozzet VAG, Boschero AC, Bosqueiro JR. Functional alterations in endocrine pancreas of rats with different degrees of dexamethasone-induced insulin resistance. Pancreas. 2008;36(3):284-93.

Rafacho A, Cestari TM, Taboga SR, Boschero AC, Bosqueiro JR. High doses of dexamethasone insuce increased β-cell proliferation in pancreatic rat islets. Am J Physiol Endocrinol Metab. 2009;296(4):E681-E9.

Rebuffé-Scrive M, Lundholm K, Bjiimtorp P. Glucocorticoid hormone binding to human adipose tissue. Eur J Clin Invest. 1985;15(5):267-71.

Rebuffé-Scrive M, Krotkiewski M, Elferson J, Bjorntorp P. Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. J Clin Endocrinol Metab. 1988;67(6):1122-8.

Rockall AG, Sohaib SA, Evans D, Kaltsas G, Isidori AM, Monson JP, Besser GM, Grossman AB, Reznek RH. Hepatic steatosis in Cushing's syndrome: a radiological assessment using computed tomography. Eur J Endocrinol. 2003;149(6):543-8.

Rodbell M. Metabolism of isolated fat cells I. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem. 1964;239:375-80.

Rose AJ, Vegiopoulos A, Herzig S. Role of glucocorticoids and the glucocorticoid receptor in metabolism: Insights from genetic manipulations. J Steroid Biochem Mol Biol. 2010;122(1-3):10-20.

Schoneveld OJLM, Gaemers IC, Lamers WH. Mechanisms of glucocorticoid signaling. Biochim Biophys Acta. 2004;1680(2):114-28.

Seckl JR, Walker BR. Minireview: 11β-hydroxysteroid dehydrogenase type 1 – a tissue-specific amplifier of glucocorticoid action. Endocrinology. 2001;142(4):1371-6.

Severino C, Brizzi P, Solinas A, Secchi G, Maioli M, Tonolo G. Low-dose dexamethasone in the rat: a model to study insulin resistance. Am J Physiol Endocrinol Metab. 2002;283(2):E367-E73.

Shi JH, Du WH, Liu XY, Fan YP, Hu XL, Zhou HY, Xu HB, Zhang XM, Xiang P, Chen FL. Glucocorticoids decrease serum adiponectin level and WAT adiponectin mRNA expression in rats. Steroids. 2010;75(12):853-8.

Shinahara M, Nishiyama M, Iwasaki Y, Nakayama S, Noguchi T, Kambayashi M, Okada Y, Tsuda M, Stenzel-Poore MP, Hashimoto K, Terada Y. Plasma adiponectin levels are increased despite insulin resistance in corticotropin-releasing hormone transgenic mice, and animal model of Cushing Syndrome. Endocr J. 2009;56(7):879-86.

Slavin BG, Ong JM, Kern PA. Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. J Lipid Res. 1994;35(9):1535-41.

Sjögren J, Weck M, Nilsson M, Ottosson M, Björntorp P. Glucocorticoid hormone binding to rat adipocytes. Biochim Biophys Acta.1994;1224(1):17-21.

Smith SR, Lovejoy JC, Greenway F, Ryan D, deJonge L, la Bretonne J, Volafova J, Bray GA. Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolism complications of obesity. Metabolism. 2001;50(4):425-35.

Speaker KJ, Fleshner M. Interleukin-1 beta: a potential link between stress and the development of visceral obesity. BMC Physiol. 2012;12:8. doi: 10.1186/1472-6793-12-8.

Staels B, van Tol A, Chan L, Verhoeven G, Auwerx J. Variables effects of different corticosteroids on plasma lipids, apolipoproteins, and hepatic apolipoprotein mRNA levels in rats. Arterioscler Thromb. 1991;11(3):760-9.

Stahn C, Löwenberg M, Hommes DW, Buttgereit F. Molecular mechanisms of glucocorticoid action and selective glucocorticoid receptor agonists. Mol Cell Endocrinol. 2007;275(1-2):71-8.

Swierczynski J, Goyke E, Wach L, Pankiewicz A, Adamonis W, Sledzinski Z, Aleksandrowicz Z. Comparative study of the lipogenic potential of human and rat adipose tissue. Metabolism. 2000;49(5):594-9.

Swierczynski J, Zabrocka L, Goyke E, Raczynska S, Adamonis W, Sledzinski Z. Enhanced glycerol 3-phosphate dehydrogenase activity in adipose tissue of obese humans. Mol Cell Biochem. 2003;254(1-2):55-9.

Taylor AH, Raymond J, Dionne JM, Romney J, Chan J, Lawless DE, Wanke IE, Wong NCW. Glucocorticoid increases rat apolipoprotein A-I promoter activity, J Lipid Res. 1996;37(10):2232-43.

Trayhurn P, Beattie JH. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. Proc Nutr Soc. 2001;60(3):329-39.

Trayhurn P, Wood IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. Biochem Soc Trans. 2005;33(Pt 5):1078-81.

Van Cauter E, Leproult R, Kupfer DJ. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J Clin Endocrinol Metab. 1996;81(7):2468-73.

Villanueva EC, Myers MG. Leptin receptor signaling and the regulation of mammalian physiology. Int J Obes (Lond). 2008;32 Suppl 7: S8-S12.

Volpe JJ, Marasa JC. Hormonal regulation of fatty acid synthetase, acetyl-CoA carboxylase and fatty acid synthesis in mammalian adipose tissue and liver. Biochim Biophys Acta. 1975;380(3):454-72.

Voshol PJ, Rensen PC, Van Dijk KW, Romijn JA, Havekes LM. Effect of plasma triglyceride metabolism on lipid storage in adipose tissue: studies using genetically engineered mouse models. Biochim Biophys Acta. 2009;1791(6):479-85.

Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev. 2000;21(6):697-738.

Wajchenberg BL, Giannella-Neto D, da Silva MER, Santos RF. Depot-specific hormonal characteristics of subcutaneous and visceral adipose tissue and their relation to the metabolic syndrome. Horm Metab Res. 2002;34(11-12):616-21.

Wang Y, Voy BJ, Urs S, Kim S, Soltani-Bejnood M, Quigley N, Heo YR, Standridge M, Andersen B, Dhar M, Joshi R, Wortman P, Taylor JW, Chun J, Leuze M, Claycombe K, Saxton AM, Moustaid-Moussa N. The human fatty acid synthase gene and de novo lipogenesis are coordinately regulated in human adipose tissue. J Nutr. 2004;134(5):1032-8.

Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE. Enlarged subcutaneous abdominal adipocyte size, but no obesity itself, predicts Type II diabetes independent of insulin resistance. Diabetologia. 2000;43(12):1498-1506.

White PC, Rogoff D, McMillan DR, Lavery GG. Hexose 6-phosphate dehydrogenase (H6PD) and corticosteroid metabolism. Mol Cell Endocrinol. 2007;265-266:89-92.

Willi SM, Kennedy A, Wallace P, Ganaway E, Rogers NL, Garvey WT. Troglitazone antagonizes metabolic effects of glucocorticoids in humans: effects on glucose tolerance, insulin sensitivity, suppression of free fatty acids, and leptin. Diabetes. 2002;51(10):2895-2902.

Wilson JPD, Galton DJ. The effect of obesity and Cushing's disease on the incorporation of glucose into lipid and esterification of palmitate by human adipose tissue. Eur J Clin Invest. 1972;2(6):379-83.

Wise EM, Ball EG. Malic enzyme and lipogenesis. Proc Natl Acad Sci U S A. 1964;52:1255-63.

Wong RHF, Sul HS. Insulin signaling in fatty acid and fat synthesis: a transcriptional perspective. Curr Opin Pharmacol. 2010;10(6):684-91.

Zakrzewska KE, Cusin I, Stricker-Krongrad A, Boss O, Ricquier D, Jeanrenaud B, Rohner-Jeanrenaud F. Induction of obesity and hyperleptinemia by central glucocorticoid infusion in the rat. Diabetes. 1999;48(2):365-70.

Zechner R, Strauss J, Frank S, Wagner E, Hofmann W, Kratky D, et al. The role of lipoprotein lipase in adipose tissue development and metabolism. Int J Obes Relat Metab Disord. 2000;24 Suppl 4: S53-S6.