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RESEARCH ARTICLE

The role of nitric oxide in obstructive sleep apnea-induced insulin resistance

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ABSTRACT

Background: Insulin resistance (IR) is a characteristic feature in patients who are developing Type 2 diabetes in the prediabetic stage. Obstructive sleep apnea (OSA) is a breathing disorder characterized by frequent episodes of reduced airflow due to obstruction in the upper airway while sleeping. OSA is associated with intermittent hypoxia and predisposition to increased IR. **Aims and Objectives:** This study investigates the extent of IR in prediabetics and OSA patients and also compares serum nitric oxide (NO) levels in the three study groups. **Materials and Methods:** Three groups each comprising of 50 people were selected: Group I - control group; Group II - prediabetic patients; and Group III - OSA patients. Fasting blood glucose, insulin, and NO levels were measured in these subjects and IR calculated. **Results:** Insulin levels and IR were significantly higher in OSA and prediabetic patients when compared to control subjects. There was no significant difference in insulin levels ($P > 0.05$) between OSA and prediabetic groups. OSA group had significantly lower level of NO compared to both control and prediabetic groups. **Conclusion:** High insulin levels and IR in prediabetics indicate that they are prone to develop Type 2 diabetes. The same findings in OSA patients could mean that they are also prone to develop Type 2 diabetes. NO levels are significantly low in OSA patients. Low NO level has been associated with hypoxia; this molecule with its wide array of actions may be involved in glucose homeostasis.

KEY WORDS: Insulin Resistance; Prediabetes; Obstructive Sleep Apnea; Hypoxia; Nitric Oxide

INTRODUCTION

The adult prevalence of obstructive sleep apnea (OSA) worldwide ranges within 2–4%. In India, overall prevalence is 9.3% in the total population, being about 13.5% in men and 5.6% in women.[1] Patients with OSA have disturbed sleep at night, which is estimated to produce a ≥3% drop in oxygen saturation. This hypoxia can lead to a number of health consequences some of them being daytime sleepiness, sympathetic overactivity, blood pressure surges during sleep, endothelial damage, and nocturnal as well as daytime hypertension. In addition, there is a release of acute-phase proteins and reactive oxygen species that exacerbate insulin resistance (IR). Studies have shown that OSA is an essential risk factor for the development of hypertension, cardiovascular disease, and metabolic derangements such as insulin insensitivity/resistance and low glucose tolerance.[2,3]

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Type 2 diabetes has a strong genetic predisposition. In the early prediabetic stage, these patients require a higher concentration of insulin to maintain normal glucose tolerance. This decreased ability of insulin to act effectively on target tissues to bring about

glucose homeostasis is described as IR.[4] Eventually, insulin secretion cannot be sustained, and the defect progresses to a state of inadequate insulin secretion and overt diabetes. Both IR and impaired insulin secretion contribute to Type 2 diabetes.

IR is present in prediabetics as well as in patients with metabolic syndrome and OSA. Both metabolic syndrome and OSA could have common intermediary pathways that increase cardiovascular mortality and morbidity.[5,6] Nitric oxide (NO) is an important signaling molecule which has many widespread actions.[7] Hypoxia has been shown to produce IR; in turn, IR has been shown to be decreased by oxygen therapy.

Hence, we hypothesize that decreased NO level could be the underlying pathophysiological link for the development of IR in patients with OSA.

Aim

The aim of this study is to compare the insulin level, IR, and NO level in controls, prediabetics, and OSA patients.

MATERIALS AND METHODS

The cross-sectional study was conducted among three groups, each comprising of 50, carried out in the Department of Physiology, ACS Medical College and Hospitals, Dr. MGR Educational and Research Institute University, Summa Institute of Sleep Medicine and Saveetha Institute of Medical and Technical Sciences, in Chennai, Tamil Nadu, India, during January 2015–December 2016. The participants were in the age group of 30–60 years of both sexes. The study commenced after getting approval from the Institutional Human Ethical Committee, Saveetha University - IHEC No-008/12/2014/IEC/SU, dated - December 18th, 2014, Chennai, Tamil Nadu. The participants received a detailed explanation about the procedure and their cooperation and willingness were obtained with an informed consent.

Selection of Participants

Group I participants were matched for age and sex with the other two groups. They were normal, healthy people who were not taking any prolonged medication and had normal blood chemistry.

Group II participants were categorized as being pre-diabetics according to the American Diabetes Association criteria. Their selection was based on their HbA1C of 5.7–6.4%, fasting blood glucose of 100–125 mg/dl, or oral glucose tolerance test 2 h blood glucose of 140 mg/dl–199 mg/dl.

Group III participants had apnea/hypopnea index more than five who were newly diagnosed as having OSA based on the polysomnogram, which is the "gold standard" diagnostic tool for OSA. In the polysomnogram, OSA was confirmed by a single overnight cardiorespiratory sleep study using a four channel recorder and scored in accordance with the American Academy of Sleep Medicine Standards.

Exclusion criteria for the selection of patients were history of cardiac disease, hypertension, asthma, chronic obstructive pulmonary disease, history of liver disease, cancer, renal disease, active tuberculosis, and endocrine disorders such as diabetes mellitus (DM), Cushing's syndrome, and thyroid dysfunction. People with anatomical deformities such as craniofacial abnormalities and congenital cardiopulmonary disorders were also excluded from the study.

Blood Sample and Biochemical Analysis

After overnight fast, 5 ml of peripheral blood was collected through venipuncture in test tubes from all patients. From this blood, fasting blood sugar was estimated by GOD–POD method by the semi-automated analyzer (Erba-Mannheim Transasia, Germany) and insulin by enzyme-linked immunosorbent assay method by an automated analyzer (Evolis BIORAD, France). Homeostatic model assessment of IR (HOMA-IR) was calculated by the formula; fasting insulin (μIU/ml) × fasting glucose (mg/dl)/405.Estimation of NO was done by the standard method of Griess reagent by colorimetric method and readings were made at 540 nm against blank using ultraviolet (UV)-visible spectrophotometer (UV-1601, Shimadzu).

Statistical Analysis

All data were analyzed using one-way analysis of variance among the three groups. The continuous variables are expressed as a mean ± standard error. SPSS statistical software of version 9.0 was used for the analysis. Results were considered statistically significant if the $P < 0.05$.

RESULTS

Fasting Insulin Level

Fasting insulin level in control group was 5.69 ± 0.21, in prediabetics 14.06 ± 0.45, and in OSA 15.20 ± 0.67. Increased level of insulin was observed in prediabetic and OSA groups when compared to the control group ($P < 0.05$). The difference observed between prediabetes and OSA group was not statistically significant ($P > 0.05$) [Figure1].

IR

HOMA-IR in control group was 1.20±0.05, in prediabetics 3.96 ± 0.15, and in OSA 4.53 ± 0.22. Increased IR is observed in OSA

and prediabetes compared to the control group ($P < 0.05$). The difference in IR observed between prediabetes and OSA groups is statistically significant ($P < 0.05$) [Figure 2].

NO

NO level in control group was 37.68 ± 0.81, in prediabetics 40.11 ± 1.78, and in OSA 17.02 ± 0.59. Decreased level of NO observed in OSA when compared to prediabetes and control groups was statistically significant ($P < 0.05$). The small increase observed in prediabetes when compared to control is not statistically significant ($P > 0.05$) [Figure 3].

DISCUSSION

This study was carried out in three groups of people each having sample size of 50. The groups were normal controls, prediabetic patients, and people with OSA. In all participants, three concentrations were measured, namely, blood glucose, serum insulin, and serum NO. From the blood glucose and insulin concentrations, IR was calculated by the HOMA-IR method. Serum insulin level was significantly high in prediabetics and OSA patients compared to controls. IR was also significantly high in prediabetics and OSA patients compared to control subjects. Serum NO level in OSA group was significantly lower when compared to controls and prediabetics.

The presence of IR in OSA patients was found in the present study. Similar results of increased IR in sleep disturbances/OSA were also described by others. Youssef *et al*. in 2014 stated that IR in OSA is due to hypoxic stress associated with sleep.[8] Ip *et al*. in 2002 investigated the relationship between IR and sleep-disordered breathing in 270 subjects. Among them 185 were diagnosed as OSA, these patients were found to have higher levels of serum insulin and increased IR.[9] Elmasry *et al*. in 2001 reported that OSA patients have higher levels of serum insulin independent of body mass index.[10] Punjabi *et al.* showed a significant increase in fasting peripheral glucose and fasting insulin in OSA patients which can lead to Type 2 DM.[11]

IR is a high-risk factor for developing Type 2 DM. The prevalence of Type 2 diabetes in prediabetes population was estimated to be about 5–10% annually.[12] However, the prevalence and new incidence of Type 2 diabetes in OSA population were around 15–30%.[1] The underlying cause for this drastic difference in OSA population developing to Type 2 DM still remains unclear.

One difference between prediabetics and OSA patients found in the present study was that OSA patients had significantly low NO levels. Several factors are shown to be associated with altered NO levels. High arginase activity can lower NO level by reducing the substrate for NO synthase.[13] Total nitrite/nitrate levels were shown to have a positive influence on circulating NO levels.[14] Oxygen also influences NO levels in the blood. Overnight treatment through continuous

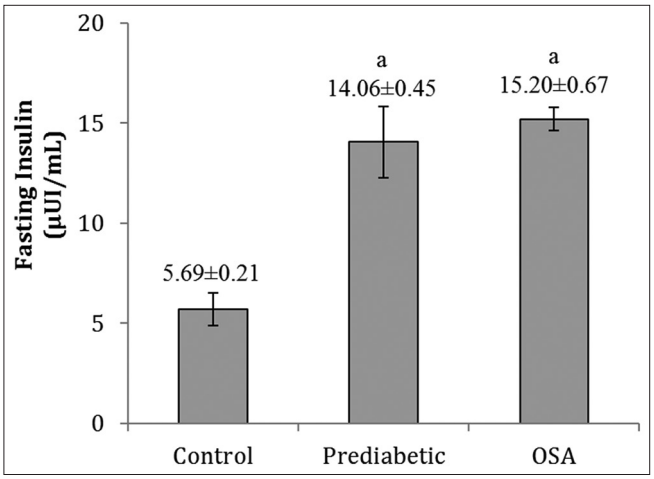

Figure 1: Fasting insulin: Values are presented as mean (±standard error). [a]Indicates significantly different from control group

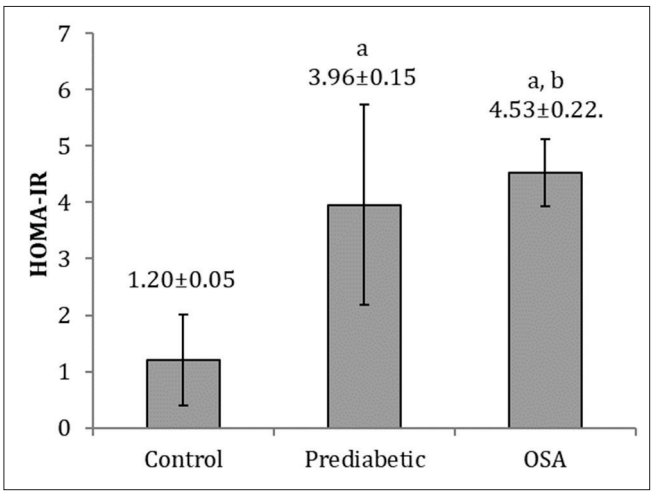

Figure 2: Insulin resistance: Values are presented as mean (±standard error). [a]Indicates significantly different from control group; [b]Indicates significantly different from prediabetic group

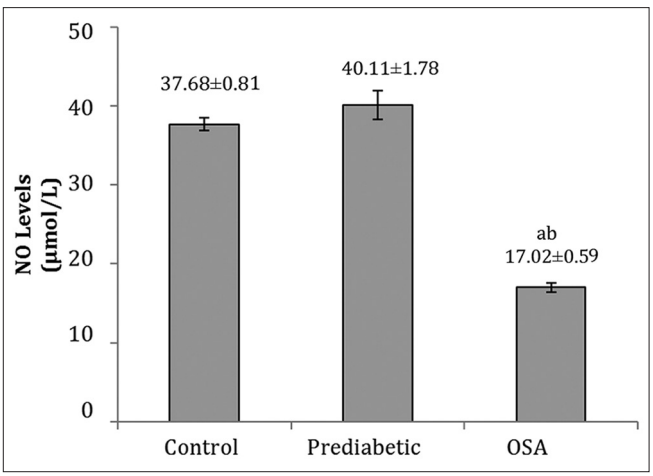

Figure 3: Nitric oxide: Values are presented as mean (±standard error). [a]Indicates significantly different from control group. [b]Indicates significantly different from prediabetic group

positive airway pressure (CPAP) therapy corrects hypoxia in OSA patients and maintains the serum NO levels, thereby reducing irreversible vascular modifications.[15] Sherpa population acclimatized to high altitude for several centuries has higher blood NO levels.[16]

IR is well known to precede Type 2 diabetes, but mechanisms underlying IR in OSA remain unclear. Suggested mechanisms for this association include increased sympathetic activity, obesity, oxidative stress, and chronic low-grade inflammation contributing to metabolic alterations.[17] The release of cortisol and other stress hormones may be a trigger for generating IR.[18] Leptin deficiency probably plays an important role in the insulin and glucose responses to intermittent hypoxia.[19] Increased oxidative stress may enhance IR due to dysfunction of beta cells of the pancreas.[20]

The molecular mechanisms involved in the actions of glucose within cells are of relevance to the present study. These also involve IR and NO, both of which are related to OSA. NO in physiological amounts is beneficial to the cell. Decreased NO availability leads to a number of features of diabetes and might lead to the development of IR. The redox balance in the cell is disrupted by increased adiposity and hyperglycemia and leads to impairment of the PI3K-Akt pathway. This pathway has two main actions, promoting entry of glucose into cells by GLUT4 mechanism and production of NO. The decrease in NO production resulting from impairment of this pathway leads to IR and endothelial dysfunction (hypertension and atherosclerosis). A reciprocal relationship seems to exist between NO and IR/endothelial dysfunction. NO might act as a regulatory factor for the downstream signaling molecules linking GLUT4 translocation and glucose uptake.[21] Thus, NO plays a vital role in glucose homeostasis.[21,22] Oxygen is one of the cofactors for the activity of NO synthase, and therefore, adequate oxygen is necessary for NO production.[23] Since it is well known that oxygen is essential for normal mitochondrial function, we speculate that hypoxia in OSA patients may lead to alteration in their ROS production and consequent decrease in NO levels. This would explain the diabetogenic and vascular effects of the metabolic syndrome which is often present in OSA patients as well as the increased tendency of OSA patients to develop Type 2 diabetes.

The major strength of the present study is the number of subjects (50) in each group and the demonstration of IR and reduced NO levels in OSA patients. The three associated factors hypoxia, IR, and reduced NO could account for the increased susceptibility of OSA patients to develop Type 2 diabetes. This association explains the efficacy of CPAP therapy and also suggests that treatment modalities such as reducing fat stores by exercise and increasing the intake of nitrates and nitrites in the diet may benefit OSA patients.

Limitations are lack of supporting experimental evidence to discover the specific reason for reduction of NO in OSA patients. The association of eNOS polymorphism and IR in obese individuals has been demonstrated.[21] If one or more of these factors is shown to exist in OSA patients, it may provide the required supporting evidence. Correction of hypoxia by CPAP therapy, regular exercise, and inclusion of nitrate/nitrite-rich food in the diet could be tried in OSA patients and IR and NO levels repeated to find out the beneficial effects of these measures.

CONCLUSION

Increased IR was found in both prediabetic and OSA patients and low serum NO levels in OSA patients alone. Hypoxia associated with OSA may account for the low NO level and rapid progression to Type 2 diabetes in this group.

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