Parameters of glucose metabolism in patients with obstructive sleep apnea

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Diploma thesis

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Foremost, I would like to express my deep sense of gratitude to my mentor Assist. Prof. Joško Božić, MD, PhD, who helped me finish my medical studies offering his continuous advice and encouragement, both as a professor and as a friend, throughout writing my thesis.

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Luka, I thank you with all my heart for your unconditional love proving to me I am a real lucky girl.

This diploma thesis is dedicated to my grandma, Oma Irmi, who taught me to never give up.

1. INTRODUCTION

Disturbances in sleep are among the most frequent health complaints physicians encounter in everyday practice. An occasional night of poor sleep, often in the setting of stress or excitement triggered by life and environment, is both common and without lasting consequences (1). Beside insomnia, sleepiness or tiredness during the day, the most common and serious in terms of morbidity and mortality is obstructive sleep apnea (OSA) (1,2). OSA is a common disorder affecting at least 2-4% of the adult population and is increasingly recognized by broad population (3).

1.1. Sleep-related breathing disorder

There are four major categories of sleep-related breathing disorders: obstructive sleep apneas (OSAS), central sleep apnea (CSA) syndromes, sleep-related hypoventilation disorders, and sleep-related hypoxemia disorder (4).

Sleep apnea is the cessation of breathing during sleep. As a result, the patient suffers from hypoxemia and sleep disruption, consequently leading to excessive daytime sleepiness (5). Repetitive cessations of breathing may be due to either an occlusion of the airway (OSA), absence of respiratory effort (CSA), or a combination of those two.

CSA syndromes include those in which airflow is diminished or absent in an intermittent or cyclical fashion resulting from a reduced or absent respiratory effort. Primary CSA is a disorder of unknown cause characterized by recurrent episodes of cessation of breathing during sleep without associated ventilatory effort (4).

As defined by the most recent version of the American Academy of Sleep Medicine (AASM) scoring manual, sleep-related hypoventilation must be established by demonstration of elevated PCO2 by blood gas or, more commonly, by proxy measures such as end-tidal or transcutaneous CO2, while obesity hypoventilation requires demonstration of daytime hypercapnia. Sleep-related hypoventilation due to an underlying medical condition may result from pulmonary airway or parenchymal disease, such as chronic obstructive pulmonary disease or idiopathic pulmonary fibrosis, as well as extrinsic factors such as chest wall disorder, or neuromuscular disease (4).

1.2. Obstructive sleep apnea

1.2.1. Definition of obstructive sleep apnea

OSA is characterized by repetitive cessation of breathing or partial upper airway obstruction. Related to those events, reduced blood oxygen saturation is present, frequently accompanied by snoring and sleep disruption. As a result, excessive daytime sleepiness or insomnia may occur. Five or more respiratory events (apneas, hypopneas, or respiratory effort-related arousals) per hour of sleep are required for diagnosis (6).

1.2.2. Prevalence of obstructive sleep apnea

OSA is estimated to occur in one to 5% of adult men and is about half as common in women (7,8). Furthermore, up to 5% of adults in Western countries are likely to suffer from undiagnosed OSA (7). Moreover, children may also present with OSA, usually associated with tonsil or adenoid enlargement (9). However, alarmingly the prevalence of OSA shows a raising pattern with aging. Current estimates determined that 10% of men between the age of 30–49-years and even 17% of the 50–70-year-old men suffer from moderate to severe sleep-disordered breathing (10).

1.2.3. Clinical Picture of obstructive sleep apnea

As a consequence of sleep fragmentation, excessive daytime sleepiness is one of the most frequent symptoms of OSA, and one that significantly affects quality of life. Symptoms may be both subtle, such as drowsiness occurring during periods of inactivity, or severe with episodes of falling asleep during activities such as driving (8). Furthermore, impaired vigilance, disrupted cognitive function, difficulties in concentrating, unrefreshing nocturnal sleep, nocturia, and nocturnal chocking may be present as well. Partners often report loud snoring in all lying positions, which may be punctuated by silence of apneas (11). Asymptomatic patients are often recognized and correctly diagnosed after complaining about lack of energy, fatigue and tiredness (11). Importantly, early detection can save lives, especially since recent studies have shown a strong association between OSA and cognitive functions in traffic and motor vehicle accidents (7).

1.2.4. Risk factors for obstructive sleep apnea

OSA is associated with anatomical risk factors mostly by the narrowing effect on the upper airway (8). Obesity, as a major risk factor, may predispose the development of OSA by fat deposition in the region of the upper airway resulting in alterations in airway function, as well as alterations in balance between ventilator drive and load (12). Furthermore, obesity indirectly contributes to airway collapsibility by reducing tracheal caudal traction which promotes reduction of the airway (8). Studies have shown a direct relationship between BMI and apnea severity, a 10% increase in weight was associated with a six-fold increase in risk for development of OSA during a four year follow up period (8,12). Another reason for narrowed airways is nasal congestion or obstruction, possibly caused by allergic rhinitis or anatomic variations of nose structure resulting in a two-fold increase in risk for OSA (13). As for many disorders, smoking and alcohol may promote the development of OSA. While in smoking, apnea is potentiated by airway inflammation, alcohol consumption relaxes the muscles which lead to an increase in upper airway resistance (12).

1.2.5. Diagnostic approach

In order to confirm OSA, one should eliminate other reasons, such as shift work as a major cause of tiredness in patients over the age of 40, as well as depression which may be presented by sleepiness as one of the first sign. Additionally, narcolepsy, idiopathic hypersomnolence and most importantly, adverse effects of certain drugs as potential cause, must be excluded (9).

1.2.5.1. Anamnesis, heteroanamnesis and questionnaires

A careful medical history is essential for making the diagnosis. Firstly, the duration, severity and consistency of the sleeping disorder should be enquired, followed by the estimation of the patient´s subjective opinion about the waking function and daytime activity. Of great value is information gained by a family member or bed partner, since further symptoms and signs can be obtained and recognized. Those include snoring or episodes of inattentive behavior in traffic due to tiredness or inobservance, which may be embarrassing or even unaware to the patient (9).

The Epworth Sleepiness Scale Score (ESS) is not a perfect measure for the detection of a sleep-related disorder (9,14). However, it might help to assess the patient´s health condition. The ESS asks the respondent to rate on a four-point scale (0-3) their usual chances of having dozed off or fallen asleep while engaged in eight different activities that differ widely in their potential vigilance and weariness. Also, it is not based on the person's subjective feelings of alertness or drowsiness at some particular time, nor does it measure the amount and duration of the sleep during the day. The maximum Epworth Score which can be achieved is 24. It is of utter clinical importance to recognize patients with a score greater than 11 indicating excessive sleepiness, and requiring further investigations. Scores between 16-24 determine severe daytime sleepiness and need be followed by immediate clinical examination (15).

Another helpful questionnaire is the Berlin questionnaire. It consists of eleven questions divided into three categories incorporating snoring, daytime somnolence, hypertension and BMI as main predictors of sleep disorder. Patients are scored as being high-risk for obstructive sleep apnea when having a positive score on two or more categories. On contrary, patients who scored positive in only one category are in the low-risk group. Possible difficulties in the use of this type of questionnaire, may be low quality due to imprecision and heterogeneity (16, 17).

Finally, the Snoring, Tiredness, Observed Apnea, and High Blood Pressure (STOP) questionnaire, a concise and easy-to-use screening tool for OSAS with high sensitivity, has been developed originally to evaluate patients in preoperative clinical circumstances. However, it can be used together with the patient´s body mass index, age, neck size, and gender, and consequently be used as a highly sensitive test for individuals with moderate to severe OSA (18). It consists of a 4-item questionnaire which can classify patients as being at high risk of having OSA if they answer yes to two or more questions (14).

1.2.5.2. Physical examination

As previously mentioned, obesity is the most common risk factor for OSA. Hence, measurement of body weight and height, and the resulting determination of Body mass index (BMI) as well as the waist-to-hip ratio, can be one of the first steps in the clinical examination.

According to recent studies, a neck circumference, measured at the superior border of the cricothyroid membrane in the upright position, of at least 40 cm has a sensitivity of 61% and a specificity of 93% for OSA regardless of gender (8).

Anatomical structures, such as jaw structure, the oropharynx and nose, as well as the tongue and tonsils should be assessed to obtain an overview over the upper airway patency (8). Pulmonary function test may be both normal and abnormal, since lung disease neither precludes nor increases the potential of sleep apnea (19).

In addition to airway examination, blood pressure measurements should be made, since patients with obstructive sleep apnea have a higher risk of adverse cardiovascular events. Finally, blood glucose levels and potential diagnosis of diabetes can provide information about the metabolic state, which is commonly associated with apneas and hypopneas due to insulin resistance independent of obesity (9).

1.2.5.3. Assessment by polysomnographic and home-sleep testing

The gold standard for evaluation of sleep and sleep-related breathing is the polysomnography (PSG). PSG consists in its simplest form of an electroencephalogram (EEG), electrooculogram (EOG), and an electromyogram (EMG), providing the basic information needed to classify the sleep stage and its process (19). Apart from the mentioned measurements, further key items which are routinely measured are oxygen saturation, heart function by electrocardiogram, body position and limb movement (20). The only objective measure for evaluating a person´s sleep is to perform PSG and subsequently the examination of brain wave activity. Scoring criteria depend upon EEG bandwidth activity, EEG events, eye movement activity and the level of muscle tone. Consequently, the sleep disorder can be analyzed quantitatively and provide the exact objective severity of the underlying disturbance (19).

Besides PSG, home sleep testing by portable monitoring can be used in some indications, such as patients for whom in-laboratory PSG is impossible due to immobility, safety, or critical illness. Furthermore, portable monitoring is used as an alternative diagnostic test for OSA on the premise of lower costs and faster deployment (21). The recording includes oxygen saturation, airflow, respiratory effort, heart and pulse rate, and body position. It is essential that visual evaluation is performed in order to prevent misclassification of sleep

apnea severity. In addition, patients should not suffer from other significant sleep or comorbid disorders (e.g. heart failure, stroke, diabetes mellitus, severe cardiac arrhythmias), since quality of measured values may not be sufficient and correct. Finally, the main characteristic of home sleep testing is that it cannot differentiate between central and obstructive respiratory events with certainty (22).

1.2.6. Treatment of obstructive sleep apnea

OSA should be approached as a chronic disease requiring long-term, multidisciplinary management in which the patient acts as an active participant in the decision on treatment type and disease regulation (3). Treatment options include, rectifiable behavioral changes, such as weight loss and alcohol reduction. The withdrawal or replacement of sedative drugs, which impair airway tone, may be crucial (9). Continuous positive airway pressure (CPAP) providing pneumatic splinting or custom made oral appliances, such as mandibular repositioning splint, can improve the anatomic barrier leading to sleep apnea. Finally, obtainment of sleep apnea reduction can be achieved by different types of surgical intervention (3).

1.2.6.1. Continuous positive airway pressure (CPAP)

The gold standard treatment for patients with moderate to severe OSA is CPAP. The mechanism of action is to provide an open airway by applying a pressure of five to twenty mmHg which is inhaled through a mask. Patients can be treated with fixed-pressure CPAP machines set at a specific pressure value, or with a self-adjusting device. The main side effect is airway drying which can be solved by usage of an integral heated humidifier (9). Since the average sleep duration is around seven hours, studies have shown that a minimum of four hours of sleep with CPAP are needed in order to gain beneficial results for individuals with OSA (23).

1.2.6.2. Oral appliances

As a leading alternative to CPAP, mandibular repositioning splints are widely used for the treatment of OSA. Those devices are used intraorally, attached to the upper and lower dental arches holding the mandible in advanced position (20). Tongue retaining devices hold only the tongue in a forward position providing open airway and free passage of airflow (3). Oral appliances are mostly used by patients who cannot tolerate CPAP, and unfortunately, long-term use is often followed by high dropout rates.

Mandibular advancement device (MAD) is the most commonly used and investigated oral appliance for OSA treatment. It protrudes the mandible and thereby reduces the collapsibility of the upper airway during sleep. It has been demonstrated that MAD treatment improves the sleep assessment variables in mild to moderate OSA patients and reduces subjective symptoms and excessive daytime somnolence (24).

Another option is a device which holds the tongue from restricting the upper airway. The tongue retaining device (TRD) consists of a mouthpiece that covers the entire upper and lower dental arches, with a defined mandibular protrusion. It pulls the tongue slightly forward due to the negative pressure created by the displacement of air from the lingual compartment of the device and consequently opens the airway (25).

Contraindications for oral appliances are periodontal diseases, disorders of the temporomandibular join, as well as missing teeth (25).

1.2.6.3. Surgical intervention

If OSA is caused by anatomic or structural obstruction of the airway, surgical correction may be a treatment choice, especially when CPAP has failed to improve the disorder. However, OSA surgeries are rarely curative for patients, but may improve clinical outcome to some degree. Additionally, patients should always consider several surgical options, the likelihood of success and set a clear goal by considering both risks and benefits of the procedure (3).

1.3. Pathophysiologic changes in obstructive sleep apnea

A majority of patients with OSA have coexisting risk factors for cardiovascular and cerebrovascular diseases, in particular the factors that comprise the metabolic syndrome as central obesity, hypertension, dyslipidemia, and insulin resistance or glucose intolerance. However, sleep apnea itself can lead to atherosclerosis by nocturnal activation of sympathetic nervous system resulting in an increase of free fatty acids via stimulation of lipolysis (26).

The presence of various types of metabolic dysfunction in subjects with OSA, and the association of OSA and metabolic syndrome was highlighted as “syndrome Z” in the late 1990s. It is an approach to the explanation of similar risk factors, such as obesity, hypertension, as well as dysregulation of glucose, and the overlapping clinical consequences of both disorders which cause a vicious cycle (27).

During sleep, there are organized patterns of sleep stage-related changes in blood pressure and sympathetic activity which are disrupted in OSA. Apneic episodes result in progressive increases in sympathetic nerve activity. In contrast to sleep in healthy individuals, when blood pressure and sympathetic nerve activity decline significantly during non-REM sleep, both sympathetic activity and blood pressure reach very high levels during sleep in patients with OSA (28). As a result, metabolism of glucose is increased by glycogen breakdown and gluconeogenesis.

The severity of hypoxemia in OSA is related to the degree of glucose intolerance and insulin resistance acting through mechanisms of oxidative stress to mediate alterations in glucose metabolism. Recurrent arousals and sleep loss lead to alterations in hypothalamic-pituitary-adrenal axis, leading to altered cortisol levels, decreased pancreatic beta-cell activity, elevated growth hormone levels, and alterations in neuroendocrine control of appetite (29).

Impaired glucose metabolism is the main pathologic component of diabetes mellitus. That is why the strong association between OSA and diabetes is explained by many hypotheses. First, they both share the same risk factors, including obesity, visceral adiposity, and advanced age, finally resulting in cardiovascular disorders and events. Studies have shown that by inducing sleep deprivation, one can cause a state of glucose intolerance (30). Furthermore, the cross-sectional relationship between sleep apnea and fasting glucose as well as insulin resistance has shown the complex mechanism by which diabetes and sleep apnea cause a vicious cycle. The exact mechanism is not understood, since co-founding factors like

obesity, underlying vascular disorders and the lack of data, often lead to insufficient scientific results. Nevertheless, the high prevalence of OSA in patients with diabetes (and *vice versa*), suggests a potential way how to improve everyday life and the disease´s outcome (31).

2. OBJECTIVES

AIMS:

1. Determine the differences in biochemical and glucose metabolism parameters in OSA patients compared to control group
2. Determine the prevalence of glucose metabolism disorders in OSA patients compared to control group

HYPOTHESIS:

1. Disorders of glucose metabolism (insulin resistance, impaired fasting glucose and impaired glucose tolerance) will be more prevalent in OSA patients compared to control group

3. MATERIALS AND METHODS

3.1. Ethical background of data collection

All data which were used for this thesis were gathered at the Sleep Medicine Center in Split and was approved by the Ethics Committee of University of Split School of Medicine. All performed procedures were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration. Informed consent was obtained from all individual participants included in this study.

3.2. Subjects

Patients included in this study, were newly diagnosed male patients with obstructive sleep apnea at the Sleep Medicine Center in Split. The diagnosis of OSA was defined in accordance with the guidelines established by the American Academy of Sleep Medicine (AASM) and European Sleep Research Society (ESRS) (32,3). All subjects, both patients with OSA and controls, completed the Epworth Sleepiness Questionnaire prior to study begin. Subjects who met one or more of the following criteria were excluded from the study: diagnosed diabetes mellitus, severe cardiovascular, neurological, psychiatric, respiratory or renal disease, active malignant disease; regular use of drugs that could interfere with glucose metabolism, use of sedatives or narcotics, alcohol and drugs abuse; history of any OSA treatment prior to the study enrollment; and female gender. Taking into account the exclusion criteria, twenty-five men were included in the study group.

The control group consisted of 25 subjects from a pool of healthy male volunteers matched with the OSA patients for age and BMI. Potential subjects were excluded if they had Epworth Sleepiness Scale (ESS) score higher than 9. The Snoring, Tiredness, Observed apnea and high blood Pressure (STOP) questionnaire, a screening tool for OSA with high sensitivity and specificity for determining the risk for OSA, has been used to identify subjects with high risk for OSA. Subjects with a STOP questionnaire score ≥ 2 were excluded from the study due to the risk for the development of OSA (14).

Subjects included in this study underwent a detailed initial medical history interview, physical examination, and anthropometric measurements. Body weight and height were measured followed by the calculation of body mass index (BMI). Waist circumference was measured at the mid-point between the inferior tip of the ribcage and the superior aspect of the iliac crest, with the subjects standing in the upright position. Neck circumference was

measured in the mid-way of the neck, between mid-cervical spine and mid-anterior neck (below laryngeal prominence), with the subjects standing in the upright position. Arterial blood pressure was measured at least twice (after 10 min of rest), in a sitting position with a standard mercury sphygmomanometer and appropriate cuff size.

3.3 Sleep assessment

Full-night attended polysomnography (PSG) was performed, recording electroencephalography, electrooculography, mental and tibial electromyography, electrocardiography, nasal airflow, pulse oxymetry, thoracic and abdominal movements and snoring intensity (Alice 5LE, Philips Respironics, Eindhoven, Netherlands). The collected data were evaluated in accordance with the published AASM and ESRS guidelines (33). In case the recording of the patient´s sleep phase lasted less than 6 hours, a second PSG was undertaken.

The self-administrated questionnaire, the Epworth Sleepiness Scale (ESS), validated in Croatian language, was used to measure excessive daytime sleepiness (14). The apnea-hypopnea index (AHI) was defined as the average number of apneas plus hypopneas per hour of sleep. Apnea was defined as a complete cessation of respiratory airflow for a minimum duration of 10 s whereas hypopnea was defined as a decrease in airflow by more than 50% from baseline for at least 10 s, combined with a reduction in hemoglobin oxygen saturation of at least 3%. Oxygen desaturation index (ODI) was calculated as a number of significant oxygen saturation (SpO2) drops of 3% or more per hour of sleep (33).

3.4. Blood sampling and laboratory analysis

After performing PSG and evaluation of questionnaires, patients underwent laboratory analysis. After a fasting period of 12 hours, venous blood samples were collected through a polyethylene catheter inserted in a forearm vein. All blood samples were analyzed by the same experienced biochemist and at the same laboratory, with the same method for each assay following standard procedure. Fasting plasma insulin levels were determined by electrochoemiluminescence immunoassay (ECLIA) method (Roche Diagnostics GmbH,

Mannheim, Germany). Fasting plasma glucose levels were measured using photometry with hexokinase method (Abbott, Chicago, USA) and HbA1c levels were measured by turbidimetric inhibition immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). Other laboratory assays were performed by routine laboratory methods.

Each subject underwent a 75-g oral glucose tolerance test (OGTT) shortly after the collection of the first fasting blood sample, during which plasma glucose and plasma insulin levels were determined at 0 and 120 minutes. Impaired fasting glucose (IFG) was defined as fasting plasma glucose from 5.6 to 6.9 mmol/L and impaired glucose tolerance (IGT) as plasma glucose in the range of 7.8–11 mmol/L 2 h after the glucose load (33). Insulin resistance was assessed by the homeostatic model assessment index of insulin resistance (HOMA-IR) calculated as the product of the fasting plasma insulin concentration (mU/L) and fasting plasma glucose concentration (mmol/L) divided by 22.5 (34).

3.5. Statistical analysis

Statistical analysis was performed using statistical software MedCalc for Windows, version 11.5.1.0 (MedCalc Software, Mariakerke, Belgium). Continuous data were presented as mean ± standard deviation, whereas categorical variables were presented as whole numbers and percentages.

The Kolmogorov-Smirnov test was used to assess normality of data distribution. The comparison between OSA patients and the control group for parameters of glucose metabolism were tested using t-test for independent samples. The difference in prevalence of glucose metabolism disorders between OSA patients and control group were assessed by Fisher's exact test. The statistical significance was set at $P<0.05$.

4. RESULTS

Table 1 describes the baseline parameters which were measured in control group and patients with OSA. Both groups consisted of twenty-five men, with no significant difference in age (P=0.579). There were no statistically significant differences in anthropometric parameters between the groups except in neck circumference (39.6±2.7 cm in control group vs. 45.8±3.1 cm in OSA group; P<0.001) (Table 1).

Table 1. Baseline characteristics of control and OSA group

Parameters	Control group (n=25)	OSA group (n=25)	P*
Age (years)	51.4±9.8	49.9±9.18	0.579
Body height (cm)	181.3±5.1	182.2±6.3	0.581
Body weight (kg)	94.9±8.0	98.4±11.1	0.207
BMI (kg/m^2)	28.87±3.0	29.61±2.78	0.370
Neck circumference (cm)	39.6±2.7	45.8±3.1	<0.001
Waist circumference (cm)	103.4±8.4	106.9±10.7	0.204
Systolic blood pressure (mmHg)	131±9.2	135.8±11.7	0.113
Diastolic blood pressure (mmHg)	85.6±7.1	87.2±5.2	0.367

* t-test for independent samples
Data are presented as mean± standard deviation
BMI - body mass index

The analyses of polysomnographic data and the Epworth Sleepiness Scale (ESS) scores are presented in Table 2. The mean AHI in our OSA patients was high (47.3±23.9 events/h), because most of patients included in study had severe OSA (AHI>30 events/h). In addition, there was significant difference in excessive daytime somnolence measured by the ESS score between control and OSA group (3.9±1.8 vs. 9.1±3.9; P<0.001) (Table 2).

Table 2. Polysomnographic and sleep questionnaire analysis of OSA patients

Parameters	Control group (n=25)	OSA group (n=25)
AHI (events/hr)	nd	47.3±23.9
Mean SpO_2 (%)	nd	92.0±4.9
ODI (events/hr)	nd	43.3±26.1
Total sleep time (hours)	nd	6.24±1.9
ESS score	3.9±1.8	9.1±3.9*

Data are presented as mean± standard deviation
nd - not determined
* t-test for independent samples (P<0.001)
AHI - apnea-hypopnea index, SpO_2 - arterial oxygen saturation, ODI - oxygen desaturation index, ESS score - Epworth Sleepiness Scale score

The results of biochemical parameters of OSA patients and control subjects are presented in Table 3. Apart from the measured vales of HDL cholesterol, which were 1.5±0.3 mmol/L in the control group and 1.3±0.3 mmol/L in patients with OSA (P=0.007), there were no statistically significant differences between the groups (Table 3).

Table 3. Biochemical parameters of control and OSA group

Parameters	Control group (n=25)	OSA group (n=25)	P*
Triglycerides (mmol/L)	1.3±0.8	2.0±1.3	0.052
Cholesterol (mmol/L)	5.6±1.2	5.9±1.4	0.591
HDL cholesterol (mmol/L)	1.5±0.3	1.3±0.3	0.007
LDL cholesterol (mmol/L)	3.6±1.2	3.8±1.2	0.400
AST (U/L)	21.9±16.4	25.6±10.6	0.348
ALT (U/L)	33.3±16.2	32.4±15.0	0.839
GGT (U/L)	38.9±25.6	47.2±23.0	0.233

* t-test for independent samples
Data are presented as mean±standard deviation

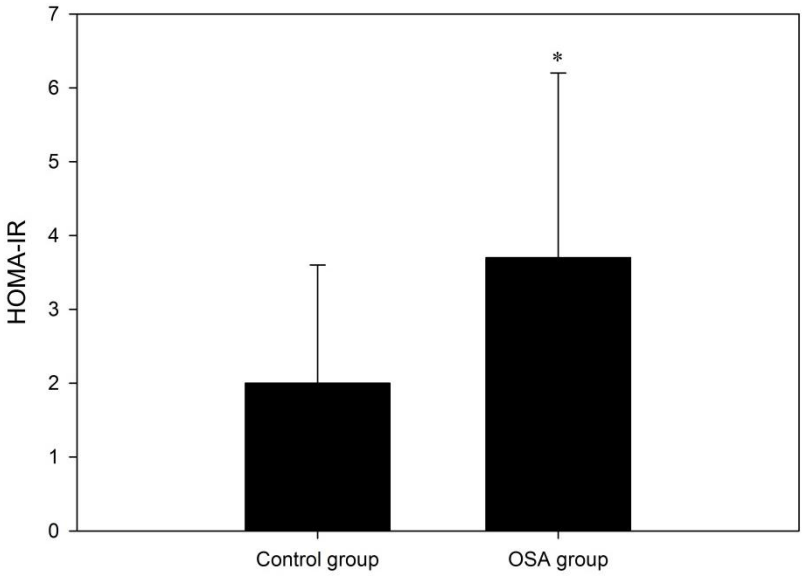
Regarding the parameters of glucose metabolism, they are summarized in Table 4. Fasting plasma glucose and fasting plasma insulin were significantly higher in OSA group in comparison with control group. There were no significant differences between groups in the postprandial plasma glucose and insulin levels (after 120 min of OGTT), as well in HbA1c levels (Table 4).

Table 4. Parameters of glucose metabolism in control and OSA group

Parameters	Control group (n=25)	OSA group (n=25)	*P**
Fasting insulin (pmol/L)	61.4±30.0	106.2±72.6	0.006
Postprandial insulin (pmol/L)	429.5±401.5	494.2±403.7	0.572
HbA1c (%)	5.4±0.4	5.6±0.3	0.124
Fasting glucose (mmol/L)	5.0±0.4	5.2±0.5	0.023
Postprandial glucose (mmol/L)	5.5±1.2	5.7±1.4	0.590

* t-test for independent samples
Data are presented as mean±standard deviation

The homeostatic model assessment index of insulin resistance (HOMA-IR) was significantly higher in the OSA group in comparison to control group (3.7±2.9 vs. 2.0±1.6; P=0.006) (Figure 1).



**P*=0.006
HOMA-IR - homeostatic model assessment index of insulin resistance

Figure 1. Insulin resistance index in control and OSA group

The impairment of glucose metabolism in OSA patients compared to the subjects in the control group is presented in Table 5. In OSA group more patients had impaired fasting glucose (IFG) and insulin resistance (IR) in comparison with the control group. Interestingly, there was no statistically significant difference in the prevalence of impaired glucose tolerance (IGT) between groups (Table 5).

Table 5. Prevalence of glucose metabolism disorders in control and OSA group

Parameters	Control group (n=25)	OSA group (n=25)	*P**
Impaired fasting glucose (IFG)	1 (4)	7 (28)	0.020
Impaired glucose tolerance (IGT)	2 (8)	3 (12)	0.974
Insulin resistance (IR)	4 (8)	14 (52)	0.007

* Fisher's exact test
Data are presented as number (%)
IFG was defined as fasting plasma glucose in range of 5.6 to 6.9 mmol/L; IGT was defined as glucose 2 h after glucose load in range of 7.8 to 11 mmol/L; IR was defined as homeostatic model assessment index of insulin resistance (HOMA-IR)>2.5

5. DISCUSSION

OSA emerged as an important disorder manifesting in a raising pattern over the past fifty years, followed by a steadily increasing research output concerning this topic and the potential correlation of sleep characteristics with glucose metabolism in those subjects has been investigated more and more (35). The present study was made in order to confirm the recent investigations concerning this health issue by comparing it with results achieved from patients with OSA at the Clinical Hospital in Split.

Beside growing evidence from epidemiological and clinically based studies suggesting an independent relationship between OSA and abnormal glucose tolerance, there is affirmation that insulin resistance plays a major role in this complex relationship (36). It has been showed that insulin resistance, as pivotal factor in the pathogenesis of a metabolic syndrome, which generally includes hyperinsulinemia, glucose intolerance, dyslipidemia, central obesity and hypertension, is highly prevalent in patients with OSA (37).

The results of the present study showed a complex relationship between blood glucose levels and obstructive sleep apnea. The analysis showed higher values of fasting insulin levels, as well as fasting glucose levels in plasma in patients with OSA when compared to subjects in the control group. In accordance with this data, the calculated homeostatic model assessment index of insulin resistance was also higher in patients with OSA than in controls.

Although OSA is associated often with both obesity and metabolic syndrome, the sleeping disorder itself is independently connected to lower insulin sensitivity, resulting in increasing risk for glucose intolerance and diabetes type 2 (38). There are several mechanisms triggering this effect. Intermittent hypoxia and sleep fragmentation induce sympathetic nervous system activation, followed by systemic changes and increased activity of the hypothalamic-pituitary-adrenal-axis, leading to nocturnal hypersecretion of cortisol and finally, progression to glucose intolerance, at least partially (39). One study has shown that in non-obese, non-diabetic apneic men, the severity of nocturnal hypoxia was associated with decreased insulin sensitivity (40). Analysis of sleep-disordered breathing showed that the degree of sleep-related hypoxemia was strongly associated with measured parameters of glucose tolerance and insulin resistance, whereas disruption of nocturnal sleep continuity, as assessed by the frequency of arousals, was found to be associated only with insulin resistance, not glucose tolerance (41).

Since there is increasing evidence that OSA carries an independent risk for cardiovascular and cerebrovascular diseases, the possible connection between those two entities might help to reduce this risk.

A link between OSA and insulin resistance appears early, as something that might be called “pre-diabetes”. The transition from the early metabolic abnormalities that precede diabetes, measured by IFG and IGT, to the actual development of diabetes itself may take many years. Unfortunately, most patients do not persist in this intermediate state of abnormal glucose regulation, but rather proceed to the evolvement of diabetes. Even though, the natural history of both IFG and IGT seems variable, with only twenty-five percent of people progressing to diabetes, while fifty percent remain in their abnormal glycemic state, over an observational period of 3–5 years, with longer observation, the majority of patients end up with the full range of symptoms and sequelae od diabetes (42).

In the present study, this constellation was partially confirmed. Interestingly, prevalence of IGT did not show statistical difference between healthy individuals and patients with obstructive sleep apnea. However, IFG was more pronounced in OSA patients, as well as insulin resistance which were present in more than fifty percent of patients.

Although both IFG and IGT are insulin-resistant states, they differ in their site of insulin resistance as well as in their pathophysiologic mechanisms contributing to disturbances in glucose homeostasis (43). People with isolated IFG predominantly have hepatic insulin resistance and normal muscle insulin sensitivity, whereas individuals with isolated IGT have normal to slightly reduced hepatic insulin sensitivity and moderate to severe muscle insulin resistance. Additionally, the combination of hepatic insulin resistance and defective insulin secretion in isolated IFG, results in excessive fasting hepatic glucose production accounting for fasting hyperglycemia which finally leads to a vicious cycle and metabolic syndrome in the end (42).

Apart from fasting glucose and insulin levels, HOMA-IR showed a significant difference in control group and subjects with OSA. Several studies showed that disturbed respiratory function during sleep and minimum oxygen saturation were significant parameters influencing HOMA-IR (44). Intermittent hypoxia, as the hallmark of OSA, contributes in healthy humans to elevated oxidative stress, resulting in an increase in cytokine levels and insulin resistance (45). Moreover, even mild recurrent events of oxyhemoglobin desaturation less than 4% are associated with impaired metabolic function (46). Among 270 non-diabetic

subjects, from Hong Kong Sleep Clinic, HOMA-IR was higher in those with OSA and rise with increasing OSA severity, in both obese and non-obese subjects (44).

Study of Punjabi et al. showed that AHI and average oxygen saturation were independently associated with higher fasting glucose and OGTT two-hours-results, which persisted even in subjects with normal weight (47). Additionally, an AHI higher than 5 was associated with an increased risk of glucose intolerance independently of the effect of obesity, in middle-aged, overweight or mildly obese men (48). Even though the present study did not find significant difference in the measured levels of HbA1c between subjects with OSA and controls, in 2014, an analysis of 5,294 non-diabetic participants in the pan-European ESADA study, showed the relationship between glycemic health and severity of sleep disordered breathing, with a significantly higher adjusted HbA1c level in patients with high AHI compared to the lower AHI (49).

Since studies are still unsatisfactory in establishing an etiologic link of the precise and complex mechanism behind the metabolic changes resulting from OSA, an alternative approach would be to mitigate sleep apnea with continuous positive airway pressure (CPAP) treatment and explore changes in glucose metabolism (50). On the basis of this idea, one study concluded, that good adherence to long-term CPAP treatment can significantly reduce HbA1c levels, but has no effect on markers of insulin resistance (51). The beneficial effects of CPAP which is the gold standard for OSA widely depends on obesity, diabetes as underlying disease and the severity of obstructive sleep apnea, as well as the duration of treatment and its implementation (52).

Future investigation will have to include more participants in order to obtain better or precise scientific results. Although, the control group fulfilled both ESS and STOP questionnaires, and underwent all laboratory measurements, a limitation to the study is the missing data which was not collected by polysomnography in controls compared to patients with OSA. Moreover, more precise results might be achieved by dividing patients into groups according to severity of sleep apnea. Also, the exact correlation might further be investigated by explicitly testing the effect of CPAP and other methods used in treating OSA. By this way, the effect of sleep fragmentation and oxygen desaturation could be directly related to the underlying mechanism of glucose metabolism and its components. At last, longer follow-ups of patients with OSA may lead newer aspects of this multifaceted disorder.

6. CONCLUSIONS

1. Fasting plasma glucose and insulin levels were significantly higher in OSA patients group compared to control group.
2. Homeostatic model assessment index of insulin resistance (HOMA-IR) was significantly higher in OSA patients group compared to control group.
3. There were no significant differences in the postprandial plasma glucose and insulin levels between groups.
4. Impaired fasting glucose and insulin resistance were more prevalent in OSA patients group compared to control group, while there was no difference in prevalence of impaired glucose tolerance between groups.

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8. SUMMARY

Title: PARAMETERS OF GLUCOSE METABOLISM IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

Objectives: Obstructive sleep apnea (OSA) by its main characteristic of recurrent intermittent hypoxemia is associated with glucose metabolism alterations and insulin resistance, with increased risk for development of diabetes. The aim of the study was to compare parameters of glucose metabolism in non-diabetic OSA patients with respective controls.

Materials and methods: A total of 25 male newly diagnosed OSA patients and 25 matched control subjects underwent an oral glucose tolerance test (OGTT) and blood sampling for determination of parameters of glucose metabolism. Insulin resistance was assessed by the homeostatic model assessment index of insulin resistance (HOMA-IR).

Results: Fasting plasma glucose level (5.2±0.5 vs. 5.0±0.4 mmol/L; *P*=0.023) and fasting plasma insulin level (106.2±72.6 vs. 61.4±30.0 pmol/L; *P*=0.006) were significantly higher in OSA group in comparison with control group. There were no significant differences between groups in the postprandial plasma glucose and insulin levels. HOMA-IR was significantly higher in the OSA group in comparison to control group (3.7±2.9 vs. 2.0±1.6; *P*=0.006). In OSA group more patients had impaired fasting glucose (28% vs. 4%; *P*=0.020) and insulin resistance (52% vs. 8%; *P*=0.007) in comparison with the control group. There was no statistically significant difference in the prevalence of impaired glucose tolerance between groups.

Conclusion: In conclusion, this study confirmed the evidence of glucose metabolism disorders in patients with OSA.

9. CROATIAN SUMMARY

Naslov: PARAMETRI METABOLIZMA GLUKOZE U BOLESNIKA S OPSTRUKCIJSKOM APNEJOM TIJEKOM SPAVANJA

Ciljevi: Opstrukcijska apneja tijekom spavanja (OSA) zbog svoje glavne značajke intermitentne hipoksemije povezana je s promjenama metabolizma glukoze i inzulinskom rezistencijom te s povećanim rizikom za razvoj dijabetesa. Cilj ovog istraživanja bio je usporediti parametre metabolizma glukoze u OSA bolesnika koji ne boluju od dijabetesa s odgovarajućim kontrolama.

Materijali i metode: Ukupno 25 muških novodijagnosticiranih OSA bolesnika i 25 kontrolnih ispitanika podvrgnuto je oralnom testu opterećenja glukozom (OGTT) i uzorkovanju krvi za određivanje parametara metabolizma glukoze. Inzulinska rezistencija procijenjena je homeostatskim modelom procjene inzulinske rezistencije (HOMA-IR).

Rezultati: Jutarnje plazmatske koncentracije glukoze (5,2±0,5 vs. 5,0±0,4 mmol/L; *P*=0,023) i inzulina (106,2±72,6 vs. 61,4±30,0 pmol/L; *P*=0,006) bile su značajno više u OSA skupini u usporedbi s kontrolnom skupinom. Nije bilo značajnih razlika između skupina u koncentracijama glukoze i inzulina postprandijalno. HOMA-IR bio je značajno veći u OSA skupini u usporedbi s kontrolnom skupinom (3,7±2,9 vs. 2,0±1,6; *P*=0,006). Skupina pacijenata s OSA-om imala je značajno veću prevalenciju poremećene glukoze natašte (28% vs. 4%; *P*=0,020) i inzulinske rezistencije (52% vs. 8%; *P*=0,007) u usporedbi s kontrolnom skupinom. Nije bilo statistički značajne razlike u prevalenciji poremećene tolerancije glukoze između skupina.

Zaključak: Zaključno, ova studija potvrdila je dokaze o poremećajima metabolizma glukoze u bolesnika s OSA-om.

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