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Effects of acute intermittent hypercapnic hypoxia on insulin sensitivity in piglets using euglycemic clamp

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Abstract

Continuous hypoxia is associated with insulin resistance, altered glucose metabolism, and increased sympathetic nervous activity. This study examined the effect of 2 successive exposures to intermittent hypercapnic hypoxia (IHH) on glucose metabolism and insulin sensitivity in neonatal piglets. Piglets were assigned to 2 groups. One group was exposed to 2 × 90 minutes of hypercapnic hypoxia (8% O_2, 7% CO_2), intermittently in 6-minute cycles alternating with 6-minute air. The second group was given 2 × 90 minutes of air. Blood pressure, blood gases, glucose, insulin, and lactate were measured during exposures. Insulin sensitivity was assessed using the euglycemic clamp before and after the exposures. Piglets in the IHH group exhibited reduced PO_2 (from 111.4 ± 14.2 to 43.3 ± 21.7), increased PCO_2 (from 33.6 ± 1.9 to 49.4 ± 5.4), and lactic acidosis. Compared with air, IHH decreased blood glucose (control [CON] 4.44 ± 0.72 mmol/L vs IHH 2.67 ± 1.2 mmol/L, $P = .007$), insulin (CON 12.5 ± 7.4 μU/mL vs IHH 3.6 ± 3.1 μU/mL, $P = .03$), and mean arterial pressure (CON 143.0 ± 7.9 mm Hg vs IHH 112.5 ± 9.5 mm Hg, $P < .001$) over 90 minutes. Maximal insulin-stimulated glucose disposal was not different between the groups on either day, nor was endogenous glucose production. Overall, exposure to hypoxia in an intermittent pattern reduced sympathetic drive as indicated by blood pressure and did not alter insulin sensitivity, resulting in decreases in blood glucose and insulin. We speculate that an intermittent hypoxic stimulus results in failure of initiation of compensatory responses to increased energy requirements that would usually be observed during sustained exposure to hypoxia.

1. Introduction

To provide a context for this physiological study, positive correlations have been demonstrated between obstructive sleep apnea (OSA) and markers of the metabolic syndrome. In adults, where the association is best characterized, many clinical factors associated with OSA are features of the metabolic syndrome. The higher prevalence of diabetes and other cardiovascular disease risk factors including obesity, insulin resistance, and hypertension in patients with vs without OSA raises questions about whether OSA is, in effect, a component of the metabolic syndrome [1].

Human studies investigating the pathophysiological links between OSA and metabolic dysfunction are confounded by contributions of obesity [1,2]. Obesity is a major risk factor for both OSA and metabolic dysfunction. However, the presence of OSA appears to increase metabolic disturbances. Obese hypertensive men with OSA have significantly higher fasting insulin, glucose, and hemoglobin A_{1c} levels compared with those with similar anthropometry but without OSA [3]. Increased leptin resistance has also been demonstrated under similar circumstances [4]. When results are adjusted for body mass index, the severity of glucose intolerance or insulin resistance still correlates to the severity of OSA in that when OSA is present in addition to obesity, it appears to add significantly to the risk for diabetes [5].

In children, where OSA is also common, studies published to date support an influence of OSA on metabolism even during childhood. De la Eva et al [6] found that the severity of OSA correlated with fasting insulin

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levels in obese children. Kohyama et al [7] found that the severity of sleep disordered breathing positively correlated with hemoglobin A_{1c} as a measure of glycemic control.

Other than obesity, the 2 major components of OSA that have been considered likely candidates for altering glucose metabolism are blood gas (oxygen) disturbances and activation of the sympathetic nervous system. Recurring apneas are associated with episodic hypoxia; and at the termination of apneas, there is frequently an arousal and increased sympathetic nervous system activity [8]. Hypoxia and sympathetic nervous system activity both cause altered glucose metabolism. For example, a study in newborn calves demonstrated that exposure to hypoxia for 2 hours resulted in large increases in blood lactate, glucose, and insulin; and insulin receptor binding activity was also reduced, suggesting that hypoxia induced insulin resistance [9].

To better understand the acute impact of OSA on blood glucose metabolism during early development, this study examined the effects of 1 day of exposure to intermittent hypercapnic hypoxia (IHH) on glucose metabolism in piglets. In addition to acute measurements of glucose and insulin during the IHH protocol, the primary outcome measure for metabolic function was insulin sensitivity, which was measured directly under conditions of maximal insulin stimulation using the euglycemic-hyperinsulinemic clamp.

2. Methods

2.1. Animals

Large White-Dura crossbred piglets were obtained from a commercial piggery at 3.3 ± 2.2 days of age. Piglets were housed in a temperature-regulated animal facility under a 12-hour light/dark cycle. Dark hours were 6:00 AM to 6:00 PM so that all studies took place during a normal sleep period for the piglets.

Piglets underwent aseptic surgery at 7 days of age. Anesthesia was induced and maintained using a face mask delivering 1% to 5% isofluorane in 30% nitrous oxide, remainder O_2. Polyvinyl catheters were inserted into the piglets' left femoral artery and femoral vein and tunneled subcutaneously to exit on the ipsilateral flank. The catheters were protected by jackets, which were worn for the duration of the study. Analgesics (1 mg meloxicam) and antibiotics (52 mg cephalexin) were administered intramuscularly on the day of surgery and for 2 days after. The study protocol commenced a minimum of 36 hours after surgery. Ethics approval for the study was obtained from the Animal Ethics Committee of the University of Sydney.

2.2. Study protocol

Each piglet underwent 3 days of study and was randomly assigned to either a control (CON) (n = 6) or IHH (n = 7) treatment group for the day-2 protocol. Maximum insulin-stimulated glucose disposal and endogenous glucose production (EGP) were measured using the euglycemic clamp before treatment (day 1) to establish baseline metabolic function for all piglets. On day 2, piglets were subjected to treatment with either IHH or CON gas mixes. On day 3, the clamp measurements were repeated.

2.3. Euglycemic-hyperinsulinemic clamp and EGP

Euglycemic clamps commenced at 8:00 AM in conscious, 14-hour–fasted piglets in a sling restraint. An arterial blood sample was obtained immediately before the study to measure fasting insulin and glucose concentration and to assess background isotopic enrichment of glucose.

To asses EGP and glucose utilization in the basal state, a primed (6 μCi) continuous (0.12 μCi/min in saline) infusion of 3-^{3}H-glucose (Amersham Life Sciences, Buckingham, United Kingdom) was administered for 90 minutes at a constant rate via the femoral vein (delivery volume, 10 mL/h). Tracer steady state was achieved after 50 minutes; and arterial blood samples were taken at 60, 70, 80, and 90 minutes to measure plasma specific activity of glucose.

After the basal period, glucose production was assessed in response to insulin stimulation. Insulin (porcine insulin, Actrapid; Novo Nordisk, Sydney, Australia) was added to the basal infusate (0.25 U/[kg h] in 0.12 μCi/min 3-^{3}H-G), and delivery was maintained at the same rate (10 mL/h). A glucose infusion (10% glucose) was delivered at a variable rate using an adjustable volumetric pump. The 2 solutions were infused simultaneously into the femoral vein via a 3-way connector. To maintain steady state isotope enrichment for the duration of the study, 3-^{3}H-G was added to the glucose infusate in an amount calculated to give 50% of plasma specific activity achieved in the final basal period. Arterial blood samples were taken every 10 minutes to monitor blood glucose levels on an automated analyzer (YSI 2700; Yellow Springs Instruments, Yellow Springs, OH), and the glucose infusion rate was adjusted to maintain blood glucose at each piglet's fasting level. Glucose steady state was achieved after 170 (±21) minutes; and blood samples were taken at 180, 190, 200, and 210 minutes for measurement of glucose specific activity (described below).

2.4. Intermittent hypercapnic hypoxia

The treatment on day 2 took place using protocols established in previous studies [10]. Briefly, piglets were semirestrained in a hammock inside a temperature-controlled Perspex box (manufactured in house). The piglets were not fasted, and studies took place during a normally dark (sleep) period. Each piglet underwent two 90-minute treatment sessions separated by a 90-minute rest interval. During each 90-minute treatment session, piglets in the IHH group were exposed to cycles of 6 minutes of hypercapnic hypoxia (8% O_2, 7% CO_2, balance N_2) alternating with 6 minutes of air to achieve a total of 45 minutes of hypercapnic hypoxia per session. Gases were delivered through a firmly sealed full face mask via a 3-way tap, allowing easy switching between reservoir bags containing air or the hypercapnic hypoxia

Table 1
Physical characteristics of piglets

Piglet characteristic	CON	IHH
Arrival age (d)	3.1 ± 2.1	3.5 ± 2.5
Weight at start of study (kg)	2.3 ± 0.17	2.3 ± 0.6
Weight at end of study (kg)	2.3 ± 0.26	2.4 ± 0.27

Values are mean ± SD.

mix. Control piglets were exposed to 90 minutes of air, with tap switching continued at 6-minute intervals to ensure equivalence of the study environments apart from the inspired gas concentrations.

Blood pressure was measured for the duration of treatment via a disposable pressure transducer (TruWave, Edwards Lifesciences, Irvine, CA) connected to the arterial cannula. Arterial blood samples were taken for glucose, insulin, lactate, and blood gas analysis at 4 time points: baseline, in the last 10 minutes of the first exposure, after 90 minutes of rest, and in the last 10 minutes of the second exposure. Blood gas tensions, pH, base excess, and hemoglobin were measured in an automated blood gas analyzer (Nova Stat Profile 4; Nova Biomedical, Waltham, MA); and all values were corrected to the rectal temperature of the animal that was recorded when the samples were taken.

2.5. Analysis of blood samples

To determine the specific activity of glucose, plasma was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and immediately centrifuged. Aliquots (100 μL) of the supernatant were evaporated to dryness to remove tritiated water. Dry residues were then dissolved in water (100 μL) and added to 1 mL of scintillation fluid and counted in a scintillation counter.

Plasma insulin levels were measured by standard radioimmunoassay using a porcine insulin kit (Linco Research, St Charles, MO). Plasma glucose was determined using the glucose oxidase method. Lactate was measured in whole blood using an automated analyzer (YSI 2700, Yellow Springs Instruments).

2.6. Calculations

According to published methods, a [3-^{3}H]-glucose infusion was used to determine EGP [11]. Briefly, during steady state in both basal and insulin-stimulated conditions, the glucose appearance rate (Ra) equals the rate of peripheral glucose disposal (Rd), which can be calculated by dividing the tracer infusion rate (Ra∗, disintegrations per minute [dpm] per minute) by the plasma specific activity of glucose (SAg, dpm/mg). In the basal state, Ra equals the rate of EGP:

$$\mathrm{Ra} = \mathrm{Rd}, \text{ where } \mathrm{Rd} = \mathrm{Ra}^*/\mathrm{SAg}$$

During insulin stimulation, the rate of glucose appearance equals the rate of EGP plus the rate of exogenous glucose infusion:

$$\mathrm{EGP\ (Ra)} = \mathrm{Rd} - G_{\mathrm{inf}},$$

where G_{inf} is glucose infusion rate (milligrams per minute).

2.7. Statistical analysis

All analyses were conducted using Statview (SAS, Cary, NC) statistical program. Mean differences were calculated for before and after treatment for all parameters, and sample *t* tests (2-tailed) and analysis of variance were performed. Results are presented as mean ± SD, and statistical significance was set at $< .05$.

3. Results

3.1. Piglet characteristics

A total of 13 piglets completed the study. The average age at arrival was the same for both the CON group (n = 6) and the IHH group (n = 7), and there was no difference in the weight of the piglets at the start or at the completion of the protocol (Table 1).

3.2. Intermittent hypercapnic hypoxia

3.2.1. Blood gases

The effectiveness of the IHH treatment protocol on day 2 was assessed by measurement of arterial blood gas tensions. Before treatment on day 2, there was no difference in blood gas tensions, pH, or HCO_3 between the 2 groups (Table 2). For the CON group, there was no change across the 90-minute period. The treatment group exposed to 90 minutes of IHH results showed a 50% drop in PO_2, a 9.6–mm Hg

Table 2
Blood gas values during exposure, corrected to rectal temperature

Measure	CON				IHH			
	Baseline	1st 90 min	90-min rest	2nd 90 min	Baseline	1st 90 min	90-min rest	2nd 90 min
PO_2	108.4 ± 17.5	112.5 ± 9.7	102.1 ± 7.3	103.7 ± 10.6	111.4 ± 13.2	53.6 ± 14.7 *	97.5 ± 18.4	43.3 ± 21.7 *
PCO_2	33.3 ± 3.3	33.7 ± 2.1	32.2 ± 0.8	33.6 ± 1.9	33.6 ± 2.5	43.2 ± 7.1 *	30.0 ± 1.6	49.4 ± 5.9 *
pH	7.4 ± 0.05	7.4 ± 0.03	7.4 ± 0.05	7.4 ± 0.002	7.4 ± 0.03	7.2 ± 0.3 †	7.4 ± 0.05	7.0 ± 0.2 †
HCO_3	21.7 ± 1.4	23.5 ± 1.1	21.6 ± 1.9	22.8 ± 0.5	21.6 ± 2.6	16.3 ± 6.6 †	19.9 ± 3.0	14.1 ± 6.4 †

Values are mean ± SD for each group.

* $P \le .005$.

† $P \le .05$ for CON vs IHH and IHH baseline vs second 90-minute session.

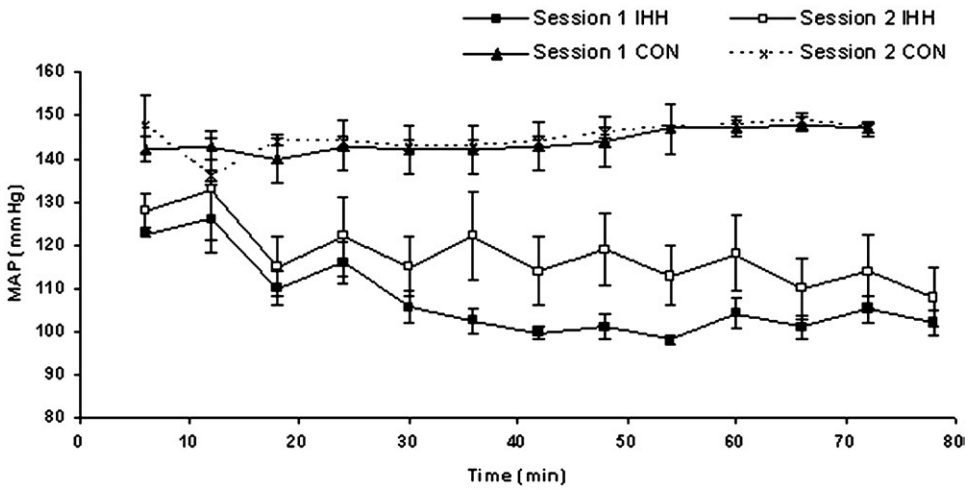

Fig. 1. Mean arterial blood pressure during IHH and CON treatment sessions on day 2. Graph shows average mean arterial pressure for each 6-minute cycle. Results are expressed as mean ± SD for each group of piglets (P =.003 for IHH session 1 vs session 2 and $P < .001$).

increase in PCO_2, and lactic acidosis to pH 7.2. These changes returned to CON values during the 90-minute rest period; but there was greater deterioration during the second 90-minute IHH exposure, with a further 10% reduction in PO_2, a greater increase in PCO_2 (19.4 mm Hg), and more severe acidosis with pH 7.0 (Table 2).

3.2.2. Blood pressure responses

There was no difference in blood pressure between the groups at the start of the study. There was no change in mean blood pressure across time or between studies for the CON piglets (Fig. 1). Blood pressure fell over time in the IHH-exposed animals and was significantly lower than baseline during the recovery periods after IHH exposure cycles between 18 and 42 minutes (P = .01). Blood pressure was significantly higher during the second session in the IHH-exposed animals (mean value, 107.3 ± 8.9 vs 117.8 ± 7.1 mm Hg in exposures 1 and 2, respectively; P = .003). However, because of the fall in blood pressure over time, the IHH-exposed animals showed an overall lower blood pressure than the CON animals (112.5 ± 9.5 vs 143.0 ± 7.9 mm Hg in IHH vs CON, respectively; $P < .001$).

3.2.3. Acute changes in glucose, insulin, and lactate

None of the piglets were fasted before the treatment on day 2. Baseline measurements showed no difference in blood glucose (CON 4.89 ± 1.2 mmol/L vs IHH 6.09 ± 1.3 mmol/L, P = .1) or insulin (CON 9.6 ± 10.4 μU/mL vs IHH 9.7 ± 8.6 μU/mL, P = .9) between the groups. (Fig. 2A, B). For the CON group, there was no significant change in blood glucose during the 2 sessions of treatment. The IHH group showed a decline in blood glucose levels over the course of each 90-minute session, resulting in significantly reduced glucose levels compared with the CON group at the end of treatment (CON 4.44 ± 0.72 mmol/L vs IHH 2.67 ± 1.2 mmol/L, P = .007). Plasma insulin followed similar patterns, with no change in the CON group but a significant fall in insulin by the end of the second session in the IHH treatment group (CON 12.5 ± 7.4 μU/mL vs IHH 3.6 ± 3.1 μU/mL, P = .03).

Whole blood lactate levels did not change during the study period in CON piglets (2.04 ± 0.93 mmol/L vs 1.35 ± 0.89 mmol/L, before vs after; P = .11). However, the IHH treatment was associated with a marked increased in lactate (1.86 ± 0.82 mmol/L vs 6.12 ± 2.78 mmol/L, before vs after; P = .0007).

3.2.4. Effect of IHH on EGP and whole-body insulin responsiveness

Response to maximum insulin stimulation was assessed using the euglycemic-hyperinsulinemic clamp method. Whole-body glucose uptake and hepatic insulin sensitivity were assessed by combining the clamp with a 2-^{3}H-glucose infusion. On day 1 of the experiment, there was no difference in fasting plasma glucose or insulin between the groups (glucose: CON 5.6 ± 0.8 mmol/L vs IHH 5.2 ± 1.1 mmol/L, P = .4; insulin: CON 3.5 ± 2.8 μU/mL vs IHH 2.1 ± 1.5 μU/mL, P = .3). Basal glucose disposal also showed no difference between IHH and CON piglets (CON 5.3 ± 2.0 mg/[min kg] vs IHH 4.7 ± 1.8 mg/[min kg]). The plasma insulin during the insulin infusions reached supraphysiological levels in all clamps (422 ± 63 μU/mL), thus achieving maximum stimulus. Glucose disposal increased under insulin stimulation to a similar degree in both groups (CON 16.4 ± 8.3 mg/[min kg] vs IHH 18.1 ± 6.3 mg/[min kg]), indicating similar whole-body insulin sensitivity among all piglets (Fig. 3A). The hepatic response to maximal insulin stimulation as measured by EGP was not different between the groups in the fasting state (CON 5.3 ± 2.0 mg/[min kg] vs IHH 4.7 ± 1.8 mg/[min kg]) and was completely suppressed by insulin infusion. None of these measures were changed on day 3, indicating that 1 day of IHH exposure had no effect on basal or maximum insulin-stimulated glucose disposal in the CON or IHH

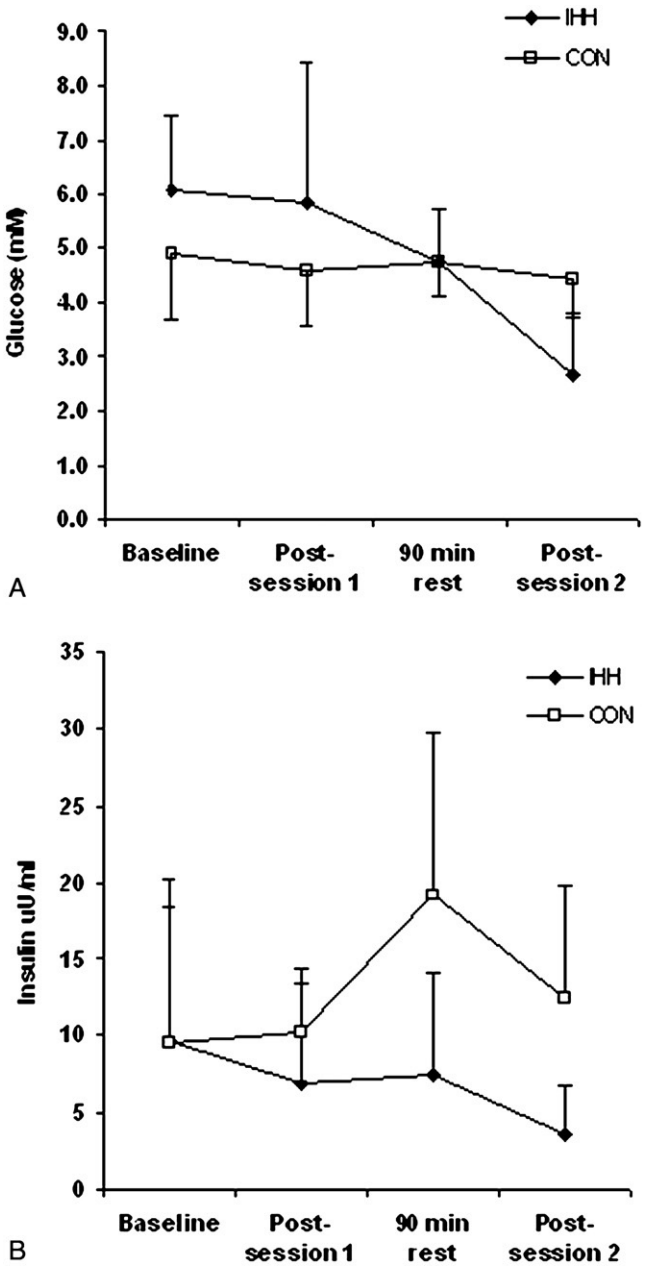

Fig. 2. Plasma glucose (A) and insulin (B) during treatment with IHH or air. Samples were taken before treatment, at the end of 90 minutes of exposure, after 90 minutes of rest, and at the end of a second 90 minutes of exposure. Results are mean ± SD (P =.007 for glucose CON vs IHH and P = .003 for insulin CON vs IHH at the end of session 2).

group. Hepatic insulin sensitivity was not altered by exposure to IHH (Fig. 3B).

4. Discussion

In this study, we demonstrated that a protocol of IHH induces acute changes in glucose homeostasis. The study aimed to examine the effects of a single exposure of IHH as well as any effects that outlasted this exposure. The immediate responses we observed during the stimulus were a fall in both insulin and glucose levels. However, using euglycemic clamp studies before and then 1 day after the protocol of IHH, we found no persisting changes in insulin sensitivity. In the current protocol, the animals had a fall in blood pressure as well as insulin and glucose, suggesting that there was an inadequate sympathetic response to the IHH protocol that we used. This delay or failure of sympathetic responses to such a (intermittent) hypercapnic hypoxic stimulus was consistent with the changes in glucose homeostasis. We speculate that, during early development,

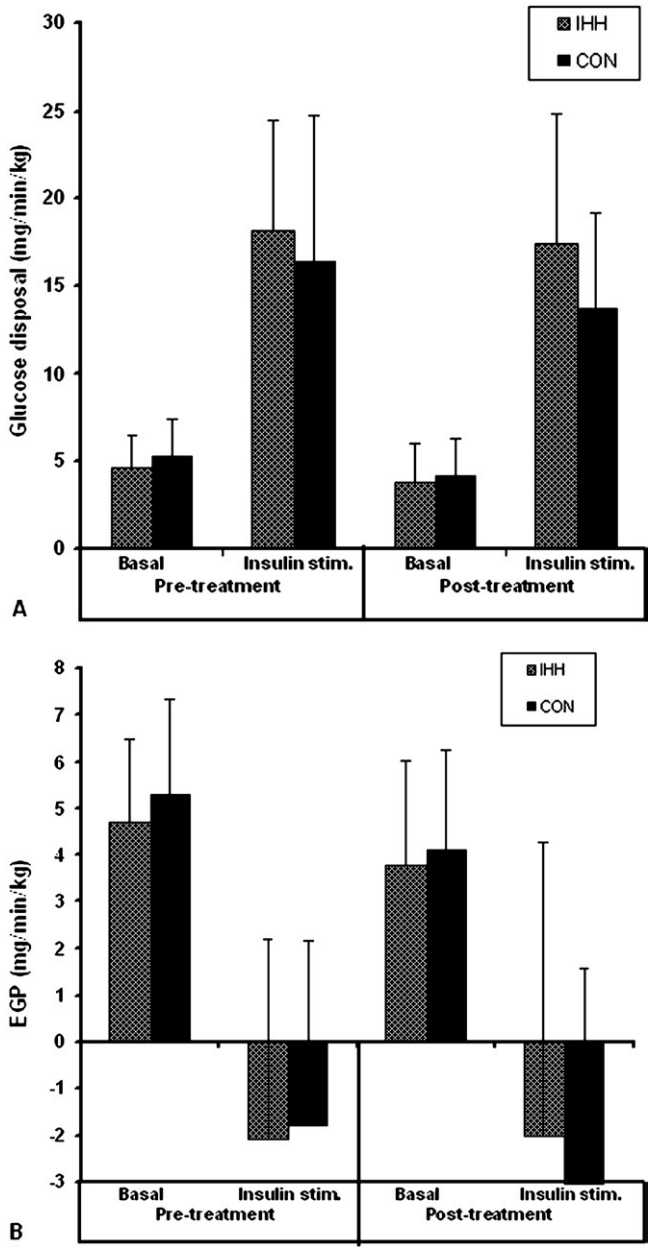

Fig. 3. Glucose disposal (A) and EGP (B) measured by euglycemic-hyperinsulinemic clamp conducted on day 1 before treatment with air or IHH and on day 3 after treatment. Results displayed as mean ± SD for each group.

exposure to IHH is associated with delays in adaptation of glucose-insulin pathways to energy deprivation. However, the responses support the hypothesis that insulin resistance is a positive adaptive response to acute energy deprivation such as occurs in hypoxia.

Exposure to hypoxia is known to produce discernible alterations to carbohydrate metabolism. Animals exposed to short-term continuous hypoxia show increases in plasma glucose and insulin and changes in substrate utilization compared with normoxic animals [12,13]. In healthy women during short exposures to hypoxia at high altitude, Braun et al [14] reported increases in plasma glucose and reduced glucose tolerance. Similar findings have been seen in men exposed to hypoxic conditions for 30 minutes using a reduced oxygen gas mixture [15]. There are less data available on the effects of intermittent hypoxic exposures on metabolism, although one study in genetically obese mice (leptin knockout) demonstrated worsening of insulin resistance and increased plasma insulin and glucose concentrations after long-term hypoxia [16]. The effect was not seen in the short exposure. There are also very little data examining the relationship between a hypercapnic stimulus and metabolic disturbance.

In the current study, piglets underwent 6 minutes of cyclic hypercapnic hypoxia, with results suggesting that glucose uptake into the tissues may have increased in response to the hypoxia. That is, blood glucose showed a steady decrease over the course of both the 90-minute treatment sessions and during the recovery period afterward. Insulin levels also decreased, in parallel with the glucose. In early experiments by Randle et al [17], incubation of skeletal muscle under hypoxic conditions resulted in increased glucose uptake into the tissue. Enhancement of the anaerobic glycolytic pathway independently of insulin was also observed. This increased flux through glycolysis is required to maintain adequate adenosine triphosphate supply in the face of reduced aerobic capacity. The lactate levels we observed support the hypothesis that anaerobic metabolism was initiated, with lactate levels in the IHH-exposed piglets from this study approximately 3-fold higher than those in the CON piglets.

It is of interest that the glucose levels continued to drop even during the 90-minute rest period after the piglets were returned to room air. It is possible that the increased rate of glucose uptake continued and that normal counterregulatory mechanisms were not initiated before the second exposure. Previous studies have demonstrated that fasted rats exposed to 48 hours of hypoxia had reduced phosphoenolpyruvate carboxykinase protein and messenger RNA and reduced gluconeogenesis compared with normoxic fasted rats, indicating a failure to adapt to fasting by the normal mechanisms of increasing glucose output [12]. Our EGP results suggest that both groups were producing the same amount of glucose in the fasting state when this was measured the next day. Unfortunately, we do not directly measure glucose uptake at time of exposure; however, falling blood glucose levels in the absence of increased insulin are strongly suggestive of up-regulation of non–insulin-dependent mechanisms of glucose uptake or of increased insulin sensitivity.

Our study results are at odds with previous observations that suggest that acute hypoxia results in insulin resistance. In newborn calves, a single 2-hour exposure to hypoxia increased blood glucose and insulin levels compared with normoxic animals. Insulin receptor binding and tyrosine kinase activity were also reduced in the liver, implying reduced hepatic insulin responsiveness [9]. Similar findings were observed in neonatal rats exposed to acute hypoxia [13]. The results of these studies and others imply the development of transient insulin resistance acutely in response to the hypoxia, which would counteract the increased glucose uptake normally seen under these conditions and maintain blood glucose levels. However, those studies did not measure insulin sensitivity directly.

As far as we are aware, our study is the first to use the criterion standard measure of hepatic and whole-body insulin sensitivity—the euglycemic-hyperinsulinemic clamp—to measure insulin sensitivity in response to acute IHH. The results of the euglycemic clamp experiments suggest that IHH exposure did not affect insulin-stimulated peripheral glucose disposal nor did it alter hepatic glucose output. The euglycemic clamp is a direct measure of whole-body insulin sensitivity and by necessity was conducted the following day, 18 hours after completion of IHH administration. It is possible that any immediate metabolic changes induced by such a short exposure to hypoxia, particularly alterations in glucose disposal, may have been normalized by this stage. Serum lactate, glucose, and insulin levels in the IHH group were back to baseline at this point, confirming this (data not shown). Although the euglycemic clamp is used as a standard measure of insulin sensitivity, the plasma insulin levels achieved during our study were supraphysiological. Therefore, it is possible that small changes in responsiveness to physiological levels of insulin may not be detected under maximal stimulation [18]. To clarify some of these questions would require future studies over longer exposure time with continued, frequent monitoring of glucose and insulin. Another possibility would be to infuse radiolabeled glucose during hypoxia to directly measure glucose uptake into tissues during the immediate exposure and insulin sensitivity again after daily exposures to this protocol.

In addition to the new methods for study that we used, possible explanations for the failure to generate insulin resistance include failure of the piglets to increase sympathetic outflow. It may also be related to the exposure pattern of the hypoxia. We selected an IHH protocol that used relatively short duration of hypoxic exposure and recovery intervals based on previous physiological studies that showed that adaptive responses to hypoxia can be altered by exposure patterns [19].

Hypoxic conditions stimulate peripheral chemoreceptors, particularly in the carotid body, which result in a cardiovascular sympathetic reflex [20]. Studies in humans

have demonstrated increased sympathetic nerve activity in response to acute hypoxia alone as well as to hypercapnic hypoxia. Indeed, several studies in humans have also demonstrated increased arterial blood pressure and surges of sympathetic activity in response to acute continuous hypoxia [8]. This is often associated with increases in arterial blood pressure due to the sympathetic adrenergic vasoconstriction triggered by this hypoxic stress [21]. Fletcher et al [22,23] demonstrated that rats exposed to intermittent hypoxia developed persisting hypertension even after removal of the stimulus [22] and that this appeared to be mediated by activation of peripheral chemoreceptors and sympathetic nervous system [23].

In our study, piglets exposed to IHH had reduced overall mean arterial pressure compared with the CON piglets. The blood pressure responses were used to provide a surrogate measure of the sympathetic response to the stimulus. It is possible that local vascular factors stimulated by the exposure also contributed to these changes. We were unable to measure sympathetic nervous system activity directly in this study; however, a review of this topic concluded that increased sympathetic nervous system activity mediates the blood pressure increases normally seen in hypoxia and in hypoglycemia. It is thought that this is a protective response that increases perfusion when substrate availability is reduced [24]. Increased sympathetic activity is also associated with insulin resistance [25]. The exact mechanism of this association is unknown, but studies have shown that the carotid body also has an important role in sensing hypoglycemia and initiation of appropriate adaptive responses [26]. Stimulation of the carotid body chemoreceptors by hypoxia has also been shown to induce similar responses, such as elevated hepatic glucose output [27] and increased cortisol release [28]. Elevated cortisol levels are associated with insulin resistance [9], but we did not measure cortisol levels in our piglets. We speculate that the blood pressure fall that we observed indicates that the piglets exposed to the IHH protocol in our study failed to initiate an increase in sympathetic activity. It would be useful to repeat the study using direct measures of sympathetic activity.

The discrepancy between our study and those demonstrating hypoxia-induced insulin resistance and increased blood pressure may also be due to the pattern of delivery of the hypoxic stimulus. Repetitive intermittent cycles of hypoxia-reoxygenation increase oxidative stress in cells, and this leads to an induction of HIF-1 gene [29]. HIF-1 regulates gene expression for many of the proteins important for the metabolic responses to hypoxia [30]. It may be that the intermittent nature of the exposure does not allow initiation of HIF-1 expression. It is also difficult to establish the contribution of the hypercapnia to the results of this study because there is very little literature examining the effect of hypercapnia on metabolism. Additional studies to compare a continuous exposure with and without hypercapnia would be helpful to elucidate the mechanisms associated with these processes. For example, the time course of exposure could affect how successful the induction of HIF-1α has been; and this is likely to be integral to the effects seen on downstream pathways such as glucose metabolism.

It is important to note that the response observed in this study is consistent with the apparent discordances between ventilatory and metabolic adaptation of piglets that we previously observed during an equivalent protocol. Our study used a relatively short and intermittent exposure to hypercapnic hypoxia, 6-minute cycles with 90 minutes of total exposure time, in contrast to longer continuous exposures in the other studies. Studies in developing mammals have established that ventilatory responses to hypoxia are dependent on the nature of the intermittent stimulus [31]. The duration of the episodes of hypoxia used in this study have been previously shown to reduce normal ventilatory and metabolic adaptive responses to hypoxia such as falls in body temperature [10]. Short-duration intermittent hypoxic episodes also had less effect on sympathetic nervous activity and blood pressure when compared with continuous hypoxia in one study [32]. That is, the pattern of intermittent exposure to hypercapnic hypoxia used in this study may not have allowed initiation of sympathetic and metabolic compensatory mechanisms that would be expected during sustained hypoxia.

5. Summary and conclusion

Rather than developing insulin resistance, piglets exposed acutely to IHH reduced their blood glucose and insulin levels. We speculate that there was a failure of the normal adaptive responses to increased energy requirements that was attributable to the characteristics of the intermittent exposure protocol because the time intervals used led to failure of the compensatory responses that would usually be observed during sustained exposure to hypoxia.

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