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Butyric Acid Suppresses Adipogenesis Triggered by Intermittent Hypoxia and Hypercapnia via sAC/cAMP/PKA Pathway Modulation

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ABSTRACT

Background: To explore whether intermittent hypoxia and hypercapnia i.e., intermittent hypoxic-hypercapnia (IHH), the main characteristics of obstructive sleep apnea (OSA), induce adipogenesis, and assess the inhibitory effect of butyric acid, (BA) on intermittent hypoxia or hypercapnia accelerated adipogenesis.

Methods: To determine the potential impact IHH on adipogenesis, cells were exposed to simultaneous transient hypoxic-hypercapnia (4% O2, 10% CO2) for 48 hours. Intracellular lipid and triglyceride accumulation of groups above was observed using an Oil Red O stain and triglyceride assay. To clarify the underlying mechanism, the expression level of the pro-adipogenic markers including peroxisome proliferator–activated receptor (PPAR)γ, CCAAT/enhancer binding protein (CEBP)α, CEBPβ and cAMP Response Element-Binding Protein (CREB) were measured. And the protein expression of soluble adenylyl cyclase (sAC) cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) was also assessed.

Results: IHH accelerated lipid accumulation, triglyceride formation, and protein expression of PPARγ, CEBPα, CEBPβ and CREB which were inhibited by butyric acid. Additionally, butyric acid suppressed the sAC/cAMP/PKA pathway protein expression in IHH-induced adipogenesis.

Conclusion: This study reveals that OSA-induced adipogenesis results from the synergistic effort of hypoxia and hypercapnia, also butyric acid could inhibit adipogenesis induced by hypoxic-hypercapnia via the suppression of sAC-dependent cAMP production and PKA activation.

INTRODUCTION

Obstructive sleep apnea (OSA) is characterized by recurring blockage of the upper airway during sleep, sleep fragmentation, intermittent hypoventilation resulting in hypercapnia and hypoxia, and extreme drowsiness during the day Bonsignore et al. (2012), Kikuchi et al. (2017). There is a substantial correlation between obstructive sleep apnea (OSA) and obesity, which is regarded as the most essential and primary modifiable risk factor for OSA and also known to contribute significantly to OSA's pathogenesis in several ways Peppard et al. (2000), Young et al. (2004). Increased neck adiposity and fat deposit in the pharyngeal wall associated with obesity reduce airway lumen size leading, which promotes airway resistance and upper airway collapsibility Young et al. (2004), Jordan et al. (2014).

In addition, obesity-related hormonal changes may contribute to the pathogenesis of OSA. Obesity is associated with leptin resistance; a hormone adipose tissues release to reduce appetite. Leptin resistance is associated with leptin resistance; a hormone adipose tissues release to reduce appetite. Leptin resistance is associated with a lowered sensitivity to hypercapnia resulting in upper airway respiratory muscles activity and poor arousal responsiveness Jordan et al. (2014), Campo et al. (2007). Furthermore, obesity decreases lung volume, especially functional residual capacity, which can result in the instability of the respiratory control system due to fluctuation in blood gases Deflandre et al. (2018), Eckert et al. (2008).

Conversely, reports show that OSA itself could worsen obesity; sleep fragmentation in OSA which is associated with excessive daytime sleepiness, can lead to reduced mood and physical activity and worsen obesity if calorie intake is not reduced. Patients with OSA often complain about recent weight gain before the onset of OSA symptoms Pillar et al. (2008), Phillips et al. (1999). Also, effective continuous positive airway pressure treatment

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has been reported to decrease visceral and subcutaneous fat accumulation and induce weight loss, however, the reports were contradictory Ong et al. (2013), Quan et al. (2008). In addition, one study indicated that CIH and CSF, with or without HFD-induced dysregulation of gut microorganisms and their metabolites, could independently or cooperatively influence the risk of lipid-related metabolic disorders Wang et al. (2022).

The accumulation of white adipose tissue (WAT) is the defining characteristic of obesity, associated with excessive food consumption and decreased physical activity. Obesity is defined clinically as a Body Mass Index (BMI)≥30 or BMI ≥27.5 in Asians. Expansion of WAT occurs either as a result of adipocyte hypertrophy which increases adipocyte size or as a result of adipocyte hyperplasia/adipogenesis, which is an increase in the number of adipocytes. While adipocyte hypertrophy is often associated with WAT expansion, some studies reveal that adipogenesis could also contribute to WAT expansion, especially in morbid obesity. Additionally, the same effectors that enhance energy intake or decrease energy expenditure also promote the differentiation of new cells to store the extra calories Rosen et al. (2014), Berry et al. (2014).

Therefore, it is acceptable to assert that adipogenesis plays a significant role in the increase of WAT and the development of obesity.

There is overwhelming evidence suggesting that ventilatory dysfunction, i.e., intermittent hypoxia and hypercapnia, in OSA accelerate adipogenesis, thereby worsening obesity. The role of intermittent hypoxia in the upregulation of adipogenesis has been a subject of great debate. Some studies show intermittent hypoxia induces lipid accumulation, intracellular triglyceride formation, and adipogenesis Su et al. (2021), Weiszenstein et al. (2016). On the contrary, Wang et al. and Musutova et al. reported that intermittent hypoxia inhibited lipid accumulation, intracellular triglyceride formation, and adipogenesis Musutova et al. (2020), Wang et al. (2018) but significantly increased lipolysis. Hypercapnia has been shown to affect several biological processes, including the migration and proliferation of fibroblast and epithelial cells, autophagy in macrophages, neutrophil and macrophage activation, ion transport in epithelial cells, and muscle atrophy in animal models Vadász et al. (2008), Wang et al. (2010), Tsuji et al. (2013).

Even though hypercapnia coincides with intermittent hypoxia in OSA incredibly obese OSA patients, most studies neglect to factor in hypercapnia in experimental models of OSA. Kikuchi et al. revealed that hypercapnia increased adipogenesis by upregulating sAC-dependent cAMP production, triggering downstream signaling cascades that led to adipogenesis. This may emphasize the vicious loop between OSA and obesity, in which OSA accelerates adipogenesis, resulting in a rise in fat deposition and a worsening of obesity, exacerbating OSA if left untreated.

Another pathophysiology that could explain the bidirectional relationship between OSA and obesity is gut dysbiosis. Intermittent hypoxia and sleep fragmentation have been associated with gut dysbiosis, which includes a reduction in short-chain fatty acid (SCFA) microbes. These SCFA producing microbes are responsible for the production of SCFA by fermentation of resistant starch in the gut. Butyric acid (BA) is a SCFA is known to confer a protective role against obesity and obesity-related diseases such as non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus Su et al. (2021), van Deuren et al. (2022), He et al. (2019), Yang et al. (2020). BA has also been reported to indirectly improve OSA by improving gut health and immune regulation. It is safe to assume that BA is could beneficial in both OSA and obesity, nevertheless, there is limited knowledge regarding the particular cellular targets and mechanisms involved in facilitating the beneficial impacts on lipid metabolism especially in the context of OSA.

The main objective of this research is to understand how OSA could aggravate obesity by accelerating adipogenesis via IHH and also to explore the role of Butyric acid in mitigatiing OSA-indused adipose tissue expansion dysfunction, particularly excessive adipogenesis. The results showed, IHH could accelerate lipid accumulation, intracellular formation, and adipogenesis. Conversely, BA had an overall inhibitory on lipid accumulation, intracellular triglyceride formation, and adipogenesis. This study reveals for the first time the inhibitory effect of BA on adipogenesis with regards to OSA. However, the mechanism of action of butyric acid on sAC-dependent cAMP production could not be ascertained as investigations are still underway.

MATERIALS AND METHODS

Reagents and antibodies

Triglyceride assay kit and oil red O staining kit were purchased from Solarbio Sciences and Technology, cAMP direct ELISA kit was purchased from Bioswap Life Science Lab, Wuhan. CEBP Beta, Phospho-PKA alpha/beta/gamma CAT (Thr198), ADCY10, PPAR gamma, CEBP alpha antibodies were provided by Affinity.

Cell culture

3T3-L1 (mouse embryonic fibroblast) cell line was purchased from Procell and cultured in preadipocyte medium (DMEM, 5% Newborn calf serum, and 1% penicillin-streptomycin) for 48 hours. Cells were differentiated with an induction medium (0.5mM 3-isobutyl-1-methylxanthine 0.5mM, 1μM dexamethasone,10ng/ml insulin) under nomoxia (20% O2 and 5% CO2), hypoxic-hypercapnia (4% O2 10% CO2 for 1 hour alternating with normoxia for 30 minutes for 6 hours daily) and butyric acid treatment (with 2.5mM or10mM of butyric acid added to induction medium and cultured in IHH condition) for another 48 hours.

The cell medium was then changed to adipocytes maintenance medium (DMEM+5%FBS+1%PS+10ng/ml insulin).

Oil Red O Staining

Oil Red O staining was conducted to assess intracellular lipid formation. A stock solution was mixed with distilled water at a 3:2 ratio and filtered after 10 minutes at room temperature. Cultured cells had their medium discarded and were rinsed with PBS. Cells were fixed with ORO fixative/formaldehyde for 10 minutes, followed by a 30-second soak in 60% isopropanol. Staining with Oil Red O working solution was performed for 10 minutes at room temperature, followed by rinsing with 60% isopropanol and distilled water. Nuclei were counterstained with Mayer's hematoxylin solution for 3-5 minutes, rinsed with distilled water, and sealed with glycerin gelatin. Observation under an inverted light microscope revealed red-stained lipid droplets and blue-stained nuclei. To quantify, culture plates were soaked in 60% isopropanol for 5 minutes, and optical density (OD) was measured at 500nm wavelength.

Triglyceride Assay

Cellular triglyceride levels were quantified using a triglyceride detection kit (Solarbio Sciences and Technology Co. Ltd). Cells were seeded in 6-well plates and subjected to various conditions (normoxia, hypoxia-hypercapnia, 2.5mM butyric acid, or 10mM butyric acid) for four days. After harvesting the cells in EP tubes, a solution comprising 60 ml each of heptane and isopropyl alcohol was added, followed by ultrasonic homogenization. The supernatant's triglyceride levels were measured at 420 nm using a microplate reader (Thermo Scientific). Triglyceride content was calculated using the formula TG (U/10^4 cell) = C*(AT- AB) ÷ (AS- AB) ÷D, where C represents standard concentration (1 mg/mL), D is the density of bacteria or cells (10^4 cell/mL), and AT, AB, and AS denote absorbance values.

c-AMP Direct ELISA Assay

cAMP direct ELISA kit (#MU30394) was purchased from Bioswap Life Science Lab, Wuhan. Cell supernatant from various group were centrifuged at 7500g for 10 minutes in a refrigerated centrifuge. A test standard of 6 different concentrations (240pmol/ml, 120pmol/ml, 60pmol/ml, 30pmol/ml 12pmol/ml, and 0pmolml) according to the manufacturer's instructions. Biotinylated anti –cAMP – antibody and HRP-Conjugate reagent was added to sample and standard test well except the blank wells and were incubated at 37℃ for 30min.

The wells were washed with was buffer five times allowing the wells to soak in wash buffer for 30s each time. The plate was patted dry on a filter paper, then add Chromogen Solution A 50µl and Chromogen Solution B 50µl to each well and incubated for 10 min at 37℃. Stop the reaction by adding stop solution and measure optical density at 450nm.

Western Blotting

Cells were washed with ice-cold PBS, followed by addition of ice-cold RIPA buffer mixed with protease inhibitor (100:1 ratio). The cells were then scraped using a cold plastic cell scraper and transferred to microcentrifuge tubes. Protein concentration was assessed using the BCA Protein Assay Kit. Subsequently, proteins were separated via SDS–polyacrylamide gel electrophoresis and transferred onto PVDF membranes (Millipore, USA). Membranes were blocked with a blocking buffer for 20 minutes, then incubated overnight at 4°C with primary antibodies targeting PPARγ, CEBPα, CEBPβ, sAC, PKA, β-actin, and tubulin, diluted at 1:1000 according to instructions. Following overnight incubation, membranes were washed with TBST and incubated with specific secondary antibodies for 1.5 hours at room temperature. Antibody binding was detected using chemiluminescence with an ECL kit.

Statistical Analysis

All statistical analyses were performed using Graph Pad prism version 9 (Graph Pad Software, Inc, La Jolla, CA, USA). Measurement data are expressed in terms of mean± standard deviation (mean ± SD) or mean ±standard error (mean ± SEM) differences between groups were compared using one-way analysis of variance<0.05 is considered statistically significant, on the contrary, P > 0.05 is considered not statistically significant. The error bars represent the standard deviation of the average from three independent experiments.

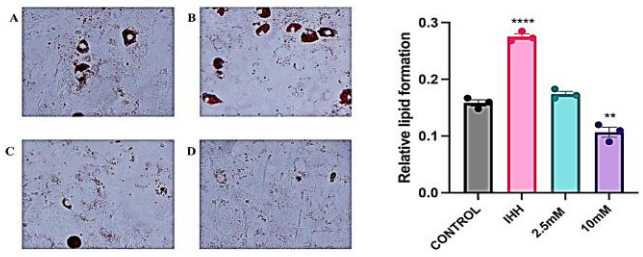
RESULTS

Butyric acid inhibited IHH-induced lipid accumulation and Triglyceride formation in differentiated preadipocytes.

3T3-L1 cells were seeded in 6-wells plate and allowed to reach confluence for 48 hours, after which the cells were induced to differentiate in an induction medium (0.5mM 3-isobutyl-1-methylxanthine 0.5mM, 1µM dexamethasone,10ng/ml insulin) for an additional 48 hours. The cells were then stained by Oil Red O dye and photographed to assess the level of lipid accumulation in the differentiating preadipocytes. The result revealed that (figure 1) treatment under IHH condition had a significantly increased level of lipid accumulation compared to control group (P<0.0001). On the other hand, there was a dose-dependent decrease in lipid accumulation in cells treated with butyric acid (2.5mM or 10mM), with 10mM having the greatest effect (P<0.01).

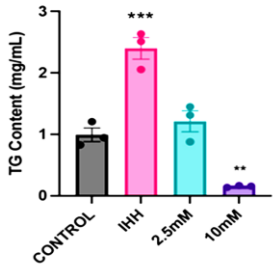
The level of intracellular triglyceride content in the differentiating preadipocytes was assessed using a TG assay kit. The result revealed that IHH also had a strong effect on triglyceride formation compared to the control group (figure 2), with a marked increase in triglyceride buildup in cells cultured under IHH conditions (P<0.001).

Figure 1: IHH accelerated lipid accumulation while BA had an inhibitory effect. 3T3-L1 Preadipocytes were allowed to differentiate in an induction medium and cultured under different conditions for 72 hours;



(A) control group: normoxic conditions (20% O2 5% CO2), (B) IHH group: 4% O2 10% CO2 for 6 hours daily, treatment group: cells were treated with (C) 2.5mM OR (D) 10mM BA for 48 hours before differentiation. Lipid accumulation detected by oil red o dye and the level of adipogenesis was corrected for cell number by dividing optical density (OD) at 500 nm after Oil Red O. Data are expressed as the relative level of lipid accumulation value between various groups. ***P<0.001 **** P<0.000.

Figure 2: IHH increased intracellular TG content while BA had an opposing effect. 3T3-L1 Preadipocytes were allowed to differentiate in an induction medium and cultured under different conditions for 72 hours;



control group: normoxic conditions (20% O_2 5% CO_2), IHH group: 4% O2 10% CO2 for 6 hours daily, treatment group: cells were treated with 2.5mM OR 10mM BA for 48 hours before differentiation. Intracellular TG contents was adjusted against total protein contents and OD value was measured at 420nm. ***P<0.001 ** P<0.01

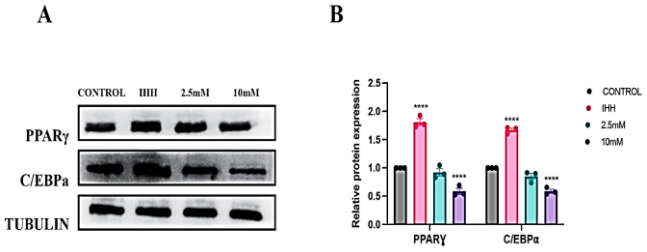
The inhibitory effect of BA was observed in a dose-dependent manner; where 2.5mM of BA did not have a significant impact in reducing triglyceride formation induced by IHH (P value was insignificant), whereas, a concentration of 10mM had a substantial effect in reducing triglyceride buildup compared to IHH group (P<0.01).

These results therefore show that, IHH could cause a significant increase in lipid accumulation and Triglyceride formation, whereas, butyric acid could mitigate these effects in a dose-dependent manner, with a higher dose having a more potent effect.

Butyric acid inhibits IHH-induced upregulation of protein expression of the Pro-adipogenic markers, PPARγ, C/EBPβ, and CEBPs.

PPARγ and C/EBPα play essential roles in activating gene promoters critical for adipogenesis, forming a vital positive-feedback loop. They also oversee the expression of genes related to lipid metabolism, insulin sensitivity, and adipokine secretion. Working together, they bind to numerous sites on promoters, controlling gene expression in adipocytes Mota de Sá et al. (2017), Wu et al. (1999), Fève et al. (2005). As shown in figure 3, IHH dramatically increased the protein expression of PPARγ and CEBPα. Compared to the control, IHH resulted in significantly higher levels of PPARγ protein expression (P<0.01) and CEBPα (P<0.001). As expected, BA inhibited the protein expression of PPARγ, and CEBPα, in a dose-dependent manner. More specifically a concentration of 10 mM had a more substantial impact on inhibition PPARγ (p<0.0001), and CEBPα (p<0.0001) than a dosage of 2.5 mM PPARγ (p<0.0001), and CEBPα (p<0.0001) in BA group compared to IHH group.

Figure 3: IHH upregulates protein expression of PPARγ, and C/EBPα whereas BA had an inhibitory effect. 3T3-L1 Preadipocytes were allowed to differentiate in an induction medium and cultured under different conditions for 72 hours;

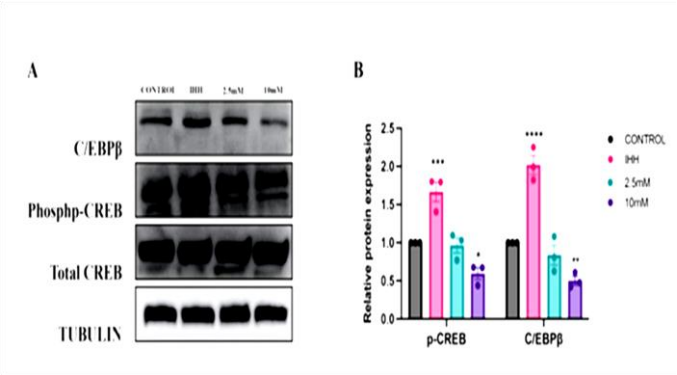


control group: normoxic conditions (20% O_2 5% CO_2), IHH group: 4% O2 10% CO2 for 6 hours daily, treatment group: cells were treated with 2.5mM OR 10mM BA for 48 hours before differentiation (A) a western blot representation shows the abundance in protein levels of PPARγ and C/EBPα, (B)relative expression of PPARγ and C/EBα normalized against β-tubulin ****P<0.0001.

CREB has been demonstrated to play a crucial role in adipogenesis, as it is both essential and capable of stimulating the expression of C/EBPβ, consequently leading to the activation of PPARγ expression Reusch et al. (2000). Therefore, the protein expression level of phosphorylated CREB and C/EBPβ were analysed using western blotting. The result showed that, compared to the control, IHH resulted in significantly higher levels of p-CREB protein expression (P<0.001) and C/EBPβ (P<0.0001). Also, BA inhibited the protein expression of p-CREB, and C/EBPβ, in a dose-dependent manner, where

a dose of 2.5mM inhibited IHH-induced protein expression to the level of control (P value was insignificant) and a dose of 10mM had a significantly more potent effect on the protein expression of p-CREB (P<0.05) and C/EBPβ (P<0.01) compared to control group. According to these findings, IHH upregulates adipogenesis by activating the pro-adipogenic transcription factors PPARγ, C/EBPα, C/EBPβ, and p-CREB. On the other hand, BA decreased IHH-accelerated adipogenesis.

Figure 4: IHH upregulates protein expression of CREB, and C/EBP whereas BA had an inhibitory effect. 3T3-L1 Preadipocytes were allowed to differentiate in an induction medium and cultured under different conditions for 72 hours;



control group: normoxic conditions (20% O_2 5% CO_2), IHH group: 4% O2 10% CO2 for 6 hours daily, treatment group: cells were treated with 2.5mM OR 10mM BA for 48 hours before differentiation (A) a western blot representation shows the abundance in protein levels of PPARγ and C/EBPα, (B)relative expression of PPARγ and C/EBPα normalized against β-tubulin ****P<0.0001 ***p<0.001.

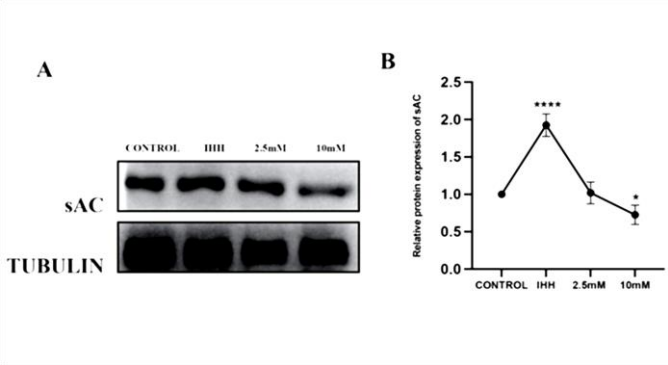
Butyric acid inhibits adipogenesis by suppressing the Production of cAMP and sAC

According to the findings of Ryota Kikuchi and colleagues, in order for hypercapnia to successfully expedite the process of adipogenesis, there must be an increase in the amount of cAMP that is synthesized by soluble adenylate cyclase (sAC). sAC generates cAMP by converting ATP to cAMP, which then stimulates downstream effectors such as protein kinase A (PKA), which then activates pro-adipogenic genes such as PPARγ and CEBPβ Petersen et al. (2008). Cells from the various groups were cultured in induction media, and the protein expression of sAC and cAMP was measured by Western blotting and direct ELISA, respectively. Figures 5 show that IHH conditions significantly increased sAC protein expression in comparison to the control group (P < 0.0001). On the other hand, BA had a dose-dependent inhibitory effect on sAC protein expression, with a concentration of 10 mM having a more significant suppressing effect on the expression of sAC (P < 0.05). Similarly, the levels of cAMP (figure 6) were increased in IHH group in compared to the control group (P < 0.001).

BA at a concentration of 2.5 mM had no discernible effect on the reduction of cAMP levels as compared to IHH group. However, a dose of 10 mM was sufficient to return cAMP levels down to that of the control group (P < 0.01).

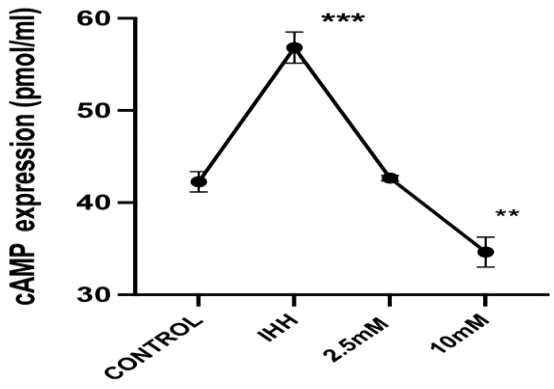
These findings suggests that IHH could accelerate the protein expression of the upstream CREB mediators sAC and cAMP whereas BA mitigated these effects.

Figure 5: BA inhibits IHH-induced protein expression of sAC. 3T3-L1 Preadipocytes were allowed to differentiate in an induction medium and cultured under different conditions for 72 hours;



control group: normoxic conditions (20% O_2 5% CO_2), IHH group: 4% O2 10% CO2 for 6 hours daily, treatment group: cells were treated with 2.5mM OR 10mM BA for 48 hours before differentiation. (A) a western blot representation showing the abundance in protein levels of sAC, (B) relative protein expression of sAC normalized against β-tubulin. ****P<0.0001 *P<0.05.

Figure 6: BA mitigates IHH-induced protein expression of cAMP. 3T3-L1 Preadipocytes were allowed to differentiate in an induction medium and cultured under different conditions for 72 hours;

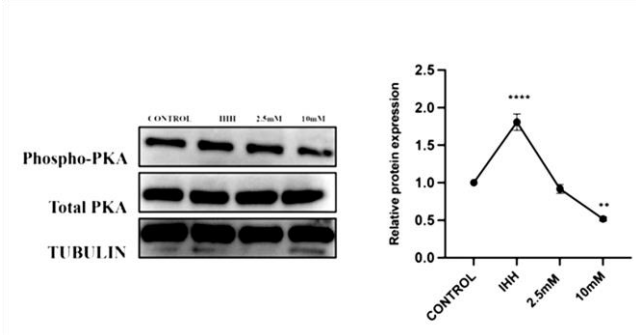


control group: normoxic conditions (20% O_2 5% CO_2), IHH group: 4% O2 10% CO2 for 6 hours daily, treatment group: cells were treated with 2.5mM OR 10mM BA for 48 hours before differentiation. The protein expression of cAMP was assessed using a direct ELISA assay kit, the figure above represents cAMP protein expression levels expressed in pmol/ml. ***P<0.001 **P<0.01

Butyric acid inhibits adipogenesis by decreasing Protein Kinase A activation.

Next, we investigated the impact of BA on protein kinase A; a significant downstream effector regulated by sAC-dependent cAMP generation, involved in the cellular processes associated with the IHH-accelerated adipogenesis by upregulating the pro-adipogenic transcription factors PPARγ and CEBPβ 2. Figure 7 shows that IHH led to an increase in the levels of phosphorylated-PKA in comparison to the control group (P<0.0001). BA at a concentration of 2.5 mM did not significantly lower the protein levels of protein kinase A when compared to the group that was exposed to IHH (P value was insignificant). However, a dose of 10 mM the effects of decreasing protein levels of protein kinase A was more evident in IHH adipogenesis (P<0.01), this underlines the relevance of PKA and the inhibitory effect of BA in IHH-induced adipogenesis.

Figure 7: BA inhibits IHH-induced protein expression of phosphorylated PKA. 3T3-L1 Preadipocytes were allowed to differentiate in an induction medium and cultured under different conditions for 72 hours;



control group: normoxic conditions (20% O_2 5% CO_2), IHH group: 4% O2 10% CO2 for 6 hours daily, treatment group: cells were treated with 2.5mM OR 10mM BA for 48 hours before differentiation. (A) a western blot representation showing the abundance in protein levels of phospho-PKA, (B) relative protein expression of sAC normalized against β-tubulin. ****P<0.0001 ***P<0.01

DISCUSSION

The bidirectional relationship between obstructive sleep apnea (OSA) and obesity is well-established, with obesity being a significant risk factor for OSA, while OSA can exacerbate obesity. This study aimed to elucidate how the hallmarks of OSA, particularly intermittent hypoxia and hypercapnia, contribute to obesity by accelerating adipogenesis. Additionally, we investigated the potential of butyric acid (BA) in mitigating OSA-induced adipose tissue dysfunction.

Our findings demonstrated that intermittent hypoxia and hypercapnia (IHH) accelerate adipogenesis by upregulating lipid accumulation, triglyceride formation, and the expression of adipogenic markers such as PPARγ, C/EBPα, and CREB. Conversely, BA exhibited an antagonistic effect on these processes, inhibiting lipid accumulation, triglyceride formation, and adipogenesis. Moreover, BA inhibited sAC-dependent cAMP production, leading to the suppression of Protein Kinase A activity, which further suppressed adipogenesis.

It is noteworthy that the experiment included hypercapnia to reflect the pathological changes in ventilation observed in OSA more accurately. We found that exposure to IHH conditions upregulated lipid accumulation, triglyceride formation, and pro-adipogenic markers in differentiating preadipocytes. This underscores the significance of considering both hypoxia and hypercapnia in understanding the pathophysiology of OSA-induced adipogenesis.

Furthermore, our study adds to the growing body of evidence suggesting that hypercapnia can promote lipid accumulation and adipogenesis in various cell types, including preadipocytes Vadász et al. (2008), Tsuji et al. (2013), Casalino-Matsuda et al. (2015), Vohwinkel et al. (2011). The induction of carbonic anhydrase isoforms during adipocyte differentiation may contribute to this upregulation in adipogenesis Cummins et al. (2014), Buck et al. (2011), Lynch et al. (1993). Further analysis revealed that IHH upregulated protein expression of sAC, cAMP, and PKA which are critical mediators in IHH-induced adipogenesis. sAC dependent cAMP production activates PKA and exchanger protein (EPAC), which in turn, upregulates expression of pro adipogenic transcription factors, such as cAMP response element binding protein (CREB), C/EBPβ, and PPARγ. These results confirm the hypothesis that simultaneous hypoxia and hypercapnia could accelerate adipogenesis. This is significant because it replicates ventilatory disturbances in a subgroup of OSA more precisely, allowing researchers to comprehend better the role of OSA in adipogenesis and how it exacerbates obesity.

Unexpectedly, BA exhibited inhibitory effects on adipogenesis under IHH conditions, contrary to its known role as a histone deacetylase inhibitor that promotes adipogenesis Yoo et al. (2006), Donohoe et al. (2012), Haberland et al. (2010). Albeit, previous research has indicated that butyric acid can inhibit adipocyte differentiation markers and inflammatory cytokines by upregulating Human Antigen R (HuR), a critical repressor of adipogenesis. HuR, a ubiquitous RNA-binding protein, exerts its regulatory function by recognizing and binding to AU-rich regions (ARE) in the 3'UTR of target mRNAs, thereby enhancing mRNA stability and translation, often outcompeting mRNA destabilizing modulators Zhang et al. (2022). Additionally, studies by Torun et al. suggest an alternative mode of action for butyric acid, specifically in modulating inflammation through the cytoplasmic translocation of HuR Torun et al. (2019). These findings imply a distinct mechanism of action for butyric acid, primarily involving post-transcriptional regulation through

alterations in mRNA stabilizing proteins, rather than transcriptional control.

Moreover, Butyric acid acts as an endogenous ligand for G-coupled receptors GPR41 and GPR43, which are expressed in various peripheral cell types and mediate diverse cellular responses. The expression of Gpr43 mRNA is noted in subcutaneous, perirenal, and epididymal white adipose tissues (WATs), as well as in 3T3-L1-derived adipocytes and mature adipocytes, suggesting a potential role in regulating obesity, food intake, and fat distribution Alvarez-Curto et al. (2016), Kimura et al. (2014). Evidence supports the interaction between adipose tissue-specific GPR43 and butyric acid in modulating adipogenesis through the regulation of cAMP production. Butyric acid demonstrates a bimodal effect on cell proliferation and survival, with low concentrations promoting cell growth and survival through increased expression of GPR41, while high concentrations inhibit cell growth, leading to apoptosis and reduced GPR41 expression Kurita-Ochiai et al. (2006). Moreover, butyric acid suppresses lipid breakdown and synthesis while enhancing glucose uptake stimulated by insulin in primary rat adipocytes. Additionally, butyrate, propionate, and acetate inhibit the growth and motility of well-differentiated human colonic cancer cells while promoting cellular differentiation Wang et al. (2012). Overall, the effects of butyric acid on various biological processes are complex and dependent on specific concentrations and contexts of application.

In clinical settings, understanding the interplay between intermittent hypoxia, hypercapnia, and adipogenesis in OSA patients is crucial for elucidating the pathophysiology of obesity in this population. Our findings highlight the overlooked role of hypercapnia in OSA-related ventilatory dysfunction and its implications for obesity research.

Adipogenesis and adipocyte hypertrophy contribute significantly to white adipose tissue expansion and ectopic fat accumulation, predisposing individuals to cardiometabolic complications. While our study focuses on adipogenesis, it's essential to acknowledge that other factors such as lipid resistance and reduced physical activity also contribute to obesity in OSA.

The inhibitory effects of BA on IHH-induced adipogenesis have important clinical implications, suggesting its potential therapeutic utility in mitigating OSA-related obesity The advantages associated with consuming dietary fiber are often attributed to the presence of SCFAs, according to literature. Research on mice has shown that consuming propionic and butyric acid through diet can help prevent obesity induced by high-fat diets by reducing food intake, improving insulin sensitivity, and increasing energy expenditure. However, less information is available about the specific cellular targets and mechanisms that play a role in mediating the positive effects on lipid metabolism. Intestinal microbial fermentation generates endogenous butyric acid that is involved in lipid metabolism. Although the body produces some butyric acid, its quantity is limited, and it has a shorter half-life in the bloodstream. Of all the short-chain fatty acids, butyrate has been identified as having several metabolic advantages. These include preventing obesity, insulin resistance, and liver steatosis induced by high-fat diets. Studies conducted on cell cultures in vitro have shown that butyrate lowers the concentration of cellular lipids and enhances lipolysis in 3T3-L1 adipocytes. In this study, our findings reveal a novel inhibitory role of butyric acid in hypoxic-hypercapnia-induced adipogenesis, thereby contributing to the current understanding of the pivotal role of butyric acid in lipid metabolism, particularly at the cellular level. Future research should further investigate the mechanisms underlying BA's actions and its therapeutic potential in OSA management.

Limitations of this study include the use of cell culture models, which may only partially reflect in vivo biological processes, and the short-term nature of the experiment. Future studies should validate these findings in animal models and explore the long-term effects of interventions on adipogenesis and metabolic processes.

CONCLUSION

In conclusion, this study underscores the intricate relationship between OSA and obesity, highlighting the role of intermittent hypoxia and hypercapnia in promoting adipogenesis. Moreover, it reveals the inhibitory effects of BA on adipogenesis, suggesting novel therapeutic avenues for managing OSA-induced obesity. Further research is warranted to validate these findings and explore potential therapeutic strategies for OSA-related metabolic dysfunctions.

DECLARATIONS

Ethical approval

For this type of study formal consent is not required.

Conflicts of interest

All authors have no competing interests and nothing to declare.

Author Contributions

Xin-tong Su and Abdullahi Rukkaiya participated in the literature research and helped draft the manuscript; Qiu-Ling Huang and Areeba Khalid participated in the literature research; Man-Huan Xu and Miao-Shang Su were the guarantor of integrity and final revision of the manuscript. All authors read and approved the final manuscript.

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