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Influência de polimorfismos no gene HSD11B1 e de sua expressão no tecido adiposo abdominal em diferentes perfis metabólicos de adultos: revisão sistemática e estudo transversal

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Dissertação para a obtenção do título de Mestre apresentada à Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas: Endocrinologia.

Orientador: Dr. Fernando Gerchman

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Sumário

Formato da Dissertação de Mestrado

Esta dissertação de Mestrado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Faculdade de Medicina da UFRGS, sendo apresentada na forma de 2 artigos científicos em inglês. Um artigo de revisão e um artigo original a serem submetidos para publicação em periódicos Qualis A Internacional na Classificação da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- (CAPES).

Lista de abreviaturas

Capítulo I – Introdução

HSD11B1 – Enzima 11-β Hidroxiesteroide Desidrogenase Tipo 1

HSD11B1 – Gene codificador da 11-β Hidroxiesteroide Desidrogenase Tipo 1

H6PD – Gene codificador da Hexose-6-fosfato Desidrogenase

IMC – Índice de Massa Corporal

Capítulo II – Artigo de revisão sistemática

11-βHSD1 – Hydroxysteroid (11-beta) Dehydrogenase Type 1

IR – Insulin Resistance

MetS – Metabolic Syndrome

T2DM – Type 2 Diabetes Mellitus

BMI – Body Mass Index

NOS – New Castle-Ottawa Scale

SAT – Abdominal Subcutaneous Adipose Tissue

VAT – Abdominal Visceral Adipose Tissue

H6PD - Hexose-6-Phosphate Dehydrogenase

Capítulo III – Artigo original

T2DM – Type 2 Diabetes Mellitus

MetS – Metabolic Syndrome

HSD11B1 – Enzyme Hydroxysteroid (11-beta) Dehydrogenase Type 1

HSD11B1 – Hydroxysteroid (11-beta) Dehydrogenase Type 1 Gene

H6PD – Hexose-6-Phosphate Dehydrogenase

SAT – Abdominal Subcutaneous Adipose Tissue

VAT – Abdominal Visceral Adipose Tissue

BMI – Body Mass Index

WC – Waist Circumference

WHtR – Waist-to-Height Ratio

BP – Blood Pressure

HDL – High-Density Lipoprotein Cholesterol

LDL – Low-Density Lipoprotein Cholesterol

RT-PCR – Real-Time Reverse Transcription PCR

RT-qPCR – Quantitative RT-PCR

SD – Standard Deviation

χ^2 – Chi-Square Test

ANOVA – Analysis of Variance

PCOS – Polycystic Ovary Syndrome

Lista de Tabelas

Lista de Figuras

Capítulo II – Artigo de revisão sistemática

Capítulo II – Artigo de revisão sistemática

Capítulo I - Introdução

Introdução

A enzima 11-β Hidroxiesteroide Desidrogenase Tipo 1 (HSD11B1) tem tanto a capacidade de atuar como desidrogenase (convertendo o hormônio ativo cortisol no hormônio inativo cortisona) quanto atuar como redutase (via inversa). A HSD11B1 é expressa principalmente no fígado, no tecido adiposo e nos músculos. Em condições fisiológicas atua, preferencialmente, como redutase, promovendo a síntese de cortisol, sendo a atividade da enzima dependente do NADPH produzido pela enzima hexose-6-fosfato desidrogenase.

Uma produção excessiva de cortisol no tecido adiposo abdominal tem sido proposta como uma característica central da síndrome metabólica. O hipercortisolismo da Síndrome de Cushing compartilha quase todas as características da síndrome metabólica, mas a doença de Cushing é rara e o nível circulante de cortisol é normal na grande maioria dos indivíduos com obesidade e diabetes tipo 2.

Existe a possibilidade de que as alterações metabólicas possam ser derivadas de um aumento dos glicocorticoides localmente disponíveis, promovido pela ação da HSD11B1. Em consequência, estudos têm procurado compreender o papel da enzima HSD11B1 na patogênese de diferentes componentes da síndrome metabólica como obesidade, resistência à insulina, hiperglicemia e dislipidemia.

Esta dissertação é composta por dois artigos, o primeiro de revisão sistemática e o segundo transversal de análise de polimorfismos e expressão gênica do *HSD11B1* e sua relação com a obesidade, síndrome metabólica e o diabetes melito.

A revisão sistemática procura agrupar os dados obtidos em trabalhos realizados a fim de esclarecer a existência de associação entre a expressão e polimorfismos no gene *HSD11B1* com obesidade, síndrome metabólica e diabetes melito tipo 2 em indivíduos com diferentes graus de tolerância à glicose.

O artigo original busca avaliar a relação entre dois polimorfismos, sendo um no *HSD11B1* e outro no gene que codifica a enzima auxiliar (*H6PD*) em 1006 indivíduos com diabetes melito tipo 2, não sendo encontrada relação individual com os parâmetros da síndrome metabólica. Quando avaliados esses polimorfismos em conjunto, foi encontrada uma relação com o índice de massa corporal (IMC).

Adicionalmente, foi realizada a análise de expressão gênica do *HSD11B1* em tecido adiposo abdominal em 28 indivíduos com diferentes graus de tolerância à glicose. Os resultados mostraram que a expressão do *HSD11B1* em tecido adiposo abdominal visceral foi inversamente relacionada com IMC, medida da cintura e razão cintura-altura.

Capítulo II - Artigo de revisão sistemática

Relationship between *HSD11B1* polymorphic variants and abdominal adipose tissue gene expression with metabolic syndrome, obesity and type 2 diabetes mellitus: a systematic review

Relationship between HSD11B1 polymorphic variants and adipose tissue gene expression with metabolic syndrome, obesity and type 2 diabetes mellitus: a systematic review.

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Short title
HSD11B1 gene expression and metabolic abnormalities.

Keywords
Diabetes mellitus; obesity; metabolic syndrome; HSD11B1; polymorphisms, gene expression

Abstract

HSD11B1 gene is highly expressed in abdominal adipose tissue and interconverts the inactive hormone cortisone in the active one cortisol. Genetic abnormalities of *HSD11B1* have been associated with the development of abnormalities of glucose metabolism and body fat distribution. To systematically review studies evaluating the relationship between abdominal adipose tissue *HSD11B1* gene expression and polymorphisms with obesity, metabolic syndrome (MetS) and type 2 diabetes (T2DM), we conducted a search of MEDLINE, SCOPUS and Cochrane Library on February 2014. Inclusion criteria were observational studies (cross-sectional, cohort or case-control), in adults, which have analyzed the relationship of genetic polymorphisms and/or abdominal adipose *HSD11B1* expression with obesity, metabolic syndrome or type 2 diabetes. Among 762 studies retrieved, 21 fulfilled inclusion criteria, (13 gene expression and 8 polymorphism studies). While abdominal adipose *HSD11B1* expression was in most studies increased with increasing BMI and abnormalities in glucose metabolism, its expression varied by the presence of MetS. While the summary data of literature suggests that abdominal adipose tissue *HSD11B1* gene is hyperexpressed in subjects with increased BMI AND abnormal glucose metabolism , conflicting data was found for *HSD11B1*gene expression and metabolic syndrome. Studies that have assessed the relationship between genetic polymorphic variants of *HSD11B1* with obesity, metabolic syndrome and type 2 diabetes found conflicting results and inclonclusive results.

Introduction

Hydroxysteroid (11-beta) dehydrogenase type 1 (11-βHSD1) is a bidirectional enzyme which is highly expressed in liver and adipose tissue. It normally converts the inactive hormone cortisone in its active form cortisol, acting generally as a reductase [1].

Overexpression of *HSD11B1* gene in adipocytes has been shown to be related to high adipose tissue cortisol concentration and the development of central obesity, insulin resistance (IR) and diabetes in mice models [2]. On the other hand, knockout mice for *HSD11B1* gene subjected to a high fat diet are protected against the development of obesity and hyperglycemia [3]. Moreover, 11-βHSD1 inhibitors have been shown to be effective in treating different aspects of metabolic syndrome (MetS), promoting weight loss, reducing IR and hyperglycemia.

Cushing's syndrome, which is caused by excess production of glucocorticoid, has clinical findings that remind in several aspects those of MetS, suggesting that they share possible pathogenic pathways which result in their metabolic abnormalities. Since overexpression of HSD11B1 in abdominal adipose tissue is related to increased adipose tissue cortisol concentrations, it is possible that polymorphic variants of this gene are related to the development of MetS [4].

In order to clarify this issue we conducted a systematic review of the literature addressing the relationship between *HSD11B1* polymorphic variants and abdominal *HSD11B1* adipose tissue expression with MetS, type 2 diabetes mellitus (T2DM) and obesity.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement was used in this report. This systematic review is registered in PROSPERO with number CRD42014008705.

Search strategy, study selection and data extraction

Observational studies (cross-sectional, cohort or case-control) which have analyzed the relationship of polymorphisms and/or *HSD11B1* gene expression with obesity, MetS or T2DM in humans adults were considered eligible. A literature search was performed in Medline, Cochrane Central and Scopus. We also manually searched the references of published studies. Study selection was not limited by language. The search strategy was based on the following Medical Subject Headings (MeSH) Terms: (((("Obesity"[Mesh]) OR "Diabetes Mellitus, Type 2"[Mesh]) OR "Prediabetic State"[Mesh])) AND ("11-beta-Hydroxysteroid Dehydrogenase Type 1"[Mesh]).

Study selection was performed by two independent investigators (FVN and VP). Disagreements were resolved by discussion between them, and when necessary a third reviewer (FG) was consulted. Data was extracted by two investigators (FVN and VP) in a standardized form. The information extracted from each individual study was as follows: design, ethnicity, polymorphisms genotyped, tissue site for biopsy and gene expression analysis, number of individuals in each group, gender distribution, body mass index (BMI), waist circumference, waist-to-hip ratio, and fasting and 2-h plasma glucoses after a 75-g oral glucose tolerance test. In order to assess the quality of the selected studies, the New Castle-Ottawa Scale (NOS) was used by the investigators [5]. NOS contains eight items categorized into three dimensions, including selection, comparability, and exposure. A series of response options is provided for each item. A star scoring system is used to allow a semi-quantitative assessment of study quality, such that the highest quality studies are awarded a maximum of one star for each item, with exception of the item related

to comparability, which allows two stars to be assigned. Total NOS score ranges from zero to nine stars.

Results

We identified 762 records (Figure 1). Based on title and abstracts, we selected 45 studies for full text examination out of which 21 fulfilled the final inclusion criteria. Studies were grouped according to the following relationships: 1) Abdominal subcutaneous and visceral *HSD11B1* gene expression and MetS [6; 7; 8]; 2) Abdominal subcutaneous and visceral *HSD11B1* gene expression and T2DM [6; 8; 9; 10]; 3) Abdominal subcutaneous and visceral *HSD11B1* gene expression and obesity [6; 7; 8; 9; 10; 11; 12; 13; 14; 15; 16; 17; 18]; and 4) Genetic polymorphisms of *HSD11B1* with obesity, MetS and T2DM [19; 20; 21; 22; 23]. The main results of these studies are presented in Tables 1 to 4. The mean quality score was 5.45 stars and the details of quality assessment were described in Table 5.

Relationship between abdominal subcutaneous and visceral *HSD11B1* gene expression with metabolic syndrome

Three studies compared the expression of *HSD11B1* in adipose tissue by the presence of MetS [6; 7; 8] (Table 1). These studies included a total of 121 participants (mean age of 41.7 years). Two of them recruited only obese subjects whereas the third one compared obese vs. lean control subjects. Two studies recruited subjects from both genders whereas one study included only women. In the first study [6] abdominal subcutaneous adipose tissue (SAT) *HSD11B1* expression was higher in 62 obese subjects with MetS compared to those without MetS and abdominal visceral adipose tissue (VAT) expression was similar between groups. Contrasting with these findings, in another study with 32 obese individuals (6 with T2DM) [8], the expression of *HSD11B1* was found to be not significant higher in both abdominal SAT and VAT in subjects without MetS compared to those with MetS. On the other hand, the abdominal VAT expression of *HSD11B1* was found to be significantly higher in 19 obese women (two with T2DM) in a case control study versus eight lean women [7] (Table 1).

Relationship between abdominal subcutaneous and visceral *HSD11B1* gene expression with hyperglycemia

Three studies analyzed the relationship between *HSD11B1* gene expression in abdominal SAT and VAT with IR, T2DM or fasting glucose (Table 2). Studies included 32 to 70 subjects (mean age of 43.2 years). These studies included both genders [6; 8; 9]. One study has found abdominal VAT expression to be positively correlated with increased HOMA-IR in morbidly obese subjects [9]. While SAT *HSD11B1* gene expression was higher in subjects with T2DM compared to normal controls (1.8 ± 0.7 vs. 1.1 ± 0.4; P<0.05) [6]. In the third study, the small number of subjects with T2DM (n=6) had a trend to have higher expression of *HSD11B1* in abdominal SAT than those without T2DM [8].

Relationship between abdominal subcutaneous and visceral *HSD11B1* gene expression with obesity

Thirteen studies relate abdominal subcutaneous and visceral *HSD11B1* gene expression with obesity (Table 3). Five studies did not find a relationship between abdominal SAT and VAT *HSD11B1* with central or generalized obesity [6; 7; 9; 16; 17]. While in one study [11] SAT and VAT *HSD11B1* abdominal gene expression was higher in obese than lean women, the same results were found in other two studies only for abdominal SAT expression of female subjects [10; 12]. In the first study [11], abdominal SAT expression was also related to waist circumference and percentage of body fat. In two other studies, *HSD11B1* gene expression was higher in abdominal VAT of obese than lean subjects [13; 14]. While in one study [8], *HSD11B1* abdominal SAT gene expression was also positively related to BMI, this relationship was not found with waist circumference. Additionally, abdominal VAT gene expression was not related with both BMI and waist circumference in the same study. In another study [15], *HSD11B1* abdominal SAT gene expression was higher in obese male and female subjects than non-obese subjects. For abdominal VAT, gene expression was higher in obese women than lean controls, but the sample size was too small to study this relationship in men. Lastly, in the study by Zha et al., not only *HSD11B1*

gene expression was higher in obese than lean subjects, but also increased abdominal SAT and VAT gene expression was related with increased BMI [18].

Relationship between genetic polymorphisms of *HSD11B1* and obesity, metabolic syndrome or type 2 diabetes mellitus

Eight studies analyzed the association between 26 different polymorphic variants at *HSD11B1* and obesity, MetS and T2DM (Table 4) [19; 20; 21; 22; 23; 24; 25; 26].

The Asian population was assessed in two studies from South Korea [19; 21], one study from Japan [20] and one from India [26]. The first study analyzed 757 individuals with and 644 without T2DM [19]. It did not find an association of four polymorphisms of *HSD11B1* with T2DM and MetS. In another study, 427 individuals with and 358 without T2DM were analyzed and it did not find a relationship of two polymorphic variants at *H11D11B1* with T2DM and MetS [21]. No relationship of seven different polymorphic variants at *H11D11B1* with MetS was found in an urban cohort of 3005 Japanese individuals with [20]. No relationship of polymorphic variants at *H11D11B1* was also found in a study in the 217 French-Canadian men [23]. In a study with 918 American Indians of the Gila River Indian Community of Arizona, polymorphic variants were also not associated with T2DM and obesity [25]. In contrast with these negative results, a study from India found a significant risk for MetS with rs12086634 polymorphism at *H11D11B1* [26]. Another study has also found a significant risk for T2DM, but not for obesity with rs846910 and rs12086634 polymorphic variant at *HSD11B1* in Pima Indians [22]. In contrast with these findings, another study showed that the polymorphic variant rs846910 at *HSD11B1* is associated with decreased insulin resistance (decreased HOMA-IR) in subjects with MetS. In the same study the rs45487298 polymorphism was associated with the same findings in control subjects without MetS [24].

Discussion

We systematically reviewed and summarized a total of 21 studies which have analyzed the relationship of human abdominal adipose *HSD11B1* gene expression and its polymorphic variants with MetS, T2DM and obesity.

The relationship between abdominal *HSD11B1* adipose tissue gene expression and MetS was analyzed in 3 studies with distinct population profiles. They found contrasting results about this relationship, which may be explained by differences in demographic and clinical characteristics of study subjects from the different studies, such as gender distribution, age, MetS prevalence, and the adoption of different MetS criteria. It has been demonstrated that the effect of *HSD11B1* in converting the inactive hormone cortisone in the active cortisol is different by the presence of MetS. Adipose *HSD11B1* activity in individuals without MetS seems to be related with fasting plasma glucose and IR in Pima Indians and Caucasians [27]. However, when individuals with MetS were investigated, these correlations disappeared or are in the opposite direction [28]. Additionally, some of these studies might be underpowered to detect differences in abdominal *HSD11B1* expression among groups with distinct metabolic profile. Based on their results it is not possible to determine a relationship between *HSD11B1* abdominal adipocyte gene expression and MetS.

The relationship between abdominal *HSD11B1* adipose tissue gene expression with IR, fasting glucose, and T2DM were analyzed in 4 studies [6; 8; 9; 10]. Their results suggested that increasing abdominal adipocyte VAT and SAT *HSD11B1* gene expression is related with IR and fasting glucose (Table 2). Although it is not possible to define which compartment of abdominal *HSD11B1* adipose tissue expression is related to metabolic abnormalities, these studies suggest that expression in abdominal adipocyte is related with IR and hyperglycemia and may have a role in the development of T2DM. The relationship between abdominal *HSD11B1* adipose tissue gene expression with central and general obesity parameters were analyzed in 13 studies. While five studies have not found a relationship of abdominal *HSD11B1* adipose tissue gene expression with obesity, eight studies have found that VAT and/or SAT expression was related to BMI, waist circumference and body fat proportion. In all these 8 studies, *HSD11B1* abdominal

adipocyte gene expression was higher in subjects with excess weight compared to lean controls.

Our collected data suggest that most polymorphic variants on *HSD11B1* are not related with MetS, obesity or T2DM [19; 20; 21; 22; 23; 25]. In one study, two polymorphic variants (rs846910 and rs12086634) of *HSD11B1* in linkage with other 3 polymorphic variants were found to be related with decreased insulin sensitivity, increased plasma glucose levels and T2DM rates [22]. The polymorphic variant rs846910 is located in the promoter region near exon 1 of the *HSD11B1* gene. Carriers of this SNP have increased enzyme activity, which is in agreement with our findings. However, rs12086634 is located in intron 3 and carriers of this polymorphic variant have decreased enzyme activity, what cannot be explained by our findings related to this SNP. Functional studies assessing how these polymorphic variants change *HSD11B1* gene functionality were not performed. Additionally, since polymorphic variants of *HSD11B1* that changes its expression and activity are expected to have a major role in modulating insulin sensitivity and adipose tissue proliferation through the regulation of intra-adipocyte cortisol production, it would be expected that the presence of these polymorphic variants would be related with obesity too, which was not demonstrated on our study [22]. The rs12086634 polymorphic variant was also found to be related with MetS in one study [26]. In contrast, this polymorphic variant was found to be related with increased insulin sensitivity in another small study (n=86). Additionally, in the same study rs45487298 was also associated with increased insulin sensitivity in control subjects without MetS [24]. This SNP is localized in intron 3 of the *HSD11B1* gene and is related with decreased enzyme expression. Since the activity of *HSD11B1* is dependent on the provision of NADPH by the co-localized enzyme hexose-6-phosphate dehydrogenase (H6PD), polymorphic variants affecting both, the effect of H6PD and 11-βHSD1, may be necessary to see an effect of their enzymatic activity, resulting in the determination of metabolic abnormalities or a healthy metabolic profile.

In conclusion, the summary of data collection of these studies suggests that intra-abdominal adipose tissue *HSD11B1* gene expression is probably related to T2DM and obesity, although these findings are not consistent while comparing different studies.

References

1   TOMLINSON, J. W. et al. Weight loss increases 11beta-hydroxysteroid dehydrogenase type 1 expression in human adipose tissue. **J Clin Endocrinol Metab,** v. 89, n. 6, p. 2711-6, Jun 2004.

2   MASUZAKI, H. et al. A transgenic model of visceral obesity and the metabolic syndrome. **Science,** v. 294, n. 5549, p. 2166-70, Dec 7 2001.

3   KOTELEVTSEV, Y. et al. 11beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. **Proc Natl Acad Sci U S A,** v. 94, n. 26, p. 14924-9, Dec 23 1997.

4   STIMSON, R. H.WALKER, B. R. Glucocorticoids and 11beta-hydroxysteroid dehydrogenase type 1 in obesity and the metabolic syndrome. **Minerva Endocrinol,** v. 32, n. 3, p. 141-59, Sep 2007.

5   STANG, A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. **Eur J Epidemiol,** v. 25, n. 9, p. 603-5, Sep 2010.

6   ALBERTI, L. et al. Type 2 diabetes and metabolic syndrome are associated with increased expression of 11beta-hydroxysteroid dehydrogenase 1 in obese subjects. **Int J Obes (Lond),** v. 31, n. 12, p. 1826-31, Dec 2007.

7   MICHALAKI, M. et al. The expression of omental 11beta-HSD1 is not increased in severely obese women with metabolic syndrome. **Obes Facts,** v. 5, n. 1, p. 104-11, 2012.

8   MUNOZ, R. et al. 11beta-hydroxysteroid dehydrogenase type 1 is overexpressed in subcutaneous adipose tissue of morbidly obese patients. **Obes Surg,** v. 19, n. 6, p. 764-70, Jun 2009.

9   BAUDRAND, R. et al. Overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in hepatic and visceral adipose tissue is associated with metabolic disorders in morbidly obese patients. **Obes Surg,** v. 20, n. 1, p. 77-83, Jan 2010.

10 ENGELI, S. et al. Regulation of 11beta-HSD genes in human adipose tissue: influence of central obesity and weight loss. **Obes Res,** v. 12, n. 1, p. 9-17, Jan 2004.

11 DESBRIERE, R. et al. 11beta-hydroxysteroid dehydrogenase type 1 mRNA is increased in both visceral and subcutaneous adipose tissue of obese patients. **Obesity (Silver Spring),** v. 14, n. 5, p. 794-8, May 2006.

12 MAKKONEN, J. et al. Increased expression of the macrophage markers and of 11beta-HSD-1 in subcutaneous adipose tissue, but not in cultured

monocyte-derived macrophages, is associated with liver fat in human obesity. **Int J Obes (Lond),** v. 31, n. 10, p. 1617-25, Oct 2007.

13   MARINIELLO, B. et al. Adipose tissue 11beta-hydroxysteroid dehydrogenase type 1 expression in obesity and Cushing's syndrome. **Eur J Endocrinol,** v. 155, n. 3, p. 435-41, Sep 2006.

14   MICHAILIDOU, Z. et al. Omental 11beta-hydroxysteroid dehydrogenase 1 correlates with fat cell size independently of obesity. **Obesity (Silver Spring),** v. 15, n. 5, p. 1155-63, May 2007.

15   PAULSEN, S. K. et al. 11Beta-HSD type 1 expression in human adipose tissue: impact of gender, obesity, and fat localization. **Obesity (Silver Spring),** v. 15, n. 8, p. 1954-60, Aug 2007.

16   SIMONYTE, K. et al. Obesity is accompanied by disturbances in peripheral glucocorticoid metabolism and changes in FA recycling. **Obesity (Silver Spring),** v. 17, n. 11, p. 1982-7, Nov 2009.

17   TOMLINSON, J. W.   et al. Expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue is not increased in human obesity. **J Clin Endocrinol Metab,** v. 87, n. 12, p. 5630-5, Dec 2002.

18   ZHA, J. M. et al. Comparison of gene transcription between subcutaneous and visceral adipose tissue in Chinese adults. **Endocr J,** v. 56, n. 8, p. 935-44, 2009.

19   KU, Y. H. et al. Regulatory effect of common promoter polymorphisms on the expression of the 11beta-hydroxysteroid dehydrogenase type 1 gene. **Horm Res,** v. 72, n. 1, p. 25-32, 2009.

20   MIYAMOTO, Y.   et al. Association study of 11beta-hydroxysteroid dehydrogenase type 1 gene polymorphisms and metabolic syndrome in urban Japanese cohort. **Diabetes Res Clin Pract,** v. 85, n. 2, p. 132-8, Aug 2009.

21   MOON, S. S. et al. Relationship of 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase gene polymorphisms with metabolic syndrome and type 2 diabetes. **Endocr J,** v. 58, n. 11, p. 949-59, 2011.

22   NAIR, S. et al. 11beta-Hydroxysteroid dehydrogenase Type 1: genetic polymorphisms are associated with Type 2 diabetes in Pima Indians independently of obesity and expression in adipocyte and muscle. **Diabetologia,** v. 47, n. 6, p. 1088-95, Jun 2004.

23   ROBITAILLE, J. et al. Molecular screening of the 11beta-HSD1 gene in men characterized by the metabolic syndrome. **Obes Res,** v. 12, n. 10, p. 1570-5, Oct 2004.

24   DUJIC, T. et al. Association between 11beta-hydroxysteroid dehydrogenase type 1 gene polymorphisms and metabolic syndrome in Bosnian population. **Biochem Med (Zagreb),** v. 22, n. 1, p. 76-85, 2012.

25   FRANKS, P. W. et al. Interaction between an 11betaHSD1 gene variant and birth era modifies the risk of hypertension in Pima Indians. **Hypertension,** v. 44, n. 5, p. 681-8, Nov 2004.

26   GANDHI, K.   et al. Association between a 11beta-hydroxysteroid dehydrogenase type 1 gene polymorphism and metabolic syndrome in a South Indian population. **Metab Syndr Relat Disord,** v. 11, n. 6, p. 397-402, Dec 2013.

27   LINDSAY, R. S.   et al. Subcutaneous adipose 11 beta-hydroxysteroid dehydrogenase type 1 activity and messenger ribonucleic acid levels are associated with adiposity and insulinemia in Pima Indians and Caucasians. **J Clin Endocrinol Metab,** v. 88, n. 6, p. 2738-44, Jun 2003.

28   KARLSSON, C. et al. Differences in associations between HSD11B1 gene expression and metabolic parameters in subjects with and without impaired glucose homeostasis. **Diabetes Res Clin Pract,** v. 88, n. 3, p. 252-8, Jun 2010.

Figure and Tables

Figure1 – Studies selection flow diagram

| Records identified through database searching (n = 760) | Additional records identified through other sources (n = 2) |

Records screened (n = 762) → Records excluded (n = 717)

Full-text articles assessed for eligibility (n = 45) → Full-text articles excluded, with reasons (n = 24):

-Other related diseases (n = 7)
-Intervention study (n = 4)
-Subjects don't match inclusion criteria (n = 4)
-Different tissue analyzed (n = 3)
-No gene expression data (n = 6)

Studies included in synthesis (n = 21)

Table 1: Relationship of *HSD11B1* abdominal adiposity gene expression and metabolic syndrome: studies subjects characteristics

Author, year	Subjects' characteristics	T2DM	Obesity	BMI (kg/m²)	MetS	VAT expression (AU)	SAT expression (AU)
Alberti, 2007	n: 62 Female: 52 (83.9%) Age: 44.4 ± 11.1 Ethnicity: nd	12 (19.4)	62 (100.0)	37.4 ± 5.1	25 (40.3)	MetS+: 2.6 ± 0.5 MetS-: 1.7 ± 1.0	MetS+: 2.0 ± 1.5 MetS-: 0.7 ± 0.4[a]
Muñoz, 2009	n: 32 Female: 21 (65.6%) Age: 40.2 ± 12.3 Ethnicity: nd	6 (18.8)	32 (100.0)	36.7 ± 3.8	16 (50.0)	MetS+: 7.5 MetS-: 10.3	MetS+: 11.6 MetS-: 12.3
Michalaki, 2012	n = 27 Female: 27 (100%) Age: 37.3 ± 9.7 Ethnicity: nd	2 (7.4)	19 (70.4)	46.1 ± 6.6	11 (40.7)	Lean controls: 27.8 ± 16.0 MetS+: 62.9 ± 24.4 MetS-: 107.2 ± 77.7[b]	MetS+: 86.5 ± 29.8 MetS-: 155.9 ± 124.9

Data are expressed in absolute and relative frequencies: n (%) or mean ± standard deviation. T2DM: type 2 diabetes mellitus; BMI: body mass index; MetS: metabolic syndrome; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue; AU: arbitrary units; nd: not described. [a]: P<0.01 vs. MetS+ group. [b]: P<0.01 vs. Lean controls.

Table 2: Relationship of *HSD11B1 a*bdominal adiposity gene expression and hyperglycemia: studies subjects characteristics

Author, year	Subjects' characteristics	T2DM	Obesity	BMI (kg/m²)	MetS	VAT expression	SAT expression
Alberti, 2007	n: 62 Female: 52 (83.9) Age: 44.4 ± 11.1 Ethnicity: nd	12 (19.4)	62 (100.0)	37.4 ± 5.1	25 (40.3)	nd	r=0.460, P<0.0001 with fasting glucose
Muñoz, 2009	n: 32 Female: 21 (65.6) Age: 40.2 ± 12.3 Ethnicity: nd	6 (18.8)	32 (100.0)	36.7 ± 3.8	16 (50.0)	r=-0.19, P=NS with fasting glucose	r=0.15, P=NS with fasting glucose
Baudrand, 2009	n = 49 Female: 35 (71) Age: 42.2 ± 10.1 Ethnicity: nd	11 (22.4)	49 (100)	42 ± 6.1	31 (63.3)	r=0.48, P=0.005 with fasting insulin	nd

Data are expressed in absolute and relative frequencies: n (%) or mean ± standard deviation. T2DM: type 2 diabetes mellitus; BMI: body mass index; MetS: metabolic syndrome; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue. nd: not described.
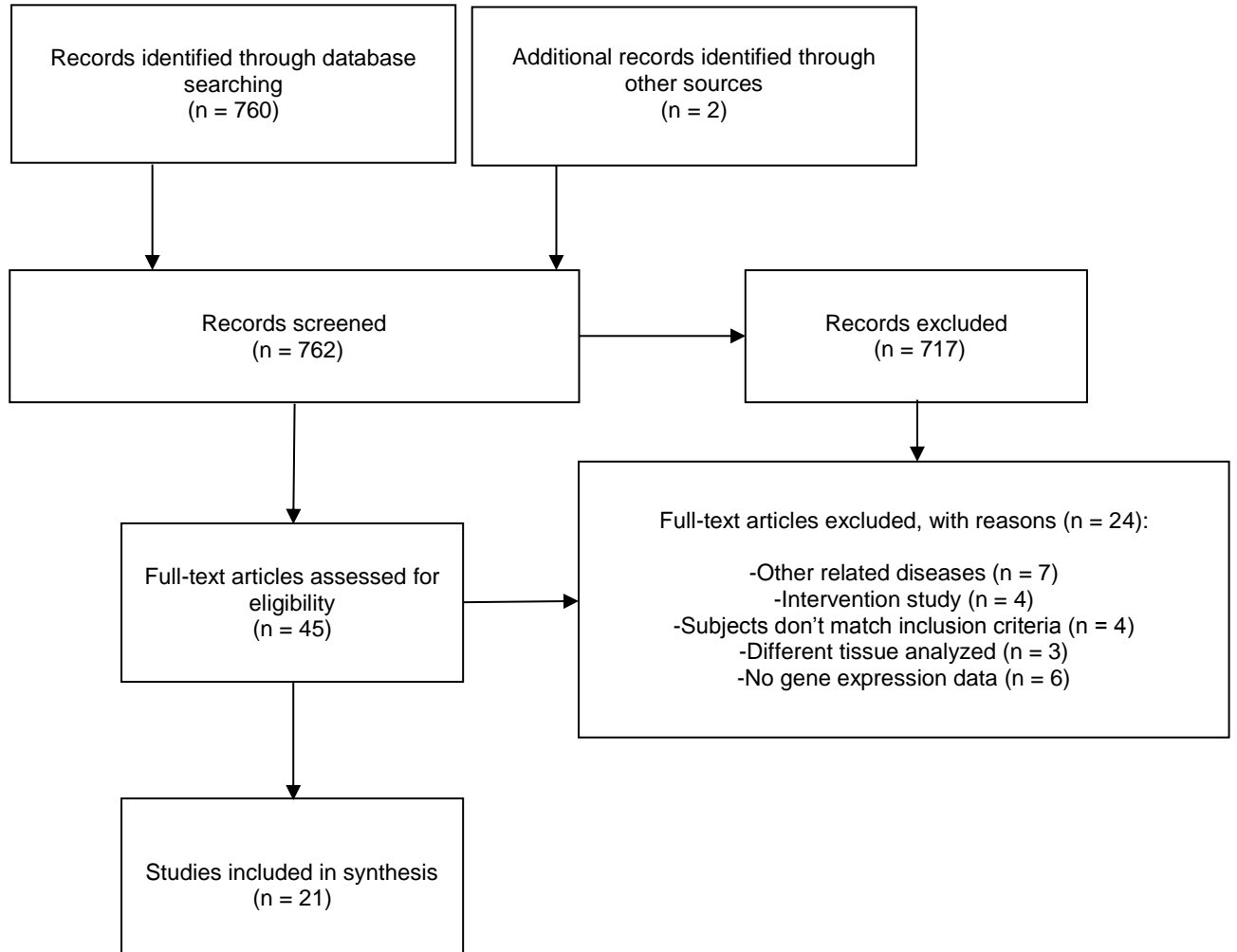
Table 3: Relationship of *HSD11B1* abdominal adiposity gene expression and obesity: studies subjects characteristics

Author, year	Subjects' characteristics	T2DM	Obesity	BMI (kg/m²)	MetS	VAT expression	SAT expression
Tomlinson, 2002	n: 32 Female: 32 (100) Age: 43 (28 – 65) Ethnicity: nd	0 (0)	11 (34.4)	28.1 ± 0.7	nd	BMI < 30kg/m²: 15.5 ± 0.3 (n-fold) BMI ≥ 30kg/m²: 14.8 ± 0.5 (n-fold)	BMI < 30kg/m²: 15.5 ± 0.4 (n-fold) BMI ≥ 30kg/m²: 15.1 ± 0.5 (n-fold)
Engeli, 2004	n: 70 Female: 70 (100) Age: 57 ± 4 Ethnicity: Caucasian	0 (0)	14 (20)	29.0 ± 3.1	nd	nd	BMI < 25kg/m²: 0.5 (AU) BMI ≥ 25kg/m²: 0.9 (AU) [a]
Desbriere, 2006	n: 22 Female: 22 (100) Age: 36.2 ± 7.5 Ethnicity: nd	0 (0)	12 (54.5)	30.2 ± 3.8	nd	BMI < 25kg/m²: 0.5 (AU) BMI ≥ 30kg/m²: 2.2 (AU)[a]	BMI < 25kg/m²: 0.5 (AU) BMI ≥ 30kg/m²: 3.6 (AU)[a]
Mariniello, 2006	n: 24 Female: 9 (64.3) Age: 42 ± 10 Ethnicity: nd	nd	8 (57.1)	34 ± 3.4	nd	BMI < 30kg/m²: 1 (n-fold) BMI ≥ 30kg/m²: 13 (n-fold)[b]	nd
Alberti, 2007	n: 62 Female: 52 (83.9%) Age: 44.4 ± 11.1 Ethnicity: nd	12 (19.4)	62 (100.0)	37.4 ± 5.1	25 (40.3)	BMI ≥ 30kg/m²: 2.1 ± 0.9 (AU)	BMI ≥ 30kg/m²: 1.4 ± 0.8 (AU)
Makkonen, 2007	n: 20 Female: 20 (100) Age: 36.5 ± 3.5 Ethnicity: Caucasian	0 (0)	10 (50)	28.7 ± 1.4	nd	nd	BMI < 25kg/m²: 0.3 ± 0.1 (n-fold) BMI ≥ 25kg/m²: 0.6 ± 0.1 (n-fold)[a]
Michailidou, 2007	n: 21 Female: 21 (100)	nd	nd	32.7 ± 1.5	nd	r=0.57 with BMI [c]	r=0.58 with BMI [a]

	Age: 35 ± 1 Ethnicity: Caucasian						
Paulsen, 2007	n: 40 Female: 20 (50) Age: 41.3 ± 9.8 Ethnicity: nd	0 (0)	20 (50)	34.7 ± 3.8	0 (0)	BMI < 30kg/m²: 0.01 (n-fold) BMI ≥ 30kg/m²: 0.04 (n-fold)[a]	BMI < 30kg/m²: 0.01 (n-fold) BMI ≥ 30kg/m²: 0.05 (n-fold)[a]
Baudrand, 2009	n = 49 Female: 35 (71) Age: 42.2 ± 10.1 Ethnicity: nd	11 (22.4)	49 (100)	42 ± 6.1	31 (63.3)	nd	nd
Muñoz, 2009	n: 32 Female: 21 (65.6%) Age: 40.2 ± 12.3 Ethnicity: nd	6 (18.8)	32 (100.0)	36.7 ± 3.8	16 (50.0)	BMI ≥ 30kg/m²: 7.8 (4.7 – 11.8) (AU)	BMI ≥ 30kg/m²: 11.4 (6.2 – 19.8) (AU)
Simonyte, 2009	n: 27 Female: 27 (100) Age: 41 ± 8.5 Ethnicity: Caucasian	3 (9.7)	27 (100)	44.6 ± 4.5	nd	BMI ≥ 30kg/m²: 11.2 ± 4.9(AU)	BMI ≥ 30kg/m²: 14.1 ± 6.4 (AU)
Zha, 2009	n: 35 Female: 17 (49) Age: 49.5 ± 12.5 Ethnicity: Chinese	0 (0)	15 (50)	25.5 ± 1.5	nd	BMI < 25kg/m²: 1 (AU) BMI ≥ 25kg/m²: 1.5 (AU)[c]	BMI < 25kg/m²: 1.2 (AU) BMI ≥ 25kg/m²: 1.5 (AU)[c]
Michalaki, 2012	n = 27 Female: 27 (100%) Age: 37.3 ± 9.7 Ethnicity: nd	2 (7.4)	19 (70.4)	46.1 ± 6.6	11 (40.7)	BMI < 25kg/m²: 27.8 ± 16 (AU) BMI ≥ 30kg/m²: 81.6. ± 46.8 (AU)[a]	BMI < 25kg/m²: nd BMI ≥ 30kg/m²: 115.7. ± 69.8 (AU)

Data are expressed in absolute and relative frequencies: n (%), median (P25 – P75) or mean ± standard deviation. T2DM: type 2 diabetes mellitus; BMI: body mass index; MetS: metabolic syndrome; VAT: visceral adipose tissue; SAT: subcutaneous adipose tissue; AU: arbitrary units. [a]: P<0.5. [b]: P<0.001. [c]: P<0.01. r = Pearson's correlation coefficient. nd: not described.

Table 4: Relationship of *HSD11B1* genetic polymorphic variants with obesity, metabolic syndrome and diabetes: studies subjects characteristics

Author, year	Subjects' characteristics	T2DM	BMI (kg/m²)	MetS	Genetic Polymorphisms	Association with metabolic parameters
Franks, 2004	n: 918 Age: 36 (28 – 46) Ethnicity: American Indians	512 (55.8)	32.7 (28 – 38)	nd	rs846910 and rs12086634	No association with obesity and diabetes
Nair, 2004	n: 706 (SNP1), 839 (SNP5) Age: nd Ethnicity: Pima Indians	SNP1: 429 (60.8) SNP5: 510 (60.8)	nd	nd	SNP1 (rs846910 and rs3334312) SNP5 (nd, rs12086634 and rs6752)	SNP1 – OR = 1.64; P = 0.01 SNP5 – OR = 1.34; P = 0.02 for T2DM (+) vs. T2DM (-)
Robitaille, 2004	n: 217 Age: 42.8 ± 7.9 Ethnicity: French-Canadian	nd	29.6 ± 4.3	nd	4478 T>G, 4437–4438insA, 10733G>C	No association with BMI, WC, HDL-c, TG, FPG, fasting insulin, BP, HOMA-IR
Miyamoto, 2009	n: 3005 Age:65.8 ± 10.3 Ethnicity: Japanese	nd	22.8 ± 2.7	431 (14.3)	rs860185, -658G>A, +1930insA, rs12086634, rs2236905, rs2298930, rs932335 and rs6752	No association with MetS
Ku, 2009	n: 1401 Age: 61.8 ± 7.2 Ethnicity: Korean	757 (54.0)	24.1 ± 3.0	nd	rs45441700, rs846908, rs701950, +1932Ginsdel, rs932335, rs13306422, rs6752, +30249T>C	No association with WHR, BMI, FPG, HbA1c, fasting insulin and HOMA-IR
Moon, 2011	n: 785 Age: 62.2 ± 0.5 Ethnicity: Korean	427 (54.4)	23.3 ± 0.2	nd	rs12086634 and rs1000283	No association with T2DM and MetS

Dujic, 2012	n: 86 Age: 47 (40 – 53) Ethnicity: Bosnian	nd	28.9 (25.7 – 31.5)	43 (50.0)	rs846910 rs45487298	rs846910 related with ↓ HOMA-IR; P = 0.011[a] rs45487298 related with ↓ HOMA-IR; P = 0.04[b]
Gandhi, 2013	n: 205 Age: 43 ± 11.5 Ethnicity: South Indian	62 (30.2)	25.7 ± 2.9	105 (51.2)	rs12086634	Associated with MetS (OR = 6.64; P < 0.0001)

Data are expressed in absolute and relative frequencies: n (%), median (P25 – P75) or mean ± standard deviation. nd: not described; T2DM: type 2 diabetes mellitus; BMI: body mass index; MetS: metabolic syndrome; WC: wiast cirfumference; HDL-c: HDL cholesterol; TG: triglycerides; FPG: fasting plasma glucose; BP: blood pressure; HOMA-IR: homeostatic model assessment for insulin resistance; HbA1c: glycated hemoglobin A1c; NPG: non-polymorphic group; PG: polymorphic group. [a]: in MetS (+) group. [b]: in control group.

Table 5: Quality score of studies used in the systematic review

Author, year	Case definition	Representativeness of the cases	Selection of controls	Definition of controls	Comparability	Ascertainment of exposure	Total
Alberti, 2007	+		+	+	+	++	++++++
Baudrand	+	+		+		++	+++++
Desbriere, 2006	+			+		++	++++
Dujic, 2011	+	+	+	+		+++	+++++++
Franks, 2004	+	+	+		+	+++	+++++++
Gandhi, 2013	+	+	+	+		++	++++++
Ku, 2009	+	+	+	+	+	+++	++++++++
Makkonen, 2007	+		+	+	+	++	++++++
Mariniello, 2006	+		+	+		++	+++++
Michailidou, 2007	+				+	++	++++
Michalaki, 2012	+			+		++	++++
Miyamoto, 2009	+	+	+		+	++	++++++
Moon, 2011	+	+	+	+	+	+++	++++++++
Munoz, 2009	+	+				++	++++
Nair, 2004	+	+	+		+	+++	+++++++
Paulsen, 2007	+			+		++	++++
Robitaille, 2004	+					+++	++++
Simonyte, 2009	+				+	++	++++
Tomlinson, 2002	+			+		++	++++
Zha, 2009	+	+		+	+	++	++++++

Capítulo III - Artigo Original

Interaction between *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms and *HSD11B1* gene expression in the protection against obesity: a cross-sectional study.

Interaction between *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms and *HSD11B1* gene expression in the protection against obesity: a cross-sectional study

Running Head

HSD11B1 and *H6PD* genes in obesity.

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Declaration of interest

The authors declare that they have no competing interests.

Novelty statement

- Hydroxysteroid (11-beta) dehydrogenase type 1 (*HSD11B1*) converts cortisone to cortisol with the NADPH provided by hexose-6-phosphate dehydrogenase (H6PD).

- While studying 1006 subjects with type 2 diabetes we showed that carriers of at least three minor alleles of polymorphic variants on *HSD11B1* and H6PD genes exhibited lower BMI compared to those with two or fewer minor alleles.

- Additionally, HSD11B1 visceral abdominal adipose tissue expression was inversely correlated with body size and central adiposity, suggesting that obese individuals may have decreased intra-abdominal VAT *HSD11B1* gene expression resulting in decreasing intra-abdominal cortisol levels as a compensatory mechanism against central and general adiposity.

Abstract

Aims: Hydroxysteroid (11-beta) dehydrogenase type 1 converts inactive cortisone to active cortisol in a process dependent of NADPH provided by hexose-6-phosphate dehydrogenase. The generation of cortisol from this enzymatic reaction may increase intra-abdominal levels of cortisol and contribute to the physiopathogenesis of obesity and metabolic syndrome (MetS). **Methods:** We firstly analyzed in a cross-sectional study the relationship of *HSD11B1* rs45487298 and *H6PD* rs6688832 polymorphisms with obesity and MetS in 1006 white participants with type 2 diabetes (T2DM). Secondly, we study how *HSD11B1* abdominal subcutaneous (SAT) and visceral adipose tissue (VAT) gene expression is related to MetS in 28 participants submitted to abdominal surgery. **Results:** Although the polymorphisms of the two genes were not individually associated with MetS features, a synergistic effect was observed between both. Carriers of at least three minor alleles exhibited lower BMI compared to those with two or fewer minor alleles after adjusting for gender and age (27.4 ± 4.9 *vs.* 29.3 ± 5.3 kg/m^2; P = 0.005; mean $\pm$ SD). Obesity frequency was also lower in the first group (24.4% *vs.* 41.6%, OR = 0.43, 95% CI 0.21 – 0.87; P = 0.019). VAT expression was inversely correlated with BMI (r=-0.435, P=0.034), waist circumference (r=-0.584, P=0.003) and waist-to-height ratio (r=-0.526, P=0.010). **Conclusions:** These polymorphisms might interact in the protection against obesity in T2DM individuals. Obese individuals may have decreased intra-abdominal VAT *HSD11B1* gene expression resulting in decreasing intra-abdominal cortisol levels as a compensatory mechanism against central and general adiposity.

Introduction

Central obesity is associated with type 2 diabetes mellitus (T2DM), hypertension and dyslipidemia. This cluster of risk factors is known as the metabolic syndrome (MetS), and also occurs in people with primary glucocorticoid excess such as those with Cushing's syndrome [1]. In view of the obvious similarities between individuals with Cushing's syndrome and those with MetS, it has been proposed that, despite normal levels of circulating cortisol, these phenotypes might arise as a consequence of tissue-specific cortisol excess due to increased activity of the enzyme hydroxysteroid (11-beta) dehydrogenase type 1 (HSD11B1) [1].

HSD11B1 is widely distributed in several tissues, but is highly expressed in liver and adipose tissue, where it may increase cortisol levels and play an important role in the pathogenesis of obesity and insulin resistance [2; 3]. Although it is a bidirectional enzyme *in vitro*, under normal circumstances it works as a reductase in most of these sites, catalyzing the conversion of hormonally inactive cortisone into its active form, cortisol [4]. The active site of HSD11B1 is located in the lumen of the endoplasmic reticulum, and the activity of this enzyme is dependent on the provision of NADPH by the co-localized enzyme hexose-6-phosphate dehydrogenase (H6PD). In the absence of H6PD, HSD11B1 acts as a dehydrogenase, inactivating cortisol [2]. Therefore, HSD11B1 activity provides a mechanism through which different cell types can utilize circulating cortisone for intracellular cortisol production, which is independent of adrenal function [5].

The role of HSD11B1 in predisposition to obesity, T2DM and MetS is well established in rodent models [5]. Transgenic mice overexpressing *HSD11B1* develop visceral obesity and exhibited insulin-resistant diabetes and dyslipidemia [6], while *HSD11B1*-knockout mice are protected from the adverse metabolic complications of obesity and high fat diet-induced hyperglycemia [7]. Moreover, HSD11B1 inhibitors improve insulin sensitivity in mice [8]. *HSD11B1* expression in humans seems to be increased in subcutaneous adipose tissue of obese people in most studies [2; 9; 10], but not in all of them [11; 12]. Some data also suggest that *HSD11B1* expression is not only increased in visceral adipose tissue of obese women, but also it correlates with fat cell size independently of body mass index [2]. Additionally, adipose HSD11B1 activity in individuals without Mets seems to be related with fasting plasma glucose and

insulin resistance in Pima Indians and Caucasians [13]. However, when individuals with MetS are investigated, these correlations disappear or are in the opposite direction [12], suggesting that HSD11B1 expression has not the same response in populations of different metabolic profile.

Polymorphic variants of the *HSD11B1* gene have been related with obesity, hypertension, insulin resistance and T2DM [14; 15; 16; 17; 18]. Because H6PD activity determines the directionality of HSD11B1 activity, it is possible that polymorphisms in the *H6PD* gene might influence the effects of *HSD11B1* polymorphisms. Indeed, Draper *et al.* [19] reported that a combination of polymorphisms in the *HSD11B1* (rs45487298:delA>insA) and *H6PD* (rs6688832:G>A) genes interacts to cause cortisone reductase deficiency. They proposed a digenic triallelic mode of inheritance in which at least three minor alleles from two *loci* are necessary for trait manifestation.

Therefore, in the present study we investigated the potential synergistic effect of *HSD11B1* (rs45487298:delA>insA) and *H6PD* (rs6688832:G>A) polymorphisms on obesity and MetS-related characteristics in white T2DM individuals and the relationship of HSD11B1 gene expression in abdominal subcutaneous (SAT) and visceral (VAT) adipose tissue with adiposity distribution in individuals with and without MetS.

Participants and Methods

Participants

We performed a cross-sectional analysis of T2DM individuals who were participating in a multicenter study that started recruiting individuals in Southern Brazil in 2002 with the aim of study risk factors for T2DM and its complications. A detailed description of this study can be found elsewhere [20]. T2DM was defined as a diagnosis of diabetes after the age of 40 years with no insulin therapy during the first year after diagnosis and no previous episodes of ketoacidosis. The fasting and/or 2-h plasma glucose after the 75-g oral glucose tolerance test criteria were used to define diabetes according to cut offs adopted by the American Diabetes Association (ADA) [21].

The *HSD11B1* (rs45487298:delA>insA) and *H6PD* (rs6688832:G>A) polymorphisms were analyzed in blood samples from 1006 white T2DM individuals. Ethnicity was self-reported and most of individuals were of European ancestry (Portuguese, Spanish, Italian and German descent).

In a second moment, we conducted a gene expression study in SAT and VAT of 28 participants with and without MetS and with different degrees of glucose tolerance who underwent elective abdominal surgery for the treatment of benign diseases at Hospital de Clínicas de Porto Alegre. After collecting and cleaning, the adipose tissues were immediately frozen in liquid nitrogen and stored at -80°C.

The study protocol was approved by the ethics committees of the participating centers, and all individuals provided written informed consent.

Sampling strategy

The sampling strategy was based in another study which compared the expression of retinol binding protein type 4 (RBP4) in visceral and subcutaneous abdominal adipose tissue in insulin resistant and sensitive individuals [22]. WinPepi program was used for the estimation of sample size with these parameters: significance level of 5%, power of 90%, 1 to 1 proportion between insulin sensitive

and resistant individuals, and means and standard deviations extracted of this study. While doing these calculations, 15 subjects are necessary to compare the relationship between subcutaneous and abdominal adipose tissue adiponectin expression according to different metabolic parameters, such as those related to obesity and MetS.

Clinical and anthropometric profiles and laboratory analyses

A standard questionnaire was used to collect information about age, age at T2DM diagnosis, and drug treatment. All individuals underwent physical and laboratory evaluations. They were weighed (barefoot and wearing light outdoor clothing) and had their height measured. Body Mass Index (BMI) was calculated as weight (kg) / height (meters^2). Obesity was defined as a BMI $\geq$30 kg/m^2. Waist circumference (WC) was taken at the midpoint between the lower costal margin and the iliac crest measured to the nearest 0.5 cm. Waist-to-height ratio (WHtR) was calculated as waist (cm) / height (cm). Blood pressure (BP) was measured twice, in the sitting position, with a 5-min rest between measurements, using a mercury sphygmomanometer (Korotkoff phases I and V with cuff adjusted for arm circumference). The mean of the two measurements was used to calculate systolic and diastolic BP. Participants who were selected for the gene expression study were submitted to a 2-hour 75-g oral glucose tolerance test with determination of plasma glucose every 30 minutes.

Participants were classified as having MetS using the new International Diabetes Federation criteria [23]. According to this criteria, MetS is defined as central obesity (waist circumference $\geq$94 cm in males and $\geq$80 cm in females) plus any two of the following factors: hypertriglyceridemia (triglycerides $\geq$1.69 mmol/l or lipid-lowering therapy), high-density lipoprotein cholesterol (HDL) <1.04 mmol/l in males or <1.29 mmol/l in females), hypertension (systolic BP $\geq$130 mmHg and/or diastolic BP $\geq$85 mmHg, or use of antihypertensive therapy), and hyperglycemia (fasting plasma glucose $\geq$5.55 mmol/l) or previously diagnosed T2DM.

Serum samples were collected for laboratory testing after a 12-h fast. Glucose levels were determined using the glucose hexokinase method (Advia 1800 analyzer, Siemens Healthcare, Munich, Germany); glycated hemoglobin (A1c), by an ion-

exchange HPLC procedure (Merck-Hitachi L-9100 analyzer, Darmstadt, Germany; reference range: 28 – 42 mmol/mol (4.7 – 6.0%); and total plasma cholesterol, HDL, and triglycerides, by enzymatic colorimetric methods (Advia 1800 analyzer, Siemens Healthcare, Munich, Germany). Low-density lipoprotein cholesterol (LDL) cholesterol was calculated using the Friedewald equation.

Genetic analyses

Polymorphism analyses

DNA was extracted from peripheral blood leukocytes by a standardized salting-out procedure. Both *HSD11B1* rs4548729883:delA>insA (83,557delA/insA) and *H6PD* rs6688832:G>A (R453Q) polymorphisms were determined using primers and probes contained in the Human Custom TaqMan Genotyping Assay 40x (Assays-By-Design Service, Life Technologies, Foster City, CA, USA). Primer and probe sequences used for genotyping the *HSD11B1* rs4548729883:delA>insA and *H6PD* rs6688832:G>A polymorphisms were: *HSD11B1*-5'-CTTACCTCCTCCTCTGAACTTTGC-3' (forward primer), *HSD11B1*-5'-TCCTCCTGCAAGAGATGGCTATATT-3' (reverse primer), *HSD11B1*-FAM-5'-CACCAAGAGCTTTT-3', *HSD11B1*-VIC-5'-CACCAAAGAGCTTTT-3', *H6PD*-5'-TCTGTCCGATTACTACGCCTACA-3' (forward primer), *H6PD*-5'-GGCCATGGAAGATATGGGATAAGAG-3' (reverse primer), *H6PD*-FAM-5'-CTGTGCGGGAGCG-3', *H6PD*-VIC-5'-CCTGTGCAGGAGCG-3'.

Reactions were conducted in 96-well plates, in a total reaction volume of 5 μl, using 2 ng of genomic DNA, TaqMan Genotyping Master Mix 1x (Life Technologies, Foster City, CA, USA), and Custom TaqMan Genotyping Assay 1x. Plates were then placed in a real-time PCR thermal cycler (7500 Fast Real PCR System; Life Technologies, Foster City, CA, USA) and heated for 10 minutes at 95°C, followed by 50 cycles of 95°C for 15 seconds and 62°C for 1 minute. Fluorescence data files from each plate were analyzed using automated allele-calling software (SDS 2.1; Life Technologies, Foster City, CA, USA). A total of 1006 individuals were recruited for this study. From this, 998 were genotyped for the *HSD11B1* rs4548729883:delA>insA and 924 for the *H6PD* rs6688832:G>A. The genotyping

success rate was 99.2% for *HSD11B1* rs4548729883:delA>insA and 91.8% for *H6PD* rs6688832:G>A with a calculated error rate based on PCR duplicates of 0%. The consensus rate was 91.1%. All amplification reactions were performed twice.

Gene Expression study

RNA was extracted from adipose tissue using Trizol$^{®}$ reagent (Invitrogen; Life Technologies, Foster City, CA, USA) following the manufacturer's protocol. Following RNA extraction, concentration and quality of the product obtained was tested using the NanoDrop 2000 Spectrophotometer (Thermo Scientific Inc., Newark, USA). Only samples with suitable purity ratios (A260/A280 = 1.9 - 2.1) were used in subsequent analyses.

Real-Time reverse transcription PCR (RT-PCR) was performed in two separate steps: first, RNA was reverse transcribed into cDNA. Second, cDNA was amplified by quantitative RT-PCR (RT-qPCR). In this procedure, 1µg of RNA was reverse transcribed to cDNA using the SuperScript$^{®}$ III First-Strand Synthesis System for RT-PCR (Invitrogen; Life Technologies, Foster City, CA, USA) following the manufacturer's protocol for the oligo (dT)$_{12-18}$ method . RT-qPCR experiments were performed in a 7500 Fast Real-Time PCR System Thermal Cycler with 7500 FAST Sequence Detection System Software (Life Technologies, Foster City, CA, USA) monitoring the increase in fluorescence of the SYBR$^{®}$ green dye [24]. Primers for *HSD11B1* (target gene) and *β-actin* (reference gene) were designed using the Primer Express 3.0 software (Life Technologies): *HSD11B1*: 5'-GCTGCCTTGCCCATGCT-3' (forward primer), 5'-CAGCCAGAGAGGAGACGACAA-3' (reverse primer); *β-actin*: 5' - GCGCGGCTACAGCTTCA - 3' (forward primer), 5' - CTTAATGTCACGCACGATTTCC - 3' (reverse primer). RT-qPCR reactions were performed using 10µL 2X Fast SYBR$^{®}$ Green Master Mix (Life Technologies, Foster City, CA, USA), 1µL (1ng/µL) of primers for *HSD11B1* or *β-actin*, 1µL (1µg/µL) of cDNA, in a total volume of 20µL. Each sample was analyzed in triplicate and a negative control was added in each experiment. The reaction conditions were: initial cycle of 20 seconds at 95°C, followed by 50 cycles at 95°C for 3 seconds and 60°C for one minute. RT-qPCR specificity was determined using melting curve analyses

and all primers generated amplicons that produced a single sharp peak during the analyses.

The measurement of *HSD11B1* expression was performed by relative quantification using the comparative ΔΔCq method [25; 26] and expressed relative to the reference gene (*β-actin*). Validation tests were carried out by amplification of the target gene (*HSD11B1*) and the reference gene (*β-actin*), using serial dilutions of cDNA samples. Target and reference gene amplifications showed similar efficiencies in all experiments (E = 95% to 105%) allowing the use of this method. The ΔΔCq method calculates changes in gene expression as relative fold changes (n-fold change) between the experimental sample and an external calibrator [25; 26].

Statistical analyses

Results are expressed as means and standard deviations (SD), percentages, or median (interquartile range). Allele frequencies were determined by gene counting and departures from the Hardy–Weinberg equilibrium were verified using the chi-square test (χ^2). Gene expression and clinical and laboratory characteristics were compared using analysis of variance (ANOVA), the unpaired Student's *t* test, Pearson's correlation or χ^2 as appropriate. The relationship of *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms with different MetS-related components were tested by general linear model univariate analyses, adjusting for covariates.

Using the digenic triallelic mode of inheritance proposed by Draper *et al.* [19; 27] we analyzed the combined effect of at least three minor alleles of the *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms in modulating different MetS-related characteristics: systolic and diastolic BP, BMI, WC, HDL, and triglyceride levels. Multiple logistic regression analysis was performed with the presence of obesity as the dependent variable and age, gender, and presence of at least three minor alleles of the two analyzed polymorphisms as independent variables.

Variables with skewed distribution, such as triglycerides, were log-transformed before analyses. All analyses were performed in the SPSS 18.0 software environment (SPSS Inc., Chicago, USA). P < 0.05 was considered significant.

Bonferroni corrections were used to account for multiple comparisons carried out on the *HSD11B1* and *H6PD* polymorphisms.

Results

Relationship between polymorphisms and MetS-related parameters

The 1006 T2DM included in the study were 58.4 ± 10.2 years old (males = 43.0%), with a known T2DM duration of 11.6 ± 8.6 years. The BMI was 29.1 ± 5.2 kg/m^2 (obesity = 40.3%), 63.6% had hypertension, and 76,3% had MetS. The A1c was 55 mmol/mol (7.2%).

Of the 998 individuals genotyped for the *HSD11B1* rs45487298:delA>insA polymorphism, 654 (65.5%) individuals were homozygous for the delA allele (delA/delA), 311 (31.2%) were heterozygous (delA/insA), and 33 (3.3%) were homozygous for the insA allele (insA/insA). Genotypes were in agreement with those predicted by Hardy–Weinberg equilibrium (P > 0.05). The insA allele frequency was 0.189. Table 1 summarizes the clinical and laboratory data of the individuals grouped according to the *HSD11B1* rs45487298:delA>insA polymorphism. Systolic and diastolic BP, BMI, WC, total cholesterol, HDL, LDL, and triglyceride levels were not significantly different among the three genotypes. It bears mentioning that none of these variables exhibited significant differences when assuming dominant (insA/insA + insA/delA *vs*. delA/delA) or recessive (insA/insA *vs*. insA/delA + delA/delA) models of inheritance for the insA allele (data not shown).

Four-hundred and sixty-four (50.2%) individuals had the *H6PD* G/G genotype, 377 (40.8%) the A/G genotype, and 83 (9.0%) the A/A genotype of the rs6688832:G>A polymorphism. All genotypes were in Hardy–Weinberg equilibrium (P > 0.05), and the A allele frequency was 0.294. Table 2 illustrates the clinical and laboratory data of the individuals grouped according to the *H6PD* rs6688832:G>A polymorphism. This polymorphism had no statistically significant relationship with the MetS-related characteristics listed in Table 2. Furthermore, none of these variables exhibited significant differences when assuming dominant (A/A + G/A *vs*. G/G) or recessive (A/A *vs*. G/A + A/A) models of inheritance for the A allele (data not shown).

Clinical and laboratory characteristics of T2DM individuals grouped according to the presence of at least three minor alleles of *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms are shown in Table 3. Taking into consideration a Bonferroni threshold of 0.0055 (P = 0.05 divided by 9 MetS-related

features analyzed in Table 3), we observed that BMI was significantly lower in carriers of at least three minor alleles of the *HSD11B1* and *H6PD* polymorphisms as compared to individuals with fewer than three minor alleles, adjusting for age and gender (P = 0.005). No significant gene–gene interaction was observed between the *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms in modulating other MetS-related features (Table 3).

As expected, the frequency of obesity was lower among individuals carrying at least three minor alleles of *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms than in the group of individuals with fewer than three minor alleles (24.4% *vs.* 41.6%, respectively; P = 0.033). Logistic regression analysis, with adjustment for age and gender, confirmed that the presence of at least three minor alleles of both polymorphisms is an independent protection factor against obesity (OR = 0.43, 95% CI 0.21 – 0.87, P = 0.019).

None of the analyzed variables exhibited significant differences among individuals carrying at least two minor alleles of *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms and individuals with fewer than two minor alleles (data not shown). Furthermore, taking into account that only three individuals had four rs45487298:delA>insA and rs6688832:G>A minor alleles, it was not possible to evaluate if the protection to obesity could increase on those individuals carrying all minor alleles of the two genes.

Relationship between *HSD11B1* gene expression in abdominal subcutaneous and visceral adipose tissue with anthropometric parameters

HSD11B1 abdominal SAT and VAT gene expressions were not different in those with or without obesity or MetS (Table 4). While *HSD11B1* SAT expression was not related with BMI, waist circumference, and waist-to-height ratio, VAT expression was inversely related to all of these parameters, while adjusting for age and gender.

In order to better understand these relationships, we stratified the sample by body size and MetS presence. *HSD11B1* VAT but not SAT expression was inversely related to BMI, WC and WHtR in participants with BMI ≥ 30kg/m² whereas both VAT and SAT expression were not related to these parameters in those with BMI < 30kg/m². When stratifying the study sample by the presence of MetS, *HSD11B1* VAT expression was inversely correlated to all these anthropometric parameters, being

statistically significant only for WC and WHtR in the MetS+ group. SAT expression was positively correlated to BMI, WC and WHtR in those without the MetS and inversely related to these parameters in those with the MetS, although these relationships did not reach statistical significance, probably because of lack of power to detect a statistically difference between groups (Table 5).

Discussion

In this study, we investigated the association of the *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms with MetS-related characteristics in white participants with T2DM. When analyzed independently, neither of these two polymorphisms was associated with any feature of MetS. However, when analyzing the rs45487298:delA>insA and rs6688832:G>A polymorphisms in combination, we observed a significant gene–gene interaction modulating the risk of obesity in T2DM individuals carrying at least three minor alleles of the two polymorphisms.

There is compelling biochemical evidence of cooperativity between H6PD and HSD11B1 enzymes within the lumen of the endoplasmic reticulum [2; 28]. Increasing or decreasing H6PD levels in cultured cells have corresponding effects on HSD11B1 activity [29]. In addition, Lavery *et al.* [30] showed that *H6PD* knockout mice have a profound switch in HSD11B1 activity from oxoreductase to dehydrogenase, increasing corticosterone clearance and resulting in a reduction in circulating corticosterone levels. This demonstrates a critical requirement of H6PD for HSD11B1 oxoreductase activity.

The effect of this interaction has been previously associated with risk of cortisone reductase deficiency [19]. As suggested by Draper *et al.* [19], this interaction might occur because both rs45487298:delA>insA and rs6688832:G>A polymorphisms seem to have functional effects. The rs45487298:delA>insA polymorphism is located in an enhancer region of intron 3 of the *HSD11B1* gene. It has been associated with decreased *HSD11B1* expression *in vivo* and after transfection of minigene constructs in cultured cells [19]. Draper *et al.* [19] also reported that in cell cultures the rs6688832:G>A polymorphism in the *H6PD* gene decreased the enzyme activity to less than 50% of normal, impairing the generation of reduced NADPH and, consequently, reducing HSD11B1 activity. Nevertheless, Lavery *et al.* [31] have not found a reduction in H6PD enzyme activity by the rs6688832:G>A variant.

Previous studies have suggested an association between different *HSD11B1* gene polymorphisms and MetS features [14; 15; 16; 17; 18]. The rs45487298:delA>insA polymorphism was associated with higher BMI, altered body composition and insulin resistance in obese U.S. children [17]. Nair *et al.* [14] reported an association between the rs846910:G>A and rs12086634:A>G polymorphisms in the *HSD11B1* gene and

risk of T2DM in Pima Indians. Nair *et al.* [14] also showed that, among participants with normal glucose tolerance, both rs846910:G>A and rs12086634:A>G polymorphisms were associated with insulin-mediated glucose uptake, and that the rs846910:G>A polymorphism was further associated with plasma insulin levels. On the other hand, they were unable to find any association between the rs45487298:delA>insA polymorphism and T2DM or other analyzed characteristic [14]. Recently, Moon *et al.* [18] reported that *HSD11B1* rs12086634:A>G and rs1000283:C>T polymorphisms were associated with MetS in T2DM individuals, while the *H6PD* rs17368528:C>T polymorphism was a risk factor for MetS in non-diabetic South Koreans.

In contrast, other studies showed no association between *HSD11B1* gene polymorphisms and body composition, glucose metabolism or MetS [32; 33; 34; 35; 36; 37]. White *et al.* [33] did not find any association between *HSD11B1* rs12086634:T>G and *H6PD* rs6688832:G>A polymorphisms (either separately or in combination) and BMI, waist-to-hip ratio, visceral adiposity, measures of insulin sensitivity or risk of polycystic ovary syndrome (PCOS) in a large population-based sample of 3551 participants from the Dallas Heart Study. Smit *et al.* [34] were not able to detect any influence of the *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms, separately or when using three or more affected alleles, on body composition, adrenal androgen production, blood pressure or glucose levels in a population-based, prospective cohort study of participants aged 55 and older. Furthermore, Draper *et al.* [27] showed no association of the *HSD11B1* rs12086634 variant (which is in complete linkage disequilibrium with the rs45487298:delA>insA variant) and *H6PD* rs6688832 variant with susceptibility to PCOS, BMI, waist-to-hip ratio or total testosterone in a UK case sample comprising 213 women with PCOS and 549 controls.

The inconsistent results reported by the studies cited herein may be at least partly explained by differences in study designs, sample sizes, ethnicity, and *HSD11B1* polymorphisms analyzed. Moreover, a number of studies that only analyzed *HSD11B1* gene polymorphisms may not have observed their association with MetS-related characteristics if this association occurs only in the presence of certain *H6PD* polymorphisms. There might also be additional polymorphisms in these two genes that were not identified in previous studies, but could have major effects on *HSD11B1* gene expression or enzyme activity. Finally, some *HSD11B1* and

H6PD polymorphisms might cause obesity and other features of MetS only in the presence of polymorphisms in a third *locus* yet to be identified.

Despite inconsistent results regarding associations between *HSD11B1* gene polymorphisms and MetS-related characteristics, a compelling evidence base argues for HSD11B1 as a major etiological factor in obesity and related features [2; 9; 10]. In addition, modulation of HSD11B1 activity has also an effect on multiple target tissues which promote insulin resistance independently of obesity [14; 38]. For example, in lean glucose-intolerant individuals, adipose HSD11B1 activity is not increased and hepatic HSD11B1 activity is maintained [39] compared with the downregulation of hepatic HSD11B1 that occurs in obesity [40]. Inhibition of HSD11B1 with oral carbenoxolone enhances hepatic insulin sensitivity [41], and has a greater effect in non-obese glucose-intolerant participants than in healthy people [42]. Moreover, the expression of HSD11B1 in myoblast cultures stimulated with glucocorticoids was negatively correlated with insulin sensitivity [43]. Consequently, HSD11B1 is a promising target for pharmacological inhibition in individuals with T2DM and/or MetS [5; 10; 44].

In this context, overexpression of the *HSD11B1* gene might determine an increase in cortisol generation and, secondarily, generate pro-obesity effects. Therefore, we hypothesize that the interaction between *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms may generate a significant decrease in HSD11B1 levels in adipocytes and other tissues; and consequently, this may decrease the risk of obesity in individuals with T2DM who carry at least three minor alleles of these polymorphisms. However, taking into account the controversial functional studies regarding the *H6PD* rs6688832:G>A effect on H6PD activity [19; 31], it seems possible that this polymorphism might be only a neutral polymorphic variant in linkage disequilibrium with an unknown causative mutation to be found elsewhere in the *H6PD* gene. In addition, in view of that some studies are suggestive of differences in regulation of glucocorticoid activity in individuals with and without impaired glucose tolerance [9; 12; 45], the present data should be interpreted with caution when translated to non-glucose-tolerant obese participants.

Data of *HSD11B1* gene expression and its association with anthropometric parameters are conflicting due to the large difference between the populations studied. Studies performed in participants without MetS have shown that *HSD11B1* abdominal adipose tissue expression is positively related with BMI and

measurements of central adiposity such as waist circumference [46; 47] whereas *HSD11B1* gene expression was not related or inversed related to anthropometric parameters in participants with MetS [11; 12].

While we found no relationships of *HSD11B1* gene expression in abdominal SAT with measurements of adiposity distribution, namely BMI, WC and WHtR, the expression of this gene in abdominal visceral adipose tissue was inversely and significantly related to these parameters in our study sample. When stratifying the sample by the presence of obesity, in both groups we have found an inverse relationship between abdominal adipose tissue *HSD11B1* gene expression and BMI, but this relationship remained significant only in those with obesity. The same findings where observed when the sample was stratified by the presence of MetS.

The results of this study suggest that *HSD11B1* abdominal adipose tissue expression in participants with obesity and/or MetS decreases in order to compensate increased HSD11B1 activity. Although we did not tested this hypothesis, we believe this may explain in part why T2DM participants carrying at least three minor alleles of *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A exhibit lower BMI than those carrying fewer than three minor alleles.

In conclusion, we have shown that the *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms might interact in protecting against obesity in T2DM individuals. Further research is required to provide functional analyses of the effects of these polymorphisms on the pathogenesis of obesity and MetS, and to confirm this result in other populations. Since HSD11B1 inhibition is now recognized as a promising pathway for pharmacological treatment of obesity, T2DM and MetS, our results might have an importance in future pharmacogenetics studies regarding the clinical testing of genetic variations that could give rise to different responses to HSD11B1 inhibitors.

References

1   STIMSON, R. H.WALKER, B. R. Glucocorticoids and 11beta-hydroxysteroid dehydrogenase type 1 in obesity and the metabolic syndrome. **Minerva Endocrinol,** v. 32, n. 3, p. 141-59, Sep 2007.

2   MORTON, N. M. Obesity and corticosteroids: 11beta-hydroxysteroid type 1 as a cause and therapeutic target in metabolic disease. **Mol Cell Endocrinol,** v. 316, n. 2, p. 154-64, Mar 25 2010.

3   COOPER, M. S.STEWART, P. M. 11Beta-hydroxysteroid dehydrogenase type 1 and its role in the hypothalamus-pituitary-adrenal axis, metabolic syndrome, and inflammation. **J Clin Endocrinol Metab,** v. 94, n. 12, p. 4645-54, Dec 2009.

4   TOMLINSON, J. W. et al. 11beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. **Endocr Rev,** v. 25, n. 5, p. 831-66, Oct 2004.

5   HOLLIS, G.HUBER, R. 11beta-Hydroxysteroid dehydrogenase type 1 inhibition in type 2 diabetes mellitus. **Diabetes Obes Metab,** v. 13, n. 1, p. 1-6, Jan 2011.

6   MASUZAKI, H. et al. A transgenic model of visceral obesity and the metabolic syndrome. **Science,** v. 294, n. 5549, p. 2166-70, Dec 7 2001.

7   KOTELEVTSEV, Y. et al. 11beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. **Proc Natl Acad Sci U S A,** v. 94, n. 26, p. 14924-9, Dec 23 1997.

8   ALBERTS, P. et al. Selective inhibition of 11 beta-hydroxysteroid dehydrogenase type 1 improves hepatic insulin sensitivity in hyperglycemic mice strains. **Endocrinology,** v. 144, n. 11, p. 4755-62, Nov 2003.

9   TOMLINSON, J. W.STEWART, P. M. Mechanisms of disease: Selective inhibition of 11beta-hydroxysteroid dehydrogenase type 1 as a novel treatment for the metabolic syndrome. **Nat Clin Pract Endocrinol Metab,** v. 1, n. 2, p. 92-9, Dec 2005.

10   STAAB, C. A.MASER, E. 11beta-Hydroxysteroid dehydrogenase type 1 is an important regulator at the interface of obesity and inflammation. **J Steroid Biochem Mol Biol,** v. 119, n. 1-2, p. 56-72, Mar 2010.

11   TOMLINSON, J. W. et al. Expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue is not increased in human obesity. **J Clin Endocrinol Metab,** v. 87, n. 12, p. 5630-5, Dec 2002.

12   KARLSSON, C. et al. Differences in associations between HSD11B1 gene expression and metabolic parameters in subjects with and without impaired glucose homeostasis. **Diabetes Res Clin Pract,** v. 88, n. 3, p. 252-8, Jun 2010.

13   LINDSAY, R. S. et al. Subcutaneous adipose 11 beta-hydroxysteroid dehydrogenase type 1 activity and messenger ribonucleic acid levels are associated with adiposity and insulinemia in Pima Indians and Caucasians. **J Clin Endocrinol Metab,** v. 88, n. 6, p. 2738-44, Jun 2003.

14   NAIR, S. et al. 11beta-Hydroxysteroid dehydrogenase Type 1: genetic polymorphisms are associated with Type 2 diabetes in Pima Indians independently of obesity and expression in adipocyte and muscle. **Diabetologia,** v. 47, n. 6, p. 1088-95, Jun 2004.

15   FRANKS, P. W. et al. Interaction between an 11betaHSD1 gene variant and birth era modifies the risk of hypertension in Pima Indians. **Hypertension,** v. 44, n. 5, p. 681-8, Nov 2004.

16   MORALES, M. A. et al. [Possible pathogenetic role of 11 beta-hydroxysteroid dehydrogenase type 1 (11betaHSD1) gene polymorphisms in arterial hypertension]. **Rev Med Chil,** v. 136, n. 6, p. 701-10, Jun 2008.

17   GELERNTER-YANIV, L. et al. Associations between a polymorphism in the 11 beta hydroxysteroid dehydrogenase type I gene and body composition. **Int J Obes Relat Metab Disord,** v. 27, n. 8, p. 983-6, Aug 2003.

18   MOON, S. S. et al. Relationship of 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase gene polymorphisms with metabolic syndrome and type 2 diabetes. **Endocr J**, Aug 23 2011.

19   DRAPER, N. et al. Mutations in the genes encoding 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. **Nat Genet,** v. 34, n. 4, p. 434-9, Aug 2003.

20   CANANI, L. et al. The fatty acid-binding protein-2 A54T polymorphism is associated with renal disease in patients with type 2 diabetes. **Diabetes,** v. 54, n. 11, p. 3326-30, Nov 2005.

21   AMERICAN DIABETES, A. Diagnosis and classification of diabetes mellitus. **Diabetes Care,** v. 33 Suppl 1, p. S62-9, Jan 2010.

22   YAO-BORENGASSER, A. et al. Retinol binding protein 4 expression in humans: relationship to insulin resistance, inflammation, and response to pioglitazone. **J Clin Endocrinol Metab,** v. 92, n. 7, p. 2590-7, Jul 2007.

23   GRUNDY, S. M. et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood

Institute Scientific Statement. **Circulation,** v. 112, n. 17, p. 2735-52, Oct 25 2005.

24   HIGUCHI, R. et al. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. **Biotechnology (N Y),** v. 11, n. 9, p. 1026-30, Sep 1993.

25   BUSTIN, S. A. et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. **Clin Chem,** v. 55, n. 4, p. 611-22, Apr 2009.

26   LIVAK, K. J.SCHMITTGEN, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. **Methods,** v. 25, n. 4, p. 402-8, Dec 2001.

27   DRAPER, N. et al. Variants implicated in cortisone reductase deficiency do not contribute to susceptibility to common forms of polycystic ovary syndrome. **Clin Endocrinol (Oxf),** v. 65, n. 1, p. 64-70, Jul 2006.

28   BANHEGYI, G. et al. Cooperativity between 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase in the lumen of the endoplasmic reticulum. **J Biol Chem,** v. 279, n. 26, p. 27017-21, Jun 25 2004.

29   ATANASOV, A. G. et al. Hexose-6-phosphate dehydrogenase determines the reaction direction of 11beta-hydroxysteroid dehydrogenase type 1 as an oxoreductase. **FEBS Lett,** v. 571, n. 1-3, p. 129-33, Jul 30 2004.

30   LAVERY, G. G. et al. Hexose-6-phosphate dehydrogenase knock-out mice lack 11 beta-hydroxysteroid dehydrogenase type 1-mediated glucocorticoid generation. **J Biol Chem,** v. 281, n. 10, p. 6546-51, Mar 10 2006.

31   LAVERY, G. G. et al. Steroid biomarkers and genetic studies reveal inactivating mutations in hexose-6-phosphate dehydrogenase in patients with cortisone reductase deficiency. **J Clin Endocrinol Metab,** v. 93, n. 10, p. 3827-32, Oct 2008.

32   MIYAMOTO, Y. et al. Association study of 11beta-hydroxysteroid dehydrogenase type 1 gene polymorphisms and metabolic syndrome in urban Japanese cohort. **Diabetes Res Clin Pract,** v. 85, n. 2, p. 132-8, Aug 2009.

33   WHITE, P. C. Genotypes at 11beta-hydroxysteroid dehydrogenase type 11B1 and hexose-6-phosphate dehydrogenase loci are not risk factors for apparent cortisone reductase deficiency in a large population-based sample. **J Clin Endocrinol Metab,** v. 90, n. 10, p. 5880-3, Oct 2005.

34   SMIT, P. et al. Lack of Association of the 11beta-hydroxysteroid dehydrogenase type 1 gene 83,557insA and hexose-6-phosphate dehydrogenase gene R453Q polymorphisms with body composition, adrenal

androgen production, blood pressure, glucose metabolism, and dementia. **J Clin Endocrinol Metab,** v. 92, n. 1, p. 359-62, Jan 2007.

35   DRAPER, N. et al. Association studies between microsatellite markers within the gene encoding human 11beta-hydroxysteroid dehydrogenase type 1 and body mass index, waist to hip ratio, and glucocorticoid metabolism. **J Clin Endocrinol Metab,** v. 87, n. 11, p. 4984-90, Nov 2002.

36   ROBITAILLE, J. et al. Molecular screening of the 11beta-HSD1 gene in men characterized by the metabolic syndrome. **Obes Res,** v. 12, n. 10, p. 1570-5, Oct 2004.

37   KU, Y. H. et al. Regulatory effect of common promoter polymorphisms on the expression of the 11beta-hydroxysteroid dehydrogenase type 1 gene. **Horm Res,** v. 72, n. 1, p. 25-32, 2009.

38   STIMSON, R. H. et al. Cortisol release from adipose tissue by 11beta-hydroxysteroid dehydrogenase type 1 in humans. **Diabetes,** v. 58, n. 1, p. 46-53, Jan 2009.

39   ANDREWS, R. C. et al. Abnormal cortisol metabolism and tissue sensitivity to cortisol in patients with glucose intolerance. **J Clin Endocrinol Metab,** v. 87, n. 12, p. 5587-93, Dec 2002.

40   STEWART, P. M. et al. Cortisol metabolism in human obesity: impaired cortisone-->cortisol conversion in subjects with central adiposity. **J Clin Endocrinol Metab,** v. 84, n. 3, p. 1022-7, Mar 1999.

41   WALKER, B. R. et al. Carbenoxolone increases hepatic insulin sensitivity in man: a novel role for 11-oxosteroid reductase in enhancing glucocorticoid receptor activation. **J Clin Endocrinol Metab,** v. 80, n. 11, p. 3155-9, Nov 1995.

42   ANDREWS, R. C.; ROOYACKERS, O.WALKER, B. R. Effects of the 11 beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone on insulin sensitivity in men with type 2 diabetes. **J Clin Endocrinol Metab,** v. 88, n. 1, p. 285-91, Jan 2003.

43   WHORWOOD, C. B. et al. Increased glucocorticoid receptor expression in human skeletal muscle cells may contribute to the pathogenesis of the metabolic syndrome. **Diabetes,** v. 51, n. 4, p. 1066-75, Apr 2002.

44   DAVANI, B. et al. Type 1 11beta -hydroxysteroid dehydrogenase mediates glucocorticoid activation and insulin release in pancreatic islets. **J Biol Chem,** v. 275, n. 45, p. 34841-4, Nov 10 2000.

45   JANG, C. et al. Altered activity of 11beta-hydroxysteroid dehydrogenase types 1 and 2 in skeletal muscle confers metabolic protection in subjects with type 2 diabetes. **J Clin Endocrinol Metab,** v. 92, n. 8, p. 3314-20, Aug 2007.

46   PAULSEN, S. K. et al. 11Beta-HSD type 1 expression in human adipose tissue: impact of gender, obesity, and fat localization. **Obesity (Silver Spring),** v. 15, n. 8, p. 1954-60, Aug 2007.

47   MICHAILIDOU, Z. et al. Omental 11beta-hydroxysteroid dehydrogenase 1 correlates with fat cell size independently of obesity. **Obesity (Silver Spring),** v. 15, n. 5, p. 1155-63, May 2007.

Tables

Table 1: Clinical and laboratory characteristics of T2DM patients according to genotypes of the HSD11B1 rs45487298:delA>insA polymorphism.

	HSD11B1 rs45487298:delA>insA polymorphism			F / P*	P†
	DelA/DelA (n = 654)	DelA/InsA (n = 311)	InsA/InsA (n = 33)		
Age (years)	58.5 ± 10.6	58.2 ± 10.0	57.3 ± 8.9	-	0.642
Males (%)	42.8	43.4	54.5	-	0.415
T2DM duration (years)	10.0 (1-48)	10.0 (1-51)	12.5 (1-46)	-	0.330
Glycated hemoglobin (%)	7.2 ± 2.1	7.4 ± 2.1	7.3 ± 2.3	-	0.909
Systolic BP (mmHg)[a]	137.7 ± 22.6	133.1 ± 20.2	131.0 ± 13.8	2.952 / 0.053	-
Diastolic BP (mmHg)[a]	84.7 ± 12.2	83.3 ± 12.8	83.0 ± 7.6	0.591 / 0.554	-
Body mass index (kg/m^2)[b]	29.2 ± 5.0	29.3 ± 5.6	28.4 ± 4.9	0.250 / 0.778	-
Waist circumference (cm)[b]	98.7 ± 12.3	98.7 ± 11.6	100.8 ± 14.4	0.141 / 0.868	-
Total cholesterol (mmol/L)[b]	5.4 ± 1.2	5.4 ± 1.3	5.0 ± 1.4	0.682 / 0.384	-
HDL cholesterol (mmol/L)[c]	1.2 ± 0.3	1.3 ± 0.3	1.1 ± 0.5	2.488 / 0.116	-
LDL cholesterol (mmol/L)[c]	3.5 ± 1.3	3.4 ± 1.3	2.9 ± 0.6	1.889 / 0.155	-
Triglycerides (mmol/L)[c]	1.6 (0.3 – 16.6)	1.7 (0.3 – 8.8)	2.6 (0.5 – 9.1)	0.280 / 0.756	-
MetS (%)	78.8	73.4	78.6	-	0.232

Data expressed as mean ± SD, median (range), or %. BP: blood pressure; MetS: metabolic syndrome; T2DM: type 2 diabetes mellitus. [*]F and P values obtained from the general linear model univariate analyses, after adjusting for: [a] age and gender, use of medication for hypertension, and BMI; [b] age and gender; [c] age, gender and use of hypolipidemic medications. [†] P values were computed by χ^2 test or ANOVA as appropriate. Only P values lower than the Bonferroni threshold (0.0055) were considered statistically significant.

Table 2: Clinical and laboratory characteristics of T2DM patients according to genotypes of the *H6PD* rs6688832:G>A polymorphism.

	H6PD rs6688832:G>A polymorphism			F / P*	P[†]
	G/G (n = 464)	G/A (n = 377)	A/A (n = 83)		
Age (years)	59.0 ± 10.3	58.0 ± 10.6	56.7 ± 10.4	-	0.125
Males (%)	44.2	44.0	38.6	-	0.621
T2DM duration (years)	11 (1-47)	10 (1-51)	10 (1-48)	-	0.043
Glycated hemoglobin (%)	7.1 ± 2.1	7.2 ± 2.1	7.4 ± 2.3	-	0.196
Systolic BP(mmHg)[a]	135.4 ± 21.3	136.9 ± 22.2	136.0 ± 24.0	1.401 / 0.242	-
Diastolic BP (mmHg)[a]	83.4 ± 11.2	85.0 ± 13.5	84.7 ± 13.0	2.656 / 0.071	-
Body mass index (kg/m^2)[b]	29.1 ± 5.2	29.4 ± 5.4	28.6 ± 5.2	1.647 / 0.193	-
Waist circumference (cm)[b]	98.6 ± 12.6	98.5 ± 11.9	98.7 ± 10.7	0.003 / 0.997	-
Total cholesterol (mmol/L)[c]	5.4 ± 1.3	5.3 ± 1.1	5.7 ± 1.1	1.353 / 0.261	-
HDL cholesterol (mmol/L)[c]	1.2 ± 0.4	1.2 ± 0.3	1.2 ± 0.3	1.339 / 0.264	-
LDL cholesterol (mmol/L)[a]	3.4 ± 1.4	3.4 ± 1.1	3.7 ± 1.0	0.657 / 0.520	-
Triglycerides (mmol/L)[c]	1.7 (0.3 – 16.6)	1.6 (0.3 – 14.0)	1.7 (0.6 – 5.3)	0.414 / 0.661	-
MetS (%)	74.4	76.7	75.4	-	0.790

Data expressed as mean ± SD, median (range), or %. BP: blood pressure; MetS: metabolic syndrome; T2DM: type 2 diabetes mellitus. [*] F and P values obtained from the general linear model univariate analyses, after adjusting for: [a] age and gender, use of medication for hypertension, and BMI; [b] age and gender; [c] age, gender, use of hypolipidemic medications. [†] P values were computed by χ^2 test or ANOVA as appropriate. Only P values lower than the Bonferroni threshold (0.0055) were considered statistically significant.

Table 3: Interaction analyses between *HSD11B1* rs45487298:delA>insA and *H6PD* rs6688832:G>A polymorphisms in T2DM patients.

	rs45487298:delA>insA – rs6688832:G>A interaction			
	< 3 minor alleles (n = 871)	≥ 3 minor alleles (n = 45)	F/ P*	P†
Age (years)	58.6 ± 10.5	54.9 ± 8.9	-	0.021
Males (%)	43.2	51.1	-	0.370
T2DM duration (years)	10 (1 – 51)	10 (1 – 46)	-	0.656
Glycated hemoglobin (%)	7.2 ± 2.1	7.3 ± 2.1	-	0.678
Systolic BP (mmHg)[a]	136.5 ± 22.1	128.8 ± 18.2	0.553 / 0.457	-
Diastolic BP (mmHg)[a]	84.3 ± 12.6	81.7 ± 7.9	0.048 / 0.826	-
Body mass index (kg/m²)[b]	29.3 ± 5.3	27.4 ± 4.9	7.856 / 0.005	-
Waist circumference (cm)[b]	98.7 ± 12.0	97.5 ± 13.5	0.526 / 0.469	-
Total cholesterol (mmol/L)[c]	5.4 ± 1.2	5.3 ± 1.2	0.179 / 0.673	-
HDL cholesterol (mmol/L)[c]	1.2 ± 0.3	1.2 ± 0.4	3.112 / 0.079	-
LDL cholesterol (mmol/L)[c]	3.5 ± 1.2	3.4 ± 1.6	0.084 / 0.775	-
Triglycerides (mmol/L)[c]	1.7 (0.3 – 16.6)	1.4 (0.6 – 9.1)	1.102 / 0.295	-
MetS (%)	75.9	68.9	-	0.398

Data expressed as mean ± SD, median (range), or %. BP: blood pressure; MetS: metabolic syndrome; T2DM: type 2 diabetes mellitus. *F and P values obtained from the general linear model univariate analyses, after adjusting for: [a] age and gender, use of medication for hypertension, and BMI; [b] age and gender; [c] age, gender, use of hypolipidemic medications. † P values were computed by χ^2 test or Student's t-test as appropriate. Only P values lower than the Bonferroni threshold (0.0055) were considered statistically significant.

Table 4: Abdominal *HSD11B1* adipose tissue gene expression stratified by the presence of obesity or MetS.

	SAT (n = 18)	VAT (n = 24)
Non obese	1.04 (0.39 – 6.05)	3.01 (0.39 – 3.74)
Obese	0.85 (0.37 – 9.54)	1.32 (0.63 – 3.47)
MetS (-)	0.9 (0.6 – 10.36)	2.39 (0.69 – 24.73)
MetS (+)	0.97 (0.3 – 5.65)	1.30 (0.39 – 3.25)

Data expressed as median (P25 – P75); SAT: subcutaneous adipose tissue; VAT: visceral adipose tissue; MetS (-): subjects without metabolic syndrome; MetS (+): subjects with metabolic syndrome. Gene expression did not differ among the groups (P>0.05). P values were computed by Student's t-test.

Table 5: Correlations between *HSD11B1* gene expression in adipose tissue and anthropometric parameters in all, non-obese, obese, MetS (-) and MetS (+) subjects.

	SAT						VAT				
	All (n = 18)	Non obese (n = 8)	Obese (n = 10)	MetS (-) (n = 6)	MetS (+) (n = 12)		All (n = 24)	Non obese (n = 11)	Obese (n = 13)	MetS (-) (n = 8)	MetS (+) (n = 16)
BMI	0.077	-0.454	0.482	0.790	-0.332		-0.435	-0.489	-0.585	-0.483	-0.411
	(0.763)	(0.258)	(0.158)	(0.061)	(0.291)		(0.034)[a]	(0.127)	(0.036)[a]	(0.225)	(0.114)
WC	0.178	0.100	0.317	0.744	-0.308		-0.584	-0.478	-0.728	-0.546	-0.597
	(0.494)	(0.813)	(0.406)	(0.090)	(0.356)		(0.003)[b]	(0.137)	(0.007)[b]	(0.161)	(0.019)[a]
WHtR	-0.014	-0.189	0.140	0.797	-0.448		-0.526	-0.122	-0.773	-0.525	-0.539
	(0.956)	(0.653)	(0.719)	(0.057)	(0.167)		(0.010)[b]	(0.721)	(0.003)[b]	(0.181)	(0.038)[a]

Data expressed as Pearson's correlation (P value). SAT: Subcutaneous adipose tissue; VAT: Visceral adipose tissue; BMI: Body Mass Index; WC: Waist circumference; WHtR: waist-to-height ratio; MetS (-): subjects without metabolic syndrome; MetS (+): subjects with metabolic syndrome. [a]: $P<0.05$; [b]: $P≤0.01$.

Conclusões

A compilação dos dados da literatura sugere que a expressão do gene *HSD11B1* em tecido adiposo abdominal relaciona-se ao diabetes melito tipo 2 e obesidade, mas com achados inconclusivos para a síndrome metabólica. Estudos dos polimorfismos do gene *HSD11B1* e sua relação com diferentes anormalidades do metabolismo apresentaram dados inconsistentes relacionados com a obesidade, a síndrome metabólica e o diabetes melito tipo 2.

O estudo do polimorfismo rs45487298:delA>insA do gene *HSD11B1* e do polimorfismo *rs6688832:G>A* do gene H6PD em 1006 pacientes com diabetes melito tipo 2 não demostrou relação com a síndrome metabólica. Entretanto, quando analisados os polimorfismos em conjunto, foi demonstrado que na presença de pelo menos 3 alelos polimórficos, o índice de massa corporal foi significativamente menor do que na presença de dois ou menos alelos polimórficos, sugerindo que a interação desses polimorfismos ou de outros que estejam em desequilíbrio de ligação sejam protetores contra a obesidade. A confirmação desses achados deve ser replicada através de estudos em diferentes populações, assim como os mecanismos moleculares que estão por trás dessa relação.

Ao se estudar a expressão gênica do *HSD11B1* em tecido adiposo abdominal de pacientes com diferentes graus de tolerância à glicose, não se observou relação da obesidade com o tecido adiposo abdominal subcutâneo. Entretanto, a expressão desse gene no tecido adiposo abdominal visceral demonstrou uma relação inversa com obesidade geral e central. Esses resultados sugerem que pacientes obesos podem apresentar uma diminuição da expressão gênica do *HSD11B1* em tecido adiposo visceral a fim de diminuir os níveis de cortisol tecidual como um mecanismo compensatório contra a obesidade.