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Article:
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Title: Possible hypoxia signaling induced alteration of glucose homeostasis in rats exposed to chronic intermittent hypoxia - Role of antioxidant (vitamin C) and Ca^{2+} channel blocker (Cilnidipine)

Abstract:

Background: Hyperglycemia founds to be a regulator of HIF-1α gene expression but the regulation of HIF-1α on glucose homeostasis is unclear. Objective: To determine whether chronic intermittent hypoxia (CIH) alters glucose regulation and such alterations can revert through treatments with either antioxidant (vitamin c) or calcium channel blocker (cilnidipine) in male albino rats. Methods: The rats were divided into six groups i.e. normoxia (21 % oxygen), CIH (10% oxygen with cycle time 3:1.5; 8h/day), normoxia with vitamin c (50 mg /100g. b.wt, orally), CIH with vitamin c, normoxia with cilnidipine (1 mg/kg/day; ip) and CIH with cilnidipine. Serum MDA, HIF-1α, fasting plasma glucose, insulin, GTT, HOMA-IR and insulinogenic index were evaluated. Results: Serum HIF-1α and MDA concentration in rats exposed to CIH increased significantly whereas simultaneous CIH with vitamin c and CIH with cilnidipine treatment show reversion of both serum HIF- 1α and MDA concentrations towards normoxic status. CIH rats showed increased fasting glucose level with unchanged plasma insulin level but both vitamin c and cilnidipine treatment improved the status. Elevated HOMA-IR and insulinogenic index along with impaired GTT were found in CIH groups although vitamin c and cilnidipine improved the glucose homeostasis in CIH exposed rats. Conclusion: CIH induces over production of reactive oxygen species as well as hyper activities of sympathetic N-type Ca^{2+} channels possibly through HIF 1-α expression and influence on insulin signaling by causing hyperglycemia, glucose intolerance and insulin resistance in rats. Simultaneous treatment with vitamin c or cilnidipine improves glucose homeostasis in CIH exposed rats.

Key words: chronic intermittent hypoxia; HIF-1α; malondealdehyde; glucose homeostasis; vitamin c; cilnidipine

1. INTRODUCTION

Hypoxia usually classified into sustained or intermittent variant and may be either acute or chronic in exposure. Normally chronic sustained hypoxia may be due to physiological or pathological reasons but chronic intermittent hypoxia (CIH) is always pathological by nature.

In general, the effect of hypoxia on the physiological system is controlled by hypoxia inducible factor-1 α (HIF-1α) protein. HIF-1α is a transcription factor and remained stable in presence of low oxygen tension and facilitates an adaptive mechanism inside the cell against hypoxia exposure. Under hypoxic status the proline residues are not hydroxylated by prolyl hydroxylase (PHD) hence hif-1α remains stable and not degraded. This stabilized HIF 1α is translocated in the nucleus where it dimerises with HIF-1β, and associates with other factors like CREB - binding protein, CBP/p300 [1-2]. Chronic intermittent hypoxia is commonly seen in obstructive sleep apnoea. It is associated with cardiovascular diseases, hypertension and impaired glucose homeostasis. Several experiments on animal models revealed a definite correlation between CIH and metabolic impairments like glucose intolerance, decrease insulin sensitivity or altered insulin secretary functions etc (Wahren J 2007; Carreras A, 2012). Study reveals that CIH influences reactive oxygen species (ROS) production which in further stimulates HIF-1 α gene expression consequently continuance of oxidative stress. This effect augments further production of HIF-1 α [3]. Relationship was established between CIH and vascular proliferation or increase of vascular reactivity or right heart failure [4].

Recent observation reveals that CIH activates NADPH oxidase which is very important for HIF-1 expression and ROS production. NADPH oxidase hyperactivities also changes intracellular calcium homeostasis and stimulate further HIF 1 production, subsequently resulting in more ROS generation. High concentration of ROS excites carotid bodies and adrenal medulla that influence on adrenergic activities via chemoreflex and alters catecholamine status [5].

A relation between increased sympathetic activation and hypoxia with consequent impaired glucose homeostasis provides a thought of possible protective counteraction with N-type calcium channel blockers which are normally used to control hypertension or heart diseases [6].

An interesting study showed that fasting glycemia become corrected after withdrawal of two weeks CIH exposure but insulin resistance and beta cell abnormalities remain unchanged [7] Although the link between CIH and altered glucose metabolism is understood but exact mechanism of impaired glucose tolerance during CIH exposure is yet to establish.

Thus the present study was undertaken to find out the CIH induced alteration of hypoxia signaling pathways and its possible impact on glucose metabolism. The objectives of the study were also to determine whether such alterations revert through treatments with either antioxidant (vitamin C) or fourth generation L/N type calcium channel blocker (cilnidipine) in male albino rats.

2. MATERIALS AND METHODS

Animals

Adult (age 60–70 days) laboratory-bred male Wister rats, weighing 180 ± 20g fed with laboratory stock diet and water ad libitum were acclimatized for 7 days to the laboratory conditions at 22°–24°C and a 12 h light: Dark cycle. The acclimatized rats were divided into six groups of six rats each. Three rats were kept in each metabolic wire cage (60 cm × 30 cm × 20 cm). Animals were taken care of as per the CPCSEA guidelines and the experimental protocol was duly approved by Institutional Animal Ethics Committee.

Experimental Groups

Group I served as control (normoxia, 21 % oxygen), group II rats were exposed to intermittent hypoxia (CIH, 10 % O_2 and 90% N_2) for 21 days, group III rats were supplemented with vitamin C (normoxia + vitamin C, dosage 50 mg / 100g. b.wt, orally) [8] for 21 days, group IV rats were exposed to intermittent hypoxia and vitamin C supplementation (CIH + vitamin C) for 21 days), group V rats were treated with calcium channel blocker cilnidipine (normoxia + cilnidipine, dosage 1 mg/kg/day; ip) [9] for 21 days (and group VI rats exposed to CIH and simultaneously treated with cilnidipine (CIH + cilnidipine) for 21 days.

Exposure of Animals to Chronic Intermittent Hypoxia

For CIH exposure, caged rats (3 per cage) were put inside a 300-liter acrylic chamber, which held a maximum of 4 cages (12 rats). The rats were placed in hypoxic chamber (10 % O_2 and 90% N_2; the cycle time of intermittent hypoxia was 180 sec for the hypoxia and 90sec for normoxia respectively) for 8 hours from 9:00-17:00 per day for 21 days [10]. The hypoxic environment was established with the inflow of a mixture of room air and nitrogen that was regulated by an oxygen analyzer (model 175518A, Gold Edition, Vacuum Med). CO_2 was absorbed by soda lime 27 granules, and excess humidity was removed by a desiccators. Temperature was maintained at 24-26°C. The chamber was opened twice a week for 1 h to clean the cages and replenish food and water. As intermittent hypoxia causes weight loss hence a dietary pair fed system between normoxic groups and intermittent hypoxic groups were maintained throughout the experimental period to keep the same body weight trajectory. All the animals were sacrificed on the 22nd day early morning between 09:00h – 11:00h to avoid diurnal rhythm on animals [11].

Gravimetry

The body weight of all the rats was recorded on the day 1 of the hypoxia treatment and the day of sacrifice in a single pan electronic balance (ATCO. Model D2RS02-W).

Biochemical analysis

Serum HIF-1α was estimated by using commercially available ELISA kit (KT-17920, Kamiya, Biomedical Company,USA) and serum malondialdehyde (MDA), a marker of oxidative stress was estimated by Kei Satoh method [12].

Glucose homeostasis: Fasting blood glucose was estimated by Accu-check active (Roche diagnostics, Germany) at 0 and 30 minutes after glucose administration (1g/kg b.wt.). Blood samples of all the six groups were collected and transferred to heparin containing microtubules which were placed in an ice bath for 20 minutes and centrifuged for 7

minutes at 4000 rpm and then plasma insulin concentrations were also measured at 0 and 30 minutes after glucose administration by ELISA kits (ERINS, Thermo Fisher Scientific, Life technologies). Homeostasis model assessment-insulin resistance (HOMA-IR) were evaluated by using the following formula:-HOMA-IR = (fasting plasma insulin (μg/L)×fasting blood glucose (mg/dL) / 22.5) [13-14]. Insulinogenic - index were estimated after glucose (1g/kg b.wt.) was administrated orally, and blood samples were drawn with heparinized capillary tubes from the retro-orbital plexus at 0 and 30 minutes for measurement of blood glucose and plasma insulin concentrations. Insulinogenic-index was calculated by using the following equation:-

Insulinogenic-index = (30 min plasma insulin-fasting plasma insulin) / (30 min blood glucose-fasting blood glucose)[13,15-16]. Oral glucose tolerance tests (OGTT) were done by evaluating fasting blood sugar level (FBS) in the rats of all groups. The rats of all six groups were orally treated with 0.35 mg/100g b. wt. of glucose. Blood samples were collected from the rat tail vein just prior to glucose administration (0.0 h) and at 0.5 h, 1.0 h, 1.5 h and 2.0 h after glucose loading. Blood glucose levels were measured immediately by using glucometer (Accu-check active, Roche Diagnostics, Mannheim,

Germany)[17].

Statistical Analysis:

Results were presented as mean ± standard deviation values for each group. Statistical analysis of the data was performed to determine the significance of inter-group differences by one-way analysis (ANOVA), followed by "post-hoc t-test".

3. RESULTS

Table 1 showed CIH exposed rats gained least body weight (%) during 21 days experimental period among all the groups. Both vitamin C supplemented and cilnidipine treated CIH exposed rats showed uniform or greater % body weight gain with normoxic rats.

Table 1 Effect of chronic intermittent hypoxia (CIH) and simultaneous supplementation of vitamin C (50 mg / 100g. b.wt, orally) and cilnidipine (1 mg/kg/day; ip) on body weight changes in rats.

Parameters	Normoxia	CIH	Nomoxia + Vitamin C	CIH + Vitamin C	Normoxia + Cilnidipine	CIH + Cilnidipine
Initial Body Weight (g)	187.5 ± 5.00^{a}	190.5 ± 8.25^{a}	185.17 ± 7.50^{a}	185.50 ± 6.25^{a}	187.75 ± 8.25^{a}	187 ± 5.00^{a}
Final Body Weight (g)	215.25 ± 12.50^{a}	200.08 ± 5.70^{b}	220.00 ± 10.50^{c}	205.50 ± 5.50^{b}	215.50 ± 15.50^{a}	215.50 ± 10.50^{a}
% Body Weight gain	12.50 ± 3.50^{a}	5.25 ± 1.00^{b}	17.50 ± 2.24^{c}	11.00 ± 2.50^{a}	13.50 ± 2.50^{a}	12.40 ± 2.00^{a}

Horizontal values are the mean ± SD of six observations in each group. In each row, values with different superscripts (a, b, c, d) are significantly different from each other ($p<0.05$).

Fig.1 shows a significant elevation of serum HIF-1α concentration in rats exposed to CIH as compared to normoxic rats. However simultaneous treatment with either vitamin C (dosage 50 mg / 100g. b.wt, orally) or cilnidipine (dosage 1 mg/kg/day; ip) on CIH exposed rats significantly decreases serum HIF-1α concentration as compared to CIH

exposed rats. Vitamin C dosage selected here is referred to as high dose and cilnidipine is a unique dihydropyridine derivative L-type Ca2+ channel blocker with an inhibitory action on the sympathetic N-type Ca2+ channels. It has superior cardioprotective, renoprotective and neuroprotective effects.

Further to note that serum HIF-1α concentration significantly reduces in cilnidipine treated CIH exposed rats as compared to vitamin C supplemented CIH exposed rats.

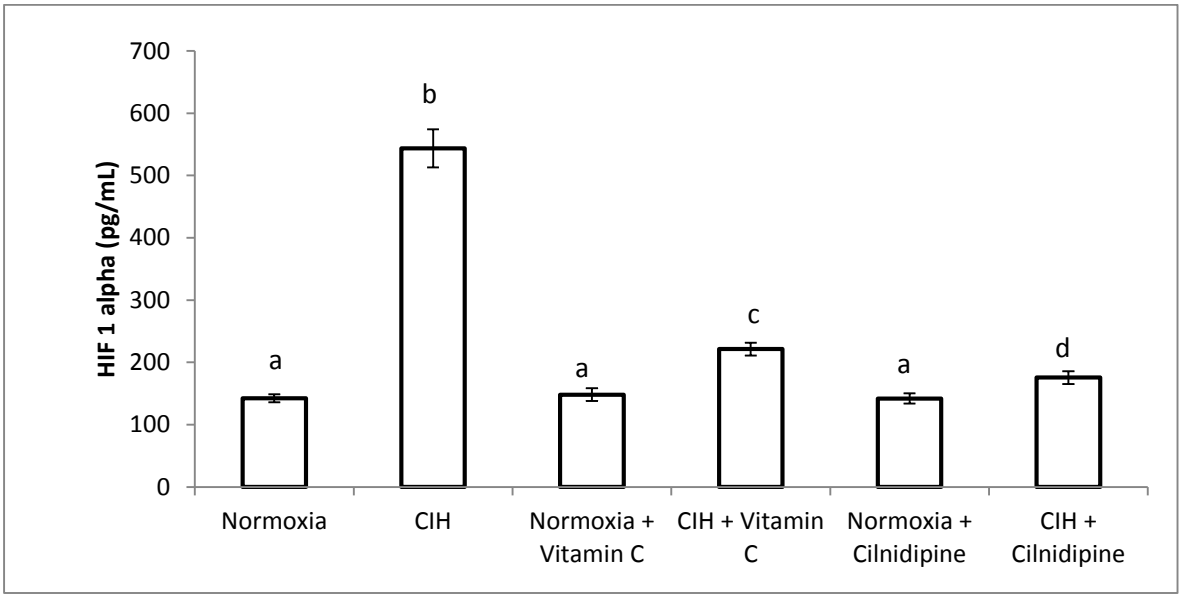

Fig.1: **HIF-1α concentration in different experimental groups exposed to chronic intermittent hypoxia (CIH) and simultaneous supplementation of vitamin C (50 mg / 100g. b.wt, orally) and cilnidipine (1 mg/kg/day; ip).** Values with different superscripts (a, b, c, d) are significantly different from each other ($p<0.05$); n = 6 in each group.

Fig.2 shows a significant increase of MDA concentration in CIH exposed rats in comparison to normoxic animals. Rats treated with either vitamin C or cilnidipine showed significant reduction of serum MDA level as compared to CIH exposed rats. Interestingly vitamin C supplemented CIH exposed rats showed significant decrease of serum MDA level in comparison to cilnidipine treated CIH exposed rats.

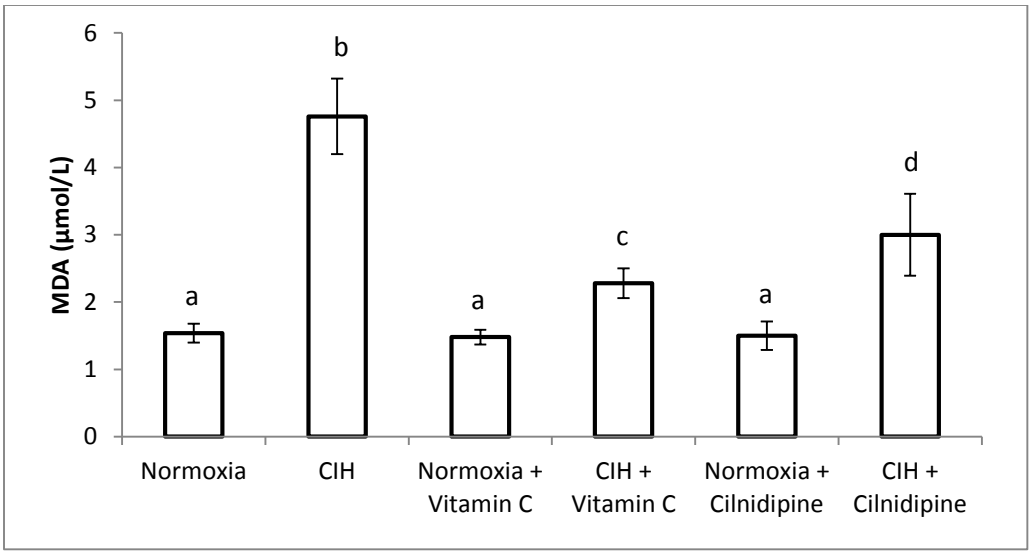

Fig. 2: Malondealdehyde (MDA) concentration in different experimental groups exposed to chronic intermittent hypoxia (CIH) and simultaneous supplementation of vitamin C (50 mg / 100g. b.wt, orally) and cilnidipine (1 mg/kg/day; ip). Values with different superscripts (a, b, c, d) are significantly different from each other ($p<0.05$); n = 6 in each group.

Table 2 shows a significant rise in fasting blood glucose concentration in rats exposed to chronic intermittent hypoxia (CIH) as compared to normoxic group rats whereas rats treated with either vitamin C or cilnidipine showed remarkable improvement in fasting blood glucose level. Fasting plasma insulin levels in all the six groups remained unchanged whether in case of CIH exposed rats or rats treated with either vitamin C or cilnidipine simultaneously. CIH group also showed significant increase of HOMA-IR and insulinogenic-index values as compared to normoxic group. In case of simultaneous vitamin C treated CIH exposed rats or cilnidipine treated CIH exposed rats, both HOMA-IR and insulinogenic-index decreased significantly as compared to CIH alone exposed rats. Fig.3 depicts a comparative analysis of percent change differences of HOMA-IR and insulinogenic – index in all the experimental groups as compared to normoxia group of rats. The results showed percent change increase of both HOMA-IR (61.8%) and insulinogenic-index (71.87%) in CIH exposed group. In case of CIH exposed rats simultaneously treated with vitamin C and cilnidipine showed remarkable improvement of percent change differences in both HOMA-IR (vitamin C, 28.8% ; cilnidipine, 23.90%) and insulinogenic – index (vitamin C, 25.0% ; cilnidipine, 6.25%).

Table 2: Effect of chronic intermittent hypoxia (CIH) and simultaneous supplementation of vitamin C (50 mg / 100g. b.wt, orally) and cilnidipine (1 mg/kg/day; ip) on glucose homeostasis (blood glucose, plasma insulin, HOMA-IR and insulinogenic response) in rats after 21 days treatment

Parameters	Normoxia	Chronic Inrtermittent Hypoxia (CIH)	Normoxia + Vitamin C	CIH + Vitamin C	Normoxia + Cilnidipine	CIH + Cilnidipine	't' value	df	'p' value
Fasting blood glucose (mg/dl)	75.00 ± 2.37[a]	128.45 ± 3.58 [b]	80.00 ± 3.30 [a]	100.00 ± 5.20 [c]	77.00 ± 2.82 [a]	91.23 ± 4.65[d]	3.54	5	0.0000
Fasting plasma Insulin (μg/L)	1.00 ± 0.17 [a]	1.01± 0.19 [a]	1.04 ± 0.11[a]	1.03 ± 0.22 [a]	1.03 ± 0.18 [a]	0.98 ± 0.14 [a]	0.58	5	0.2671
HOMA-IR	3.30 ± 0.11 [a]	5.34 ± 0.55 [b]	3.42 ± 0.35 [a]	4.23 ± 0.44 [c]	3.26 ± 0.16 [a]	4.09 ± 0.26 [d]	2.64	5	0.0015
Insulinogenic - index	0.0032 ± 0.0005 [a]	0.0055 ± 0.0005 [b]	0.0034 ± 0.0001[a]	0.0040 ± 0.0002 [c]	0.0033 ± 0.0004 [a]	0.0034 ± 0.0003 [a]	2.45	5	0.0018

Horizontal values are the mean ± SD of six observations in each group. In each row, values with different superscripts (a, b, c, d) are significantly different from each other ($p<0.05$).

The results also reveal that N-type calcium channel blocker cilnidipine regulates glucose metabolism better than antioxidant vitamin C on rats exposed to chronic intermittent hypoxia.

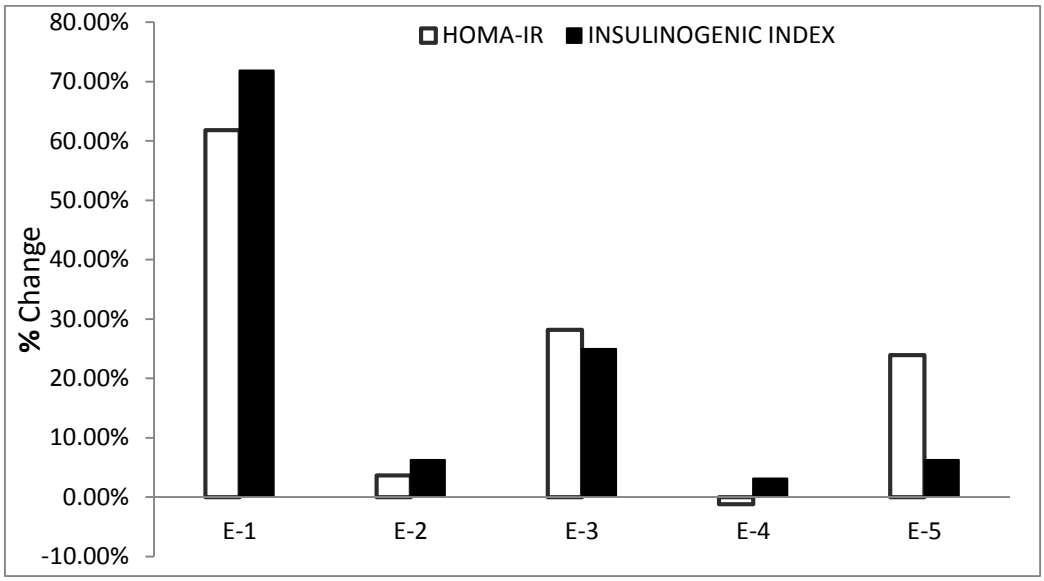

Fig. 3: Percent change chart of HOMA-IR and Insulinogenic index in rats exposed to chronic intermittent hypoxia (CIH) and simultaneous treatment with vitamin C and cildinipine. E-1, Normoxia vs CIH; E-2, Normoxia vs Vitamin C; E-3, Normoxia vs CIH + Vitamin C; E-4, Normoxia vs Cilnidipine; E-5, Normoxia vs CIH + Cilnidipine; n = 6 in each group.

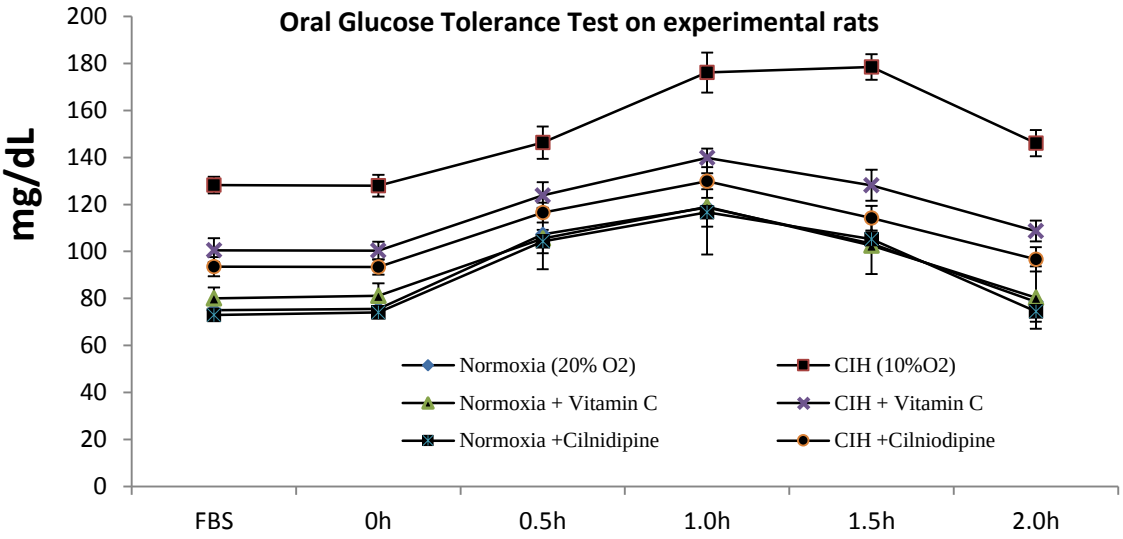

Fig. 4: Effect of chronic intermittent hypoxia (CIH) and simultaneous supplementation of vitamin C (50 mg / 100g. b.wt, orally) and cilnidipine (1 mg/kg/day; ip) on oral glucose tolerance test (OGTT) in rats (n=6 in each group) after 21 days treatment

Fig.4 shows OGTT of all the six groups of rats. CIH exposed rats showed impaired glucose tolerance by reaching the peak concentration at 1.5h and even at 2h the glucose concentration did not come back to baseline. However the CIH

exposed rats treated simultaneously with either vitamin C or cilnidipine showed better glucose tolerance in fact cilnidipine treated CIH exposed animals were having superior glucose tolerance than vitamin C treatment group.

4. DISCUSSION

The present study shows that exposure of chronic intermittent hypoxia induces hyperglycemia, glucose intolerance and insulin resistance in rats. These changes are connected with elevated levels of HIF-1α concentration and simultaneous treatment with antioxidant (vitamin C) and N-type calcium channel blocker (cilnidipine) ameliorate overall insulin sensitivities. There are variable reports on intermittent hypoxia induced glucose metabolism. Studies reveal that both chronic and acute intermittent hypoxia can increase gluconeogenesis, increase hepatic glucose output, increased sympathetic drives, increased circulating steroids and enhanced HIF-1α concentration. Increase level of HIF-1α concentration may in turn influences transcription of multiple enzymes require for gluconeogenesis [7].

Increase HIF-1α concentration due to CIH in our study indicates oxygen-sensing by carotid bodies and perhaps generate ROS besides regulating sympathetic adrenergic drive. One of the characteristic features of CIH is generation of ROS probably due to repetitive re-oxygenation which can also regulate intracellular hypoxia transcription factors [18]. The present study shows that CIH leads to formation of ROS indicated by increasing serum MDA level in CIH exposed groups and effectively counteracted by both antioxidant (vitamin C) and calcium channel blocker (cilnidipine). Upon excessive generation of ROS by CIH may possibly react with circulatory nitric oxide (NO), a crucial vasodilator and produce peroxynitrite. This mechanism impair NO dependent vasodilatation by reducing bioavailability of NO in circulatory system [19]. Several studies reported that moderate intermittent hypoxia exposure causes increase in lipid peroxidation including superoxide and hydrogen peroxide generation in different metabolically active tissues [20]. The role of HIF-1α on activation of ROS in the cell is established and it is also noticed that the binding site of these redox-regulated transcription factors are situated in the promoter region of antioxidants encoded genes [21].. It is suggested that the production of ROS during intermittent hypoxia may be due to a link between HIF-1α and NF-kβ which may be well controlled by antioxidants [22]. Vitamin C supplementation in rats exposed to CIH in our present study showed a remarkable improvement of both HIF-1α and MDA concentration probably supported this link.

The role of calcium channel blocker- cilnidipine on HIF-1α and MDA regulation are also found to be interesting. Cilnidipine is having both L- and N-type calcium channel blocking actions hence it is useful to inhibit sympathetic overdrive and reduces norepinephrine release from adrenergic nerve endings. This makes cilnidipine a cardioprotective agent. High concentration of serum HIF-1α and MDA due to CIH suggest sympathetic over activities and excessive ROS production [23]. Simultaneous treatment with cilnidipine in our study showed a remarkable improvement of both HIF-1α and MDA level which clearly indicates cilnidipine perhaps decreased sympathetic over activities and generation of excessive ROS. Our findings are further supported by the previous observation that cilnidipine influence on HIF-1α expression is dependent on O_2 concentration [24].

One of the serious implications of CIH is the balance between vasoconstriction and vasodilatation as reports said that most of the cases CIH usually induce vasoconstrictions but in some cases vasodilatation also. CIH possibly stimulate chemoreceptor activity and subsequently vasoconstriction and systemic hypertension. Beside the elevation of

chemoreflex by CIH, an impairment of baroreflex is also the possibility of for increase in vasoconstriction due to CIH [25-26].

The interesting and novel findings of this investigation are the decreased insulin sensitivities and increase serum HIF-1α concentration due to the exposure of CIH. These findings are supported by several previous observations of impaired glucose tolerance with increase insulin resistance (IR) during chronic intermittent hypoxia on animals and humans [3,27].

It is unlikely that increased sympathetic activation is entirely responsible for hypoxia induced insulin resistance, and thus our observation on increase ROS generation indicated by increase MDA level in CIH group too may be considered as influential contributor for altering insulin signaling and developing insulin resistance in rats [6].

Another interesting observation in this study is to find out the treatment of cilnidipine as a better regulator of glucose homeostasis and HIF-1α than vitamin C supplementation in spite of having higher MDA levels.. It may possibly be due to greater sympathetic imbalance rather than generation of ROS system by CIH in this study which is effectively counteracted by cilnidipine as a L-N type calcium channel blocker than vitamin C [**28**]

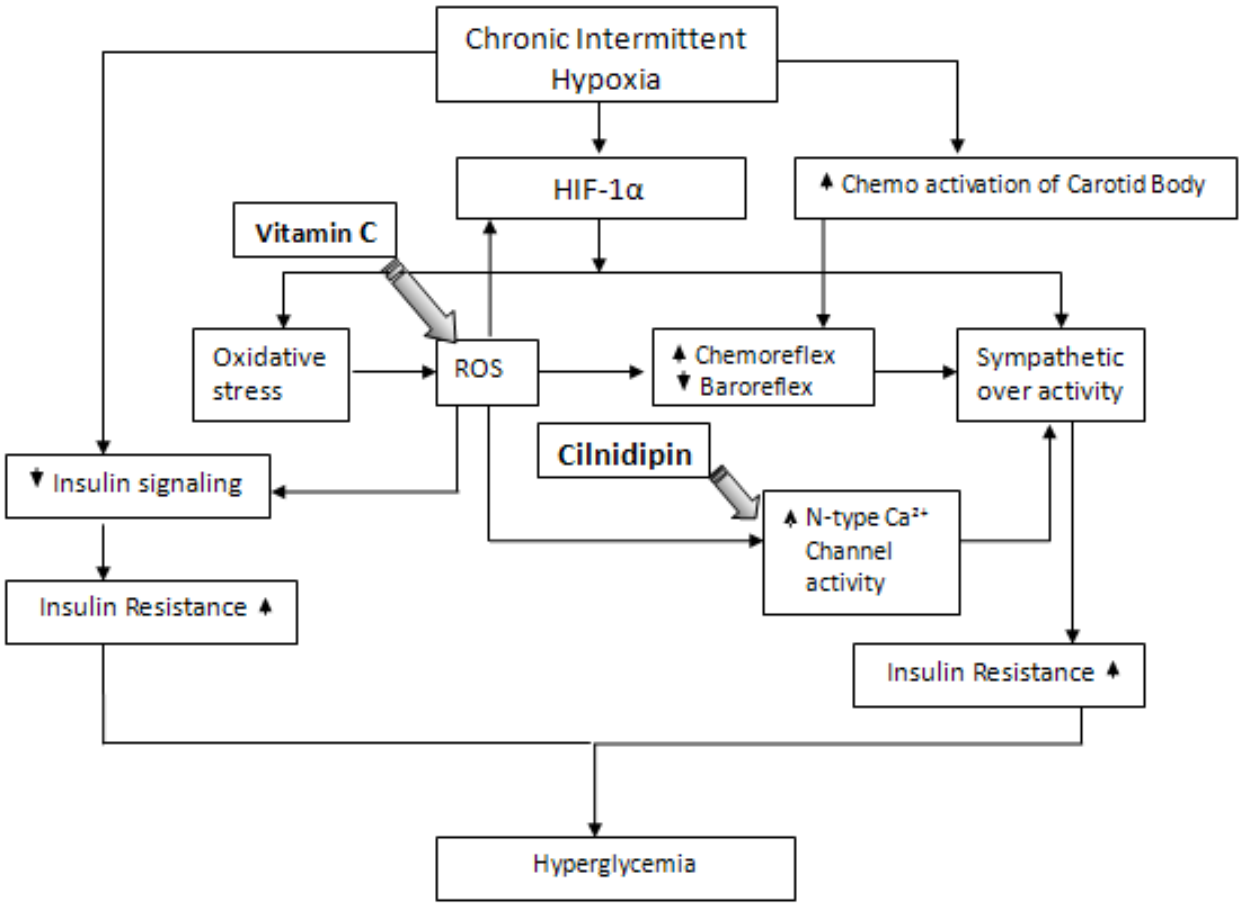

Fig.5: Schematic diagram outlining the hypothesized pathways by which chronic intermittent hypoxia leads to hyperglycemia

Hence results from this study can clearly enlightened us the role of CIH induces HIF-1α signaling pathways and altered oxidant/antioxidant balance, possibly stimulate chemoreflex and inhibit baroreflex through either ROS or direct chemoactivation of carotid bodies . It also causes sympathetic over activities and alter insulin signaling mechanisms and resultant insulin resistance. Our findings on improvement of glucose homeostasis in case of simultaneous antioxidant (vitamin C) and N- type calcium channel blocker (cilnidipine) treatment clearly support that both sympathetic over activation or oxidative stress are responsible to induce insulin resistance and alter glucose metabolism in rats (Fig.4). It has been reported that CIH can also contribute to insulin resistance through lowering GLUT 4 expression in rats [29].

The present study really clarifies certain aspects of CIH which is clinically important for patients with chronic obstructive pulmonary disease or sleep apnoea. The study reveals that CIH is linked to insulin signaling pathways, insulin sensitivities and metabolic syndrome in rats perhaps through CIH induced increase generation of ROS or sympathetic over activities which may be well controlled by treatment of antioxidant or N-type calcium channel blockers [30-31]. Sympathetic over activities may be regulated by either direct influences of CIH via chemoreceptor or through increase in HIF-1α. or through ROS (Fig.5). The role of sympathetically mediated breakdown of fat and generation of free fatty acids may also influence CIH induced insulin resistance [6]. ROS impact on insulin sensitivity may be slightly different from sympathetic actions induced by hypoxia. ROS itself may directly controls insulin signaling or stimulate N-type calcium channel and induced sympathetic over activity or even facilitate to further increase of HIF-1α expression [32]. Increase in generation of ROS due to CIH emphasized an impairment in beta cell function although the present study did not reveal any changes in insulin level due to CIH. Hence it may be attributed purely on decrease insulin sensitivities supported by increase HOMA-IR and insulinogenic-index in present study [7,33]

In conclusion, it may be stated that CIH induces impaired insulin sensitivities and resultant hyperglycemia by either direct or through HIF-1α. This disturbed glucose homeostasis may be remodeled by antioxidant like vitamin C or N-type calcium channel blocker like cilnidipine treatment.

CONFLICT OF INTEREST: The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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REFERENCES

[1]. Semenza GL. Hypoxia-inducible factor 1 and cancer pathogenesis. IUBMB Life. 2008 Sep;60(9):591-7

[2].Das KK, Saha S. Hypoxia, Lead Toxicities and Oxidative stress: Molecular interactions and antioxidant (Vitamin C) defense. Current Signaling Transduction & Therapy 2014, 9: 113-12

[3].Semenza GL[1], Prabhakar NR. HIF-1-dependent respiratory, cardiovascular, and redox responses to chronic intermittent hypoxia. Antioxid Redox Signal. 2007;9(9):1391-6.

[4].Fresquet F, Pourageaud F, Leblais V, et al. Role of reactive oxygen species and gp91phox in endothelial dysfunction of pulmonary arteries induced by chronic hypoxia. Br J Pharmacol 2006; 148 (5):714-723. doi:10.1038/sj.bjp.0706779.

[5]..Nanduri J, Vaddi DR, Khan SA, Wang N, Makarenko V, Semenza GL, et al. HIF-1α Activation by Intermittent Hypoxia Requires NADPH Oxidase Stimulation by Xanthine Oxidase. PLoS ONE 2015; 10(3): e0119762. doi:10.1371/journal.pone.0119762

[6].Peltonen GL, Scalzo1 RL, Schweder1 MM, Larson DG, Luckasen GJ, Irwin D, Hamilton KL et al. Sympathetic inhibition attenuates hypoxia induced insulin resistance in healthy adult humans. J Physiol 2012; 590 (11): 2801–2809

[7].Polak J; Shimoda LA; Drager LF; Undem C; McHugh H; Polotsky VY; Punjabi NM. Intermittent hypoxia impairs glucose homeostasis in C57BL6/J mice: partial improvement with cessation of the exposure. SLEEP 2013; 36(10):1483-1490.

[8].Husain K, Sugendran K, Pant SC, Sharma VP, Vijayaraghavan R (1992) Biochemical and pathological changes in response to hyperoxia and protection by antioxidant in rats. Indian J Physiol Pharmacol 1992; 36:97–100

[9].Kobayashi N, Mori Y, Mita S, Nakano S, Kobayashi T, Tsubokou Y, Matsuoka H. Effects of cilnidipine on nitric oxide and endothelin-1 expression and extracellular signal-regulated kinase in hypertensive rats. Eur J Pharmacol. 2001; 422 (1-3):149-57.

[10].Lin YM, Huang SK, Wang HF, Chen LM, et al. Short-term versus long-term intermittent hypobaric hypoxia on cardiac fibrosis and fas death receptor dependent apoptotic pathway in rat hearts. Chinese J Physiol 2008; 51(5): 308-316

[11].Committee for the Purpose of Control and Supervision on Experiments on Animals. CPCSEA guidelines for laboratory animal facility. Indian J Pharmacol 2003; 35:257–274

[12].Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta 1978; 90:37-43

[13].Asano T, Yoshida R, Ogata H, Kokawa K, Ogimoto M, Akehi Y, Anzai K, Ono J, Tamura K, Hidehira K & Kikuchi M. Beta-Cell Function is a Major Contributor to Oral Glucose Disposition in Obese Japanese Students. Endocr J 2007; 54:903-910.

[14] Nunes E, Peixoto F, Louro T, Sena CM, Santos MS, Matafome P, Moreira PI & Seica R. Soybean oil treatment impairs glucose-stimulated insulin secretion and changes fatty acid composition of normal and diabetic islets. Acta Diabetol 2007; 44:121- 130.

[15.]Jue Li, Takashi Kaneko, Li-Qiang Qin, Jing Wang, Yuan Wang & and Akio Sato (2003). Long-term effects of high dietary fiber intake on glucose tolerance and lipid metabolism in GK rats: Comparison among Barley, Rise and Cornstarch. Metabolism 2003; 53: 1206-1210.

[16.]Pratley RE, Weyer C. Progression from IGT to type 2 diabetes mellitus: the central role of impaired early insulin secretion. Curr Diab Rep 2002; 2:242-8

[17].Sridevi S, Chary MG, Krishna DR, Diwan PV. Pharmacodynamic evaluation of transdermal drug delivery system of glibenclamide in rats. Indian J Pharmacol 2000;32:309–31.

[18].Ryan S, Taylor CT, McNicholas WT. Selective Activation of Inflammatory Pathways by Intermittent Hypoxia in Obstructive Sleep Apnea Syndrome. Circulation. 2005;112: 2660-2667.

[19].Jordan W, Cohrs S, Degner D, Meier A, Rodenbeck A, Mayer G, Pilz J, Rüther E, Kornhuber J, Bleich S. Evaluation of oxidative stress measurements in obstructive sleep apnea syndrome. J Neural Transm (Vienna). 2006 ; 113(2):239-54.

[20].Gonchar, O. and I.Mankovska. Glutathione system adaptation to acute stress in the heart of the rats during different regimes of hypoxia training. Ukr Biokhim Zh 2007; 79: 79-85.

[21]. Gonchar, O. and I.Mankovska, Antioxidant system in adaptation to intermittent hypoxia. J Biol Sci 2010, 10(6): 345-354

[22]. Sen CK, Packer L.Antioxidant and redox regulation of gene transcription. FASEB J. 1996 ;10(7):709-20.

[23]. Oda S, Oda T, Takabuchi S, Nishi K, Wakamatsu T,Tanaka T, Adachi T, Fukuda K, Nohara R, Hirota K. The calcium channel blocker cilnidipine selectively suppresses hypoxia-inducible factor 1 activity in vascular cells. Eur J Pharmacol. 2009; 606 : 130–136

[24].Lang K., Kappel A., Goodall G.Hypoxia-inducible factor-1α mRNA contains an internal ribosome entry site that allows efficient translation during normoxia and hypoxia. Mol. Biol. Cell 2002; 13: 1792–1801.

[25.]. Fletcher EC, Orolinova N, Bader M .Blood pressure response to chronic episodic hypoxia: the renin-angiotensin system. J Appl Physiol 2002; 92, 627–633.

[26]. Lai CJ, Yang CC, Hsu YY, Lin YN & Kuo TB (2006). Enhanced sympathetic outflow and decreased baroreflex sensitivity are associated with intermittent hypoxia-induced systemic hypertension in conscious rats. J Appl Physiol 2006; 100: 1974–1982

[27].Oltmanns KM, Gehring H, Rudolf S, Schultes B, Rook S, Schweiger U, Born J, Fehm HL & Peters A. Hypoxia causes glucose intolerance in humans. Am J Respir Crit Care Med 2004; 169: 1231–1237.

[28]. Takahara A, Koganei H, Takeda T, Iwata S. Antisympathetic and hemodynamic property of a dual L/N-type Ca2+ channel blocker cilnidipine in rats. Eur J Pharmacol 2002; 434(1-2): 43–47

[29]. Tan J, Mo H, Li J, Wu Y, He X, Li B. Effects of chronic intermittent hypoxia on glucose transporter 4 expression in rat skeletal muscles. Nan Fang Yi Ke Da Xue Xue Bao. 2014; 34(7):1061-4.

[30].Schober AK, Neurath MF, Harsch IA. Prevalence of sleep apnoea in diabetic patients. Clin Respir J 2011; 5: 165–172

[31]..Lambert EA & Lambert GW. Stress and its role in sympathetic nervous system activation in hypertension and the metabolic syndrome. Curr Hypertens Rep 2011; 13: 244–248.

[32]..Biswas S, Mukherjee R, Tapryal N, Singh AK, Mukhopadhyay CK. Insulin Regulates Hypoxia-Inducible Factor-1a Transcription by Reactive Oxygen Species Sensitive Activation of Sp1 in 3T3-L1 Pre adipocyte. PLoS ONE 2013; 8(4): e62128. doi:10.1371/journal.pone.006212ereht 8

[33]..Conde SV, Sacramento JF, Guarino MP, Gonzalez C, Obeso A, Diogo LN, Monteiro EC, Ribeiro MJ. Carotid body, insulin, and metabolic diseases: unraveling the links. Front. Physiol 2014; 5:418. doi:10.3389/fphys.2014.00418