*Original Research*

# The influence of obstructive sleep apnea on the expression of *glycerol-3-phosphate dehydrogenase1* gene

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## Abstract

*Glycerol-3-phosphate dehydrogenase1* (*GPD1*) is considered to be a key enzyme that connects carbohydrate and lipid metabolism. This gene is induced in response to sleep deprivation, suggesting a potential role for this enzyme in the manifestation of obstructive sleep apnea (OSA). This study aims to examine the effects of sleep apnea, obesity and other relevant clinical parameters on *GPD1* expression in the peripheral blood of a rigorously selected sample population in order to identify a biological marker that would allow for early intervention and prevention of the disorder. Clinical and sleep parameters were assessed by a complete full-night polysomnography and the expression of *GPD1* at the mRNA level was determined. The results were compared among 20 OSA patients and 20 controls, further classified into two subgroups according to their body mass index. The expression levels of the *GPD1* gene did not differ between patients with OSA and their matched controls. The results were not affected by the clinical and biochemical measurements, the sleep parameters or the severity of nocturnal hypoxemia. On the other hand, individuals with OSA had higher levels of fasting glucose when compared with weight-matched controls ($P = 0.01$). Moreover, higher very low-density lipoprotein (VLDL) was found in the over-weight OSA patient group, and higher cholesterol levels were found in the eutrophic OSA group when compared with their respective controls ($P < 0.05$). Based on logistic regression analyses, fasting glucose levels emerged as an independent factor for OSA in both the eutrophic (odds ratio [OR] = 1.27; 95% confidence interval [CI] = 1.00–1.59) and over-weight groups (OR = 1.29; 95% CI = 1.04–1.59). Although the results from the current study corroborate the growing body of data connecting OSA to altered glucose metabolism, it does not provide evidence for the modulation of *GPD1* transcription by either OSA or its related phenotypes. This suggests that *GPD1* may not play a major role in the OSA manifestation.

**Keywords:** sleep apnea, obesity, glycerol-3-phosphate dehydrogenase1, gene expression, *GPD1*, sleep deprivation

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## Introduction

Obstructive sleep apnea (OSA) is an increasingly prevalent condition that is characterized by a recurrent partial or complete collapse of the pharyngeal airway during sleep, which results in repeated arousals and sleep fragmentation.[1] The pathophysiological mechanisms that are involved in OSA are the result of multiple risk factors.[2,3] Obesity, which has been correlated most often with this disorder, likely represents the primary risk factor for the development of OSA.[4] Similarly, there is increasing evidence that indicates a strong genetic component and a high co-morbidity for both the obesity and OSA phenotypes.[5]

Glycerol-3-phosphate dehydrogenase1 (GPD1) is a $NAD^+$-dependent cytosolic enzyme that catalyzes the conversion of dihydroxyacetone phosphate derived from glucose into glycerol-3-phosphate, which is then acylated to form triglycerides.[6] Thus, GPD1 is considered to be a key element that connects carbohydrate and lipid metabolism. As such, GPD1 may influence the manifestation of OSA either by regulating glucose metabolism or by controlling fat disposition. In fact, increased *GPD1* expression and activity have been reported in the adipocytes of obese humans and rats,[7–9] whereas *GPD1*-deficient mice exhibit decreased adiposity and body weight, despite a sufficient food supply.[10] Likewise, studies from our group and others have demonstrated that 24-h[11] or 96-h sleep deprivation[12] up-regulates the expression of the *GPD1* gene in the cerebral cortex and six other areas of the rat brain,[11] which suggests a

potential role for this enzyme not only in metabolism, but also in the response to the consequences of sleep loss. Our group has recently provided additional support for this predicted role through the demonstration that 96-h paradoxical sleep deprivation in rats also results in increased levels of the *GPD1* transcript in the blood.[13] Taken together, these data suggest that alterations in the expression level of *GPD1* in peripheral blood could serve as a useful and easily accessible biomarker that reflects the pathophysiology of the disease.

Since there has been considerable interest in identifying and understanding the role of genetic factors that could potentially be involved in the development of OSA, we decided to examine the effect of sleep apnea, both independently and in association with obesity, on the expression of *GPD1*. In addition, possible interactions between the expression level of the *GPD1* gene and relevant clinical parameters that are related to the manifestation of OSA were evaluated, in an attempt to identify a biological marker that would allow for the early intervention and prevention of the disorder.

## Methods

### Subjects

A subset of individuals was selected from the Sao Paulo Epidemiologic Sleep Study, which was a population-based survey.[14] The study protocol was approved by the local ethics Committee (CEP 0593/06), and informed consent was obtained from each research subject. The exclusion criteria included the following: hypertension, as defined by the current use of hypertensive medication; a systolic blood pressure (SBP) $>140$ mmHg and/or a diastolic blood pressure (DBP) $>90$ mmHg; diabetes, as defined by use of medication for the control of blood glucose and/or a fasting glucose level $\geq 126$ mg/dL; or a number of periodic leg movement events per hour of sleep recording (PLMS/h) $\geq 5$. The inclusion criteria included the following: being male, agreement to donate blood and the availability of high quality RNA for analysis, as well as an apnea–hypopnea index (AHI) $\geq 5$, for the patients. Our study population consisted of a total of 20 patients and 20 controls, who were selected based upon the inclusion and exclusion criteria and further subdivided according to body mass index (BMI) status. The matched controls were selected based upon age and BMI, in order to reduce or eliminate any potential confounding effects of obesity or age-associated factors.

### Polysomnography

A complete, full-night polysomnography (PSG) was performed using the EMBLA® S7000 digital system (Embla Systems, Inc, Medcare Flaga, Reykjavik, Iceland) at the Sleep Institute, São Paulo, Brazil. Sleep parameters were scored according to the criteria established by the American Academy of Sleep Medicine Manual for Scoring Sleep and Associated Events.[15] OSA was defined by an AHI $\geq 5$.

### Physical and biochemical measurements

General physical measurements and fasting blood were taken in the morning for the estimation of the levels of serum glucose, insulin, grelin and leptin as well as for the determination of the lipid profile, using Advia® 1650/2400 and Immulite® 2000 (Siemens Healthcare Diagnostics, Inc, Deerfield, IL, USA). Insulin resistance was assessed from the fasting glucose and insulin values using homeostasis model assessment (HOMA) calculations.

### RNA extraction/realtime PCR

Blood (2.5 mL/tube) was collected in PAXgene vacutainer tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland), and total RNA was isolated following the recommended procedures. Reverse transcription and realtime PCR were performed using the SuperScript III two-step qRT-PCR with Sybr Green kit (Invitrogen, Carlsbad, CA, USA) and primers for the *GPD1* and *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) genes. *GAPDH* was included as the housekeeping gene for control purposes. Independent reactions were performed in duplicate on the Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA).

### Statistical analyses

Comparisons between groups were performed using the Student's *t*-test and the Mann-Whitney test, when appropriate. Correlations between physical and biochemical measurements and the levels of GPD gene expression ($\Delta C_t$) were evaluated using linear regression analysis. To determine the independent risk factors for OSA, all variables that were found to be predictive for the disease by univariate tests with $P < 0.10$ were included in a multivariate forward stepwise logistic regression model, which was based on the likelihood ratio statistic, with a cut-off value at 0.05. Statistical analyses were performed with SPSS for Windows software (version 15.0).

## Results

### Clinical parameters

Forty male individuals, including both patients with an AHI $\geq 5$ ($n = 20$) and matched controls ($n = 20$), were classified according to their BMI as follows: eutrophic, as defined by a BMI $<25$ kg/m$^2$ or over-weight, as defined by a BMI $\geq 25$ kg/m$^2$. The demographic and clinical characteristics of the subjects as well as the results of between-group comparisons are presented in Table 1.

The comparison of the clinical and biochemical parameters of the subjects revealed that individuals with OSA have higher levels of fasting glucose when compared with weight-matched controls ($P = 0.01$ for euthropic controls; $P = 0.002$ for over-weight controls). Moreover, the over-weight OSA group had higher very low-density lipoprotein (VLDL) and HOMA levels ($P = 0.03$ and $P = 0.04$, respectively), and the eutrophic OSA group had higher levels of cholesterol ($P = 0.04$), compared with the respective

**Table 1** Univariate analysis of the demographic and clinical parameters for the two subgroups, eutrophic (BMI $< 25$) and over-weight (BMI $\geq 25$), of both patients (AIH $\geq 5$) and controls

| | Eutrophic | | | Over-weight | | |
|---|---|---|---|---|---|---|
| | Control | OSA | *P* value | Control | OSA | *P* value |
| Number of Individuals | 10 | 10 | | 10 | 10 | |
| Age (y)* | 35.3 (9.98) | 39.1 (12.3) | 0.46 | 32.3 (7.86) | 37.4 (10.12) | 0.22 |
| BMI ($kg/m^2$)* | 22.0 (2.2) | 22.1 (2.0) | 0.92 | 27.9 (2.3) | 30.3 (3.8) | 0.10 |
| AHI† | 0.9 (1) | 11.2 (15.7) | $<0.0001$‡ | 1.1 (1.6) | 29.9 (18.4) | $<0.0001$‡ |
| Systolic blood pressure (mmHg)† | 110.0 (13.5) | 120.0 (11.5) | 0.31 | 120.0 (2.5) | 122.0 (10.5) | 0.19 |
| Diastolic blood pressure (mmHg)† | 70.0 (8.5) | 80.0 (10.5) | 0.28 | 80.0 (17.0) | 80.0 (2.0) | 0.91 |
| Circumference of the neck (cm)† | 36.8 (2.1) | 36.9 (2.7) | 0.91 | 39.5 (3.8) | 40.7 (2.8) | 0.31 |
| Circumference of the waist (cm)* | 78.8 (5.6) | 80.7 (6.0) | 0.47 | 91.9 (6.8) | 97.6 (12.0) | 0.21 |
| Circumference of the hip (cm)* | 91.2 (5.0) | 91.7 (4.1) | 0.83 | 102.3 (4.9) | 107.0 (8.6) | 0.16 |
| Grelin (pg/mL)† | 631.0 (762.5) | 1,009.0 (449.0) | 0.17 | 459.0 (612.0) | 358.5 (579.8) | 0.80 |
| Leptin (ng/mL)† | 1.0 (1.6) | 1.1 (1.1) | 0.63 | 3.5 (6.3) | 5.0 (4.5) | 0.53 |
| Total cholesterol (mg/dL)* | 164.3 (25.6) | 187.4 (21.1) | 0.04‡ | 201.5 (46.7) | 207.5 (35.1) | 0.75 |
| HDL (mg/dL)* | 47.8 (10.2) | 56.5 (8.7) | 0.06 | 52.5 (9.4) | 44.2 (9.8) | 0.07 |
| LDL (mg/dL)* | 99.6 (18.3) | 111.5 (22.9) | 0.22 | 106.1 (51.0) | 130.1 (22.1) | 0.20 |
| VLDL (mg/dL)† | 15.5 (7.3) | 20.0 (14.5) | 0.58 | 18.5 (17.3) | 33.0 (19.5) | 0.03‡ |
| Triglycerides (mg/dL)† | 79.0 (37.5) | 100.0 (75.5) | 0.63 | 112.0 (99.0) | 165.0 (96.8) | 0.19 |
| Fasting glucose (mg/dL)* | 86.5 (4.6) | 94.7 (8.4) | 0.01‡ | 87.1 (7.0) | 98.2 (6.6) | 0.002‡ |
| Fasting insulin (uIU/mL)† | 5.6 (4.2) | 5.8 (4.5) | 0.85 | 9.1 (7.4) | 13.9 (7.4) | 0.17 |
| HOMA IR† | 1.1 (0.9) | 1.4 (1.1) | 0.58 | 2.0 (1.6) | 3.5 (1.9) | 0.04‡ |

AHI, apnea–hypopnea index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; HOMA IR, homeostatic model assessment-insulin resistance

*Mean (SD) and †median (IQR) for normal and skewed data, respectively

‡Compared with weight-matched control

control groups. No significant differences were found for the remaining variables.

The stepwise multivariate logistic model was used to evaluate those variables that were found to be predictive for OSA by univariate tests ($P < 0.10$). Both VLDL and cholesterol were eliminated as risk factors. In contrast, the fasting glucose level was retained as a risk factor for both eutrophic (odds ratio [OR] = 1.27; 95% confidence interval [CI] = 1.00–1.59) and over-weight individuals (OR = 1.29; 95% CI = 1.04–1.59).

Further *post hoc* pair-wise comparisons among the four different groups demonstrated that leptin levels were higher in both over-weight groups in relation to eutrophic individuals either with or without OSA ($P < 0.01$). In addition, waist and hip circumferences measurements were increased in over-weight individuals either with or without OSA, when compared with both eutrophic groups ($P < 0.01$). The neck circumference measurement, VLDL level and triglycerides level were increased in the over-weight group with OSA when compared with eutrophic control individuals ($P < 0.01$). The levels of HDL were lower in over-weight OSA patients when compared with eutrophic OSA patients ($P < 0.01$), and the levels of insulin and HOMA values were significantly higher in over-weight OSA patients when compared with both eutrophic groups ($P < 0.01$).

## GPD gene expression

The expression levels of the GPD gene were compared between the OSA patients and their weight-matched controls, and no significant differences were identified ($P > 0.05$). The lack of significant differences in the GPD levels between different groups was apparent when individuals were compared according to OSA status independent of BMI, according to BMI independent of OSA status or according to OSA severity. The distribution of the values for the expression level of the GPD gene for each of the four groups is shown in Figure 1.

Further analyses demonstrated that the various clinical and biochemical measurements, the sleep parameters and the severity of nocturnal hypoxemia also did not

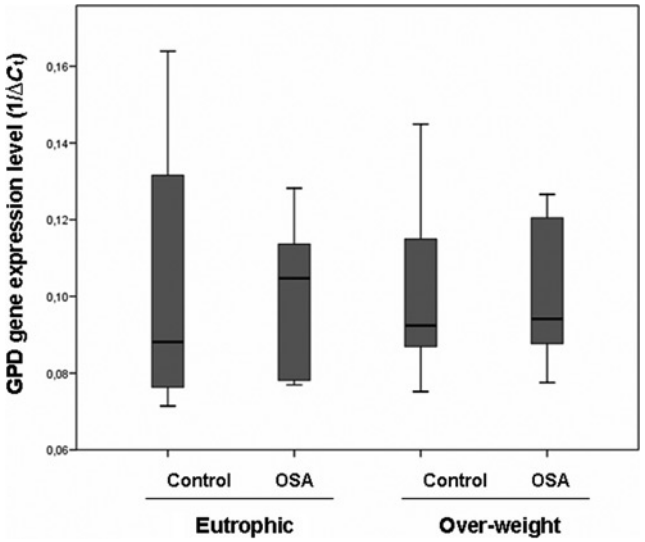


**Figure 1** Boxplot representation of the *GPD1* gene expression values, after normalization to GAPDH, for the different groups: over-weight (control individuals with BMI $\geq$25); eutrophic (control individuals with BMI $<$25); OSA (patients with BMI $<$25 and AHI $\geq$5); over-weight/OSA (patients with BMI $\geq$25 and AHI $\geq$5). All $1/\Delta C_t$ values represent the mean of the duplicates for each sample. *GPD1*, *glycerol-3-phosphate dehydrogenase1*; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; BMI, body mass index; OSA, obstructive sleep apnea; AHI, apnea–hypopnea index

significantly correlate with the level of GPD gene expression in our cohort, as determined by linear regression analysis ($P > 0.05$) (data not shown).

## Discussion

Although several previous studies have reported a significant relationship between OSA and different metabolic features, the existence of a causal link remains controversial given the lack of precise controls for confounding factors. In this study, we demonstrated that, when compared with weight-matched controls, a rigorously selected group of OSA patients did not differ in terms of circumferences of the neck, waist and hip, as well as blood pressure, leptin and ghrelin levels, as previously pointed in other studies.[16–18] As expected, given its strong correlation with absolute fat mass, percentage body fat and BMI,[5,19] leptin levels were increased in over-weight individuals, regardless of OSA status. Likewise, waist and hip circumferences were significantly higher in over-weight individuals with or without OSA. Insulin levels and HOMA values were increased only in over-weight OSA patients. These contradictory findings highlight the difficulties to distinguish between the physiological parameters that connect OSA and obesity in a vicious cycle.[5]

On the other hand, higher levels of VLDL and total cholesterol were observed in the over-weight OSA group and the eutrophic OSA group, respectively. These observations corroborate data from other studies that show a relationship between OSA and altered lipid metabolism.[17,18] Moreover, the results of the present study showed that fasting glucose levels were increased in both groups of OSA patients compared with their respective weight-matched controls and were identified, in a multivariate regression model, as an independent risk factor for OSA in our study. Several studies have shown that individuals with higher levels of fasting plasma glucose are at an increased risk for the development of type 2 diabetes,[20] even in healthy young men who fall within the normoglycemic range.[21] In addition, higher fasting glucose levels have been reported as an independent risk factor for OSA in clinical studies.[22] The multicentre Sleep Heart Health Study, which evaluated 2656 subjects, demonstrated that the AHI and average oxygen saturation during sleep were associated with both elevated fasting and 2-h glucose levels during an oral glucose tolerance test.[23] Thus, our data corroborate the growing body of evidence that indicates that OSA might be a novel risk factor for impaired glucose metabolism[24] and the development of type 2 diabetes.[16] Nevertheless, the exact biological basis of the association between OSA and diabetes remains to be elucidated. OSA is characterized by sleep fragmentation and excessive daytime somnolence,[25] and previous studies have demonstrated an increased risk of obesity and diabetes when sleep time is reduced.[5] In accordance, insulin and glucose treatment have been shown to affect the expression levels of *GDP1* in cells in a dose-dependent manner.[18] In animal models, prolonged periods of sleep deprivation have resulted in the up-regulation of this gene in the brain.[11]

To the best of our knowledge, this study represents the first evaluation of the effect of OSA on *GPD1* gene expression in a cohort that includes patients and control individuals who were matched for both age and BMI. Contrary to our hypothesis, no significant difference was found between patients with OSA and weight-matched controls with respect to *GPD1* gene expression, nor was there any detectable correlation between *GPD1* gene expression levels and AHI, BMI or other physical and biochemical parameters. This lack of significant correlation suggests that *GPD1* transcription is not affected by OSA and, therefore, that this enzyme might not have a significant impact on glucose metabolism or fat disposition, as had been previously hypothesized. One alternative explanation is that the effect of OSA on *GPD1* expression does not involve mRNA and protein synthesis or suppression, but rather a modulation of enzyme activity, as demonstrated in adipose tissue of obese humans.[8] Consequently, the results of this study should be interpreted cautiously, and a role for *GPD1* in sleep apnea/obesity should not be completely discarded. On the other hand, variations in gene expression within human blood have been shown to be related to many factors, including multiple non-disease-related sources.[26] In addition, most genes that are regulated in blood have low fold-changes and high variability,[27] which can be seen in the wide interindividual variation that is observed in our sample population.

In conclusion, chronic sleep apnea and its associated phenotypes had no significant impact on the transcriptional modulation of the *GPD1* gene. Further studies are required in order to identify and characterize specific genes and/or biological pathways that may be differentially regulated in OSA patients, with a specific focus on improving our knowledge of this prevalent condition and identifying alternative clinical approaches for the diagnosis and treatment of OSA.

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