ORIGINAL ARTICLE

Effect of Hyperoxia on Weight, Fasting Blood Glucose and Serum Peptide YY levels in Sprague Dawley Rats and its Potential Role in Treating Obesity

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ABSTRACT

Objective: To determine the effect of 30% hyperoxia on the weight, fasting blood glucose (FBG) and serum peptide YY (PYY) levels in Sprague Dawley rats and to see its potential role in treating obesity.

Study Design: Experimental, randomized control study.

Place and Duration of Study: It was carried out at the Department of Physiology, Islamic International Medical College, Rawalpindi in collaboration with National Institute of Health, Islamabad, Pakistan from April 2015 to March 2016.

Materials and Methods: Total 40 male Sprague Dawley rats of 2-4 months weighing 250-520 g were taken. They were divided into two groups of 20 each: control group A exposed to 21% oxygen and group B exposed to 30% oxygen for a period of 7 days. Before exposure to various oxygen concentrations the weight (g) of rats of both groups was taken and blood was collected for estimation of fasting blood glucose (FBG) (mg/dL) and serum peptide YY (PYY) (pg/mL).After exposure second sampling including weight, FBG and serum PYY was done. Statistical analysis was done applying SPSS 21, comparisons among the two groups were analyzed using independent sample t-test and correlation among variables was determined using Pearson's correlation coefficient. P value of <0.05 was considered significant in both analyses.

Results: Group B rats had significantly (P<0.05) increased weight (g), increased FBG (mg/dL) levels (P<0.001) and low serum PYY (pg/mL) levels (P<0.001) in comparison with group A.

Conclusion: Hyperoxia decreases PYY levels causing an increase in appetite leading to an increase in weight and FBG levels. Therefore, hyperoxia may not be useful as a treatment for obesity.

Key Words: *Fasting Blood Glucose, Hyperoxia, Peptide YY, Weight .*

Introduction

Obesity is a medical condition in which excess body fat has accumulated to the extent that it causes an adverse effect on health[1] and has become a global epidemic due to the readily available high calorie diets and prevailing sedentary life style.[2] The accumulated energy is stored mainly in the form of triglycerides in adipocytes leading to an increase in their size resulting in an increase in the overall body weight.[3] The increase in adipocyte size seen in obesity also leads to a decrease in insulin sensitivity hence raising blood glucose levels and increasing the risk of diabetes.[4]

A number of circulating hormones that play a vital role in controlling the appetite and ultimately reducing the weight of the body have been identified through recent research.[5] Peptide YY (PYY), the short 36 amino acid protein, is one such hormone. It is released from the gut and increases satiety resulting in a decreased in food consumption.[6] Much study has been carried out on the role of exogenous PYY in reducing weight, but more research needs to be conducted on raising endogenously produced PYY as its levels have shown to be decreased in obese individuals.[7]

Oxygen is an odorless and colorless gas having a normal atmospheric concentration of 21% and is widely used for treating a number of medical conditions.[8] Although exposure to a high level of hyperoxia for extensive periods has been shown to damage cells through the production of excessive free radicals,[9] low levels of hyperoxia produce lesser free radicals. These low levels of free radicals have shown to play an important role as signaling molecules that regulate various cellular processes and gene expression, such controlling fasting blood glucose levels through the production of glucose

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membrane transporters[10] and in healing wounds.[11]

The effect of hypoxia on serum PYY levels in humans has already been studied. Hypoxia has shown to decrease serum PYY levels, but as the oxygen concentration is gradually raised back to a normoxic state of 21% oxygen, the serum PYY levels increase again.[12] Studies showing the effect of hyperoxia on PYY levels, especially as a potential treatment for obesity and diabetes, still needs to be explored. The objective of this study was to see whether exposure to a low level of hyperoxia of 30% could be used as a treatment for obesity by raising endogenous PYY levels, ultimately decreasing the appetite leading to a reduction in weight and fasting blood glucose.

Materials and Methods

The experimental, randomized control study was carried out in the Department of Physiology, Islamic International Medical College, Rawalpindi in collaboration with the Animal house at National Institute of Health, Islamabad, Pakistan from April 2015 to March 2016. The study was approved by the Ethics Review Committee of Islamic International Medical College, Riphah International University. A total of 40 male Sprague Dawley rats of 2-4 months weighing 250-520 g were included in the study.[13] They were randomly divided into two groups: control group A (n=20) that was exposed to 21% oxygen and group B (n=20) that was exposed to 30% oxygen.[14] For 7 days the rats were allowed to acclimatize to the NIH Animal house environment. A standard diet in pellet form was prepared at the Animal house of NIH, Islamabad according to the guidelines given by the universities federation for animal welfare.[15] The food and water was provided ad libitum. On the morning of day 8 the first sample was collected by anesthetizing each rat of group A and B by placing it in a jar containing cotton soaked in chloroform. Its weight (g) was recorded using a weighing machine (TS200 electronic compact scale, Jiangyin Ditai electronic technology Co. Ltd., China) after which blood was drawn via intra cardiac sampling. One drop of blood was added onto the test strip of the glucometer (Easy Gluco Ultra Advance blood glucose meter, Isotech Co. Ltd., South Korea) and the fasting blood glucose (mg/dL) level was recorded. The blood was collected and stored in labeled gel tubes, protected from light and contamination and kept in a laboratory ice box at 2-8oC until shifted to the laboratory where they were tested for serum PYY (pg/mL) levels using an Enzyme-linked Immunosorbent Assay (ELISA) kit (CUSABIO Biotech Co. Ltd., China).[16]

Two transparent plastic chambers of dimensions 1.22 m x 0.72 m x 0.72 m were designed. The group A control chamber rats was not made air tight by keeping the upper two sides open allowing fresh air of a 21% oxygen concentration to enter freely. The group B chamber was made air tight with only two holes: an inlet for entry of oxygen and an outlet exit of air. Two nitrogen cylinders of 6.8 m3 and three oxygen cylinders of 4.5 m3, 3.4 m3 and 3.4 m3 were used. Two flow meters (Richu Medical Regulator YR-88E, Ningbo Beilun DB Marine Co. Ltd., China) were each attached to one nitrogen cylinder and one oxygen cylinder after which they were connected by a T-tube to allow oxygen and nitrogen to mix before supplying the chamber.[17] An oxygen sensor (CY-12C portable oxygen concentration tester, CLEVER Co. Ltd., China) was also attached within the chamber to monitor the oxygen concentration.

After 2 weeks given to the rats for replenishing their blood volume to normal levels [18] the experiment was started. To achieve an oxygen to nitrogen ratio of 3:7, the flow rate for oxygen was adjusted between 1-2 L/min and that for nitrogen between 3-4 L/minso that the chamber for group B rats was supplied with an oxygen concentration of 30 + 1%.17 Upon reaching a value of 31% on the oxygen sensor the flow rates were readjusted to bring it back to a 30% oxygen concentration. The water in the flow meters provided the required air humidity and these conditions were kept for 24 hours for 7 days [19] except for two situations where the experiment was stopped for no more than 10 min: first for supplying food and water and to clean trays for waste matter, and second to refill gas cylinders. Upon completion of 7 days of the experiment, second sample, comprising of weight, fasting blood glucose and serum PYY, was collected on the morning of day 8 similar to the method applied for the first sample collection.

The labeled gel tubes containing the blood samples were centrifuged using a centrifuge machine (EBA-20 small centrifuge, Andreas Hettich GmbH & Co. KG) at a speed of 3000 rpm for 15 min. The quantitative Enzyme-linked Immunosorbent Assay (ELISA)

method was used to measure serum PYY (pg/mL) levels. Statistical analysis was done applying the Statistical Package for Social Sciences version 21 (SPSS 21). Results were documented as mean + SEM. Comparisons among the two groups was analyzed using the independent sample t-test and correlation among the variables was done using Pearson's correlation coefficient. P value of <0.05 was considered significant for both analyses.

Results

A total of 40 male Sprague Dawley rats were included in the study. During the experiment two rats died from group A and one from group B making the survival rate 92.5%. The weight of group B rats (309.08 + 10.71 g)was significantly higher (P<0.05) than the weight of group A rats (283.75 + 5.20 g) after exposure to 30% oxygen for seven days. The rats of group B had fasting blood glucose levels of 172.71 + 8.59 mg/dL which were significantly raised (P<0.001) as compared to those of the group A rats (132.06 + 4.66 mg/dL). On comparison the serum PYY levels of the group B rats was 14.62 + 6.14 pg/mL which was significantly lower (P<0.001) than those of the group A rats (256.87 + 25.48 pg/mL). Mean ± SEM of weight (g), fasting blood glucose (mg/dL) and serum PYY (pg/mL) for the two groups of male Sprague Dawley rats exposed to different concentrations of oxygen as are displayed in Table I. No significant correlation was observed between serum PYY (pg/mL) levels, weight (g) and fasting blood glucose (mg/dL) levels in both groups using Pearson's correlation coefficient as is displayed in Table II.

Table I: Comparison of mean ± SEM of parameters (weight, fasting blood glucose and PYY) for the exposed and control groups of male Sprague Dawley rats

Parameter	Group A (21% oxygen) (n=18)	Group B (30% oxygen) (n=19)
WT (g)	283.75 ± 5.20	309.08 ± 10.71*
FBG (mg/dL)	132.06 ± 4.66	172.71 ± 8.59**
PYY (pg/mL)	256.87 ± 25.48	14.62 ± 6.14**

Weight (WT), Fasting Blood Glucose (FBG)
Peptide YY (PYY)
* = P<0.05 (value vs corresponding control)
** = P<0.001 (value vs corresponding control)

Discussion

Wasseet al, (2012) conducted an experimentin 10 male volunteers to explore how rest and exercise in a hypoxic environment influenced PYY levels. They concluded that the levels of serum PYY were lower in hypoxia as compared to normoxia [12] suggesting that as the oxygen concentration is increased from that of hypoxia to normoxia then serum PYY levels also increase. The aim of our present study was to see whether this pattern of increase in serum PYY levels was consistent after increasing the oxygen concentration beyond that of a normoxic state. According to our findings, a 7 day exposure to an oxygen concentration of 30% resulted in a significant decrease in serum PYY levels demonstrating that both hypoxia, as shown by Wasseet al (2011), and hyperoxia, as shown by our present study, lowers the levels of serum PYY.

Table II: Correlation of serum PYY (pg/mL) levels with weight (g) and fasting blood glucose (mg/dL) levels for the exposed and control groups of male Sprague Dawley rats

Parameter	Group A (21% oxygen) (n=18)	Group B (30% oxygen) (n=19)
WT (g)	-0.355	-0.343
FBG (mg/dL)	-0.012	0.163

Peptide YY (PYY), Weight (WT)
Fasting blood glucose (FBG)

Our present results showed that exposure to hyperoxia led to a significant increase in weight which was in accordance with the study carried out by Lakaniet al., (2012) who observed the effects of hypoxia, normoxia and hyperoxia on a total of 81 great sturgeon Husohuso fish and concluded that the group exposed to hyperoxia led to the greatest weight gain in the fish.[20] The significantly increased weight seen in our study could have been due to an increase in appetite caused by the significant decrease in serum PYY levels observed as supported by the studies carried out by Roth et al., (2005) who showed that fasting serum PYY levels are negatively correlated with weight.[21] On the other hand, our findings did not show a significant correlation of serum PYY levels with weight.

Stress is a situation in which the organisms' homeostasis is threatened by endogenous and exogenous stimuli[22] and cortisol is the most commonly measured indicator of stress that provides a good reflection of its duration and severity.[23] Exposure to high levels of cortisol

stimulates appetite and weight gain as well.[24] In the research by Wedemeyer, (1997) exposure to both hypoxia and hyperoxia result in oxidative stress.[25] Hence in our study an exposure to hyperoxia could have led to oxidative stress thus increasing the serum cortisol levels in the Sprague Dawley rats causing an increase in their appetite resulting in the gain of weight.

Likewise, other hormones could have also been at play such as ghrelin as shown in the study carried out by Batterham et al., (2003) to investigate the resistance of PYY in 12 obese subjects in which they concluded that PYY infusions significantly decreased plasma ghrelin levels.[26] Wren et al., (2001) have also observed an increase in appetite due to a rise in plasma ghrelin.[27] Hence it can be postulated that the decreased levels of serum PYY in our study could have led to increased levels of ghrelin which ultimately increased the appetite leading to more calorie consumption resulting in the increased weight.

Bertrand et al., (1992); Greeley et al., (1988) have demonstrated that an increase in serum PYY levels inhibits insulin secretion hence causing blood glucose levels to increase.[28,29] On the contrary, the results of our present study showed that an exposure to 30% oxygen led to a significant increase in the fasting blood glucose levels in the presence of a significant decrease in serum PYY levels. As there was no significant correlation between our findings for serum PYY and fasting blood glucose levels, it can be deduced that may be some other hormone was involved in raising the levels of fasting blood glucose which is in accordance with the study carried out by Ahren and Larsson, (1996) who infused PYY intravenously in 9 healthy adult females followed by a glucose infusion and discovered that PYY did not inhibit the acute insulin response to glucose.[30]

The significant increase in fasting blood glucose levels seen in our results can be explained from the study conducted by Antunes et al., (2014) who evaluated the association between insulin-resistance, fasting levels of PYY and ghrelin in 25 male Wistar rats and proved that increased fasting ghrelin levels were associated with insulin resistance rather than increased PYY levels ultimately leading to the increased fasting blood glucose levels.[31] Our results can also be explained by the study carried out by Adam et al., (2010) on 354 latino adolescents to determine the association between cortisol and insulin, in which they concluded that increased serum cortisol levels led to decreased insulin sensitivity and hence a raised fasting blood glucose level.[32] As we have already stated that hyperoxia induces oxidative stress and leads to an increased production of cortisol so maybe the increase in fasting blood glucose observed was due to an increase in serum cortisol levels produced on exposure to hyperoxia. As we did not measure other hormones apart from PYY, such as cortisol and ghrelin, so it remains unclear as to which hormone could have brought about the change observed in fasting blood glucose levels as seen in our study.

On the other hand, the significant increase in fasting blood glucose observed can further be elaborated by the study carried out by Stolicet al., (2002) to determine the influence of BMI, anatomical depot and body fat distribution on glucose uptake and insulin action in human adipose tissue in 68 subjects. They concluded that glucose uptake was increased in the omental adipose tissue of lean subjects, whereas it was decreased in the omental adipose tissue of obese subjects as their adipose tissue cells had developed resistance towards insulin.[33] Hence it may be postulated that the increased fasting blood glucose levels observed in our study may have resulted from an increase in adipocyte size which occurs when there was an increase in weight.

Conclusion

The conclusion derived from the results of the present study are thathyperoxia decreases serum PYY levels causing an increase in appetite leading to an increase in weight and fasting blood glucose. Therefore, hyperoxia is not a useful option for the treatment of obesity and may be a precipitating factor for the development of diabetes mellitus.

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