Fructose Feeding and Intermittent Hypoxia Affect Ventilatory Responsiveness to Hypoxia and Hypercapnia in Rats

Evelyn H. Schlenker*1, Yijiang Shi¹, Joni Wipf, Douglas S. Martin and Curtis K. Kost, Jr.

Basic Biomedical Sciences
University of South Dakota School of Medicine
Vermillion, SD, 57069

Running Title: Fructose Feeding & Intermittent Hypoxia Affect Ventilation

* Corresponding author
Basic Biomedical Sciences
University of South Dakota School of Medicine
Vermillion, SD, 57069
E-mail: eschlenk@usd.edu

Phone: 605-677-5160

FAX: 605-677-6381

¹ Both authors contributed to this manuscript equally
Abstract
We hypothesized that in male rats 10% fructose in drinking water would depress ventilatory responsiveness to acute hypoxia (10% O_2 in N_2) and hypercapnia (5% CO_2 in O_2) that would be depressed further by exposure to intermittent hypoxia. Ventilation in air and in response to acute hypoxia and hypercapnia were evaluated in ten rats prior to fructose feeding (FF), during 6 weeks of FF and after FF was removed for 2 weeks. During FF, 5 rats were exposed to intermittent air and 5 to intermittent hypoxia for 13 days. Six rats given tap water acted as control and were exposed to intermittent air and subsequently intermittent hypoxia. In FF rats, plasma insulin levels increased 3 fold in the rats exposed to intermittent hypoxia, and during washout returned to levels observed in rats exposed to intermittent air. During FF, ventilatory (V_E) responsiveness to acute hypoxia was depressed due to decreased tidal volume (V_T) responsiveness. During washout, ventilation decreased due to decreased V_T and frequency of breathing, and the ventilatory responsiveness to hypoxia in intermittent hypoxia rats did not recover. In all rats the ventilatory responses to hypercapnia were decreased during FF and recovered after washout due to an increased V_T responsiveness. In control group, hypoxic responsiveness was not depressed after intermittent hypoxia and augmented after washout. Thus FF attenuated the V_E responsiveness of conscious rats to hypoxia and hypercapnia. Intermittent hypoxia interacted with FF to increase insulin levels and depress ventilatory responses to acute hypoxia that remained depressed during washout.

Key words: Insulin, Glucose, Blood Pressure, Telemetry
Introduction

Within the last 20 years consumption of fructose especially in beverages has increased sharply in the United States (1). This increase parallels the rise in obesity and has been linked to the epidemic of metabolic syndrome X [a combination of such factors as obesity, insulin resistance, dyslipidemia, and hypertension] in adults and recently in children (1, 5, 11). Administration of fructose (either in drinking water or as part of solid food) to male rats causes elevated systolic blood pressure, insulin insensitivity, down regulation of insulin receptors in liver and skeletal muscle, increased plasma levels of triglycerides, elevated urinary levels of norepinephrine and epinephrine, elevated expression of the angiotensin AT1a receptor in the thoracic aorta and left ventricular hypertrophy (4,8,9,18,19). Underlying reasons for these physiological consequences include increased stimulation of the sympathetic nervous system, increased levels of tumor necrosis factor alpha, and stimulation of the renin-angiotensin system (2, 9, 25).

Control of breathing is abnormal in various animal models of type 2 diabetes, but they may include confounders such as obesity, diabetic neuropathy and genetic abnormalities (6, 20). In contrast, the fructose fed model rats are not obese and do not exhibit genetically determined leptin receptor insensitivity. Moreover, it is a relatively reversible model (4). That is, increases in blood pressure and dyslipidemia as a consequence of fructose feeding are reversed following removal of the fructose (4).

Of further significance is that intermittent hypoxia (IH) has been shown to contribute to the production of insulin insensitivity (3). In patients with insulin
insensitivity and obstructive sleep apnea, treatment with constant positive airway pressure, reduced their insulin insensitivity (7). In the ob/ob mouse, an animal model of type 2 diabetes, when exposed to IH insulin levels increased dramatically (14). Whether IH in fructose fed rats affects ventilation and insulin levels has not been evaluated.

The purpose of this study was to determine the effect of fructose feeding on control of breathing in rats. Previous studies in our lab noted that an animal model of obesity and type II diabetes, the obese Zucker rat, exhibited altered breathing patterns while breathing air and abnormal responses to hypoxia and hypercapnia relative to control rats (6). We hypothesized that feeding fructose to normal Sprague Dawley rats would elevate blood pressure and heart rate and depress their ventilation in response to hypoxic and to hypercapnic gas challenges, and that these responses would be reversed after washout. To determine if exposure to IH further compromised ventilation, half of the rats were exposed to IH (10% oxygen in nitrogen) for 1 ½ hour per day for 13 days and the other half to intermittent air for the same time. This protocol was determined from preliminary studies in rats (Schlenker, unpublished observations) to have the same effects on ventilatory parameters in response to hypoxia as 11 exposures of 5 minutes of hypoxia (10% O₂) interspaced with 5 minutes of air for up to 5 days. To evaluate that the effects of fructose feeding on control of breathing were specific, a separate set of experiments was carried out in rats not fed fructose but exposed to intermittent air and IH. Fructose feeding elevated blood pressure and heart rate, but attenuated the VE responsiveness of conscious rats to hypoxia and hypercapnia. IH exposure interacted with FF to increase insulin levels and depress ventilatory responses to acute hypoxia.
Methods

For this study a total of 16 adult male Sprague Dawley rats (Harlan) were utilized. Ten rats were part of the fructose study, while 6 rats served as controls, received only tap water, and were exposed to IH or intermittent air using the same protocol that was used for rats in the fructose intervention. A schematic of both studies is presented in Figure 1 and explained in more detail below.

Rats were housed individually and prior to the commencement of the experiment received water and food *ad libitum* with a 12-hour on/12 hour off lighting schedule. All procedures, noted below, except evaluation of blood pressure and heart rate, were done at the same time each day during the light hours. The University of South Dakota Animal Care and Use Committee approved the experimental protocols utilized in this study.

Evaluation of Ventilation and Metabolism in Air and after Acute Exposure to hypoxia and hypercapnia

Each rat was weighed and then placed into a clear Plexiglas cylinder measuring 22 cm in length and 15.5 cm in diameter. The front of the chamber was sealed and contained three ports: one leading to a Statham low pressure transducer which in turn connected to the Bio-Pac Data Acquisition system, a port allowing air to enter the chamber and measure inspired oxygen and carbon dioxide, and a port used to measure chamber temperature using a Cole Palmer digital thermometer. The back of the plethysmograph contained two ports: one to measure airflow through the chamber using a Gilmont Rotameter, and another to serve as a “leak” to stabilize measurements or when...
connected to Vacumed oxygen and carbon dioxide analyzers to measure fractional contents of the expired gases.

Prior to any study, the rat was accustomed to being handled and was placed into the plethsmographic chamber for about 20 minutes for 2 to 3 days. On the day of ventilatory and metabolic evaluations, the rat was placed into the plethsmographic chamber for about 30 minutes of acclimation. Then ventilation and metabolism were evaluated. Subsequently the rat was exposed to 7 minutes of 10% oxygen in nitrogen and ventilation was again evaluated. The chamber was then flushed with air for approximately 10 minutes and the ventilatory measurement repeated. Finally the rat was exposed to 5% carbon dioxide in oxygen and then the chamber was flushed with air. Subsequently the rat was removed from the plethysmograph and its body temperature was measured using a thermometer-thermocouple system (Sensortek, Clifton, NJ).

The ventilatory parameters evaluated using the barometric technique (6) included tidal volume ($V_T$) and frequency of breathing ($f$), and the product of these two parameters minute ventilation ($V_E$). CO₂ production ($VCO_2$) was determined using the flow through method and calculated as flow rate multiplied by the difference in the fractional content of expired and inspired CO₂. To determine ventilatory equivalent, a measure of how well ventilation and metabolism was matched, the ratio of $V_E$ to $VCO_2$ was calculated. Ventilation, CO₂ production and tidal volume were normalized by body weight. Ventilatory responsiveness to hypoxia or to hypercapnia was determined by subtracting the variable measured during the preceding air exposure from that during the gas
challenge. This difference was then divided by the preceding air value and multiplied by 100.

**Radiotelemetry implantation**

Four rats that were part of the fructose experiment were instrumented with radio telemetry devices (Model TA11PA-C40; Data Sciences International, St. Paul MN) to monitor blood pressure and heart rate in conscious rats. The radiotelemetry devices were implanted during isoflurane anesthesia utilizing aseptic surgical techniques as described previously (19). In brief, a portion of the aorta distal to the renal arteries was exposed through a midline abdominal incision, and the catheter of the radiotelemetry device was inserted into the aorta through a puncture wound created with a 21 gauge needle. Medical grade tissue adhesive and a cellulose fiber patch secured the catheter. The main body of the device, which contains the pressure sensor, radio transmitter, and the battery, was then sutured into the abdominal wall after the midline incision was closed. Penicillin G procain (5000 units) and heparin (50 units) were given to the rats in post-operative IM injections. Data acquisition was performed using the Dataquest LabPRO software package (Data Sciences International) with sampling parameters adjusted to 10-sec scan periods at 10-min intervals. Blood pressure and heart rate determinations were made after surgery and baseline values obtained 10 days after surgery.

**Evaluation of Glucose and Insulin Levels in the Fructose Experiment**

To determine glucose and insulin levels, 1 to 1.5 ml of jugular blood was taken after a 6 hour fast. One drop of blood was used to determine glucose levels using the OneTouch Ultra (Lifescan, Milpitas, CA) system that was calibrated twice before and
after evaluation of glucose levels in a group of rats. The rest of the blood was placed into heparinized tubes and placed on ice. The tubes were centrifuged at 5000 rpm at 4 °C for 10 min and the plasma stored at -80 °C. For evaluation of plasma insulin levels, the plasma was thawed and diluted with buffer according to directions for the Rat Insulin Enzymeimmunoassy Biotak (EIA) System (Amersham Biosciences) assay. According to data published by the manufacturer using this method normal male rat plasma insulin levels averaged 6-7 ng/ml. Determination of blood glucose and plasma insulin were conducted while the rats were exposed to fructose and then during the washout period. Glucose levels are expressed as mmol/l.

**Timeline of Experiment**

In figure 1 the timelines of both studies are presented. For the fructose study, baseline ventilatory and metabolic measurements were conducted in all 10 rats. Then 4 rats were implanted with radiotelemetry devices and baseline blood pressure and heart rate were evaluated 10 days later while the animal was in its home cage. Three days later all rats received 10% fructose in their drinking water. Measurements of water intake were conducted every 2-3 days and food intake measured weekly. One week later 5 rats were exposed to either IH (10% oxygen in nitrogen for 1 1/2 hour) or 5 to air for the same period of time in the exposure chamber. This protocol was followed for 13 days. Then ventilation, metabolism, and ventilatory responses to acute hypoxia and hypercapnia were evaluated according to the protocol described above. Finally, fructose was removed from the drinking water for 2 weeks to determine if there was an effect of removing fructose on cardiopulmonary and metabolic factors. Blood samples were taken as described above.
after the ventilatory measurements were finished during fructose feeding and at the end of the washout period.

For the control experiment, 6 rats received tap water. Ventilation and metabolism in air and ventilation in response to hypoxia and hypercapnia were evaluated at baseline, after 13 days in which rats were exposed to air for 1 ½ hours per day and 13 days after exposure to hypoxia for 1 ½ hours per day.

**Statistical analysis**

To evaluate the effects of fructose feeding and exposure to IH on ventilatory and metabolic variables, a 2 way ANOVA (group, IH or intermittent air and treatment, baseline, fructose feeding, and removal of fructose (washout)) with repeated measures was used. To evaluate the effects of intermittent air or hypoxia on hypercapnic and hypoxic responsiveness in the 6 rats that did not receive fructose paired Student *t* tests were used. In rats fitted with radiotelemetry devices and exposed to either air or to hypoxia the effect of treatment and time of day was evaluated using a 2 way ANOVA with repeated measures. Post hoc *t* tests (paired or unpaired) with Bonferroni corrections were conducted if the ANOVA’s were significant at P<0.05.

**Results**

**Bodyweights, Glucose and Insulin Levels**

Neither body weights nor 6 hour fasting glucose values were affected by fructose feeding or exposure to IH (Table 1). In contrast, insulin levels were higher in the fructose-fed and hypoxic exposed group relative to the fructose fed and air exposed group. Removal of hypoxia and fructose resulted in insulin levels similar to those in the
group of rats exposed to air (interaction of treatment and gas exposure $F_6, 15=6.02$, $P=0.0495$, effects of fructose treatment $F_1, 15 =8.31$, $P=0.028$, and gas exposures $F_1, 15 =6.91$, $P=0.039$). Thus, fructose and hypoxia appear to increase plasma insulin values that are “normalized” after removal of both hypoxia and fructose. During fructose feeding rats’ consumption of liquid was $146.9 \pm 10.0$ ml/day, which dropped to $38.5 \pm 1.6$ ml/day following removal of fructose from the drinking water. In contrast, food consumption increased from $72.9 \pm 3.6$ g/week to $135.7 \pm 4.4$ g/week following removal of fructose from drinking water.

**Effects of Fructose Feeding on Cardiovascular Variables**

In 4 rats the heart rate responses were evaluated at baseline, prior to fructose feeding, during fructose feeding, and after washout (Fig. 2). There was a significant increase ($P=0.003$) of heart rate during fructose feeding relative to baseline. Moreover, that increase returned to baseline values during washout. Heart rate was increased at all times of the day and night with fructose feeding relative to washout ($F_6, 24 =62.84$, $P<0.00001$).

Unlike the large increase of heart rate with fructose feeding, systolic blood pressure (Figure 2) showed a more modest, but significant increase of about 10 to 15 mm Hg ($P=0.01$). Following washout, systolic blood pressure returned to baseline values. Again there was an interaction between time of day and treatment ($F_6, 24 = 39.45$, $P <0.0001$). Fructose treatment increased blood pressure throughout the light and dark parts of the cycle. The highest effects were during the active periods (at night). Thus, fructose
feeding increased both heart rate and systolic blood pressure at all times and this was reversed after 2 weeks of washout when rats were given tap water to drink.

**Effects of Fructose Feeding on Metabolism and Ventilation in Air**

There were no effects of intermittent hypoxic exposure on ventilatory and metabolic variables when rats were breathing air. Thus, the data that are presented in Table 2 consist of pooled values from all 10 animals. There were no significant effects of fructose feeding relative to baseline on any of the variables. In contrast, during washout, body weight corrected VCO₂, body weight corrected Vₑ, body weight corrected Vₜ and f all decreased compared to these variables during fructose feeding. There was a greater decrease of VCO₂ relative to ventilation. Thus, the ventilatory equivalent (Vₑ/VCO₂) increased during washout relative to baseline or during fructose consumption (P<0.0001).

**Effects of Fructose Feeding on Ventilatory Responses to Hypercapnia and to Hypoxia**

There were no effects of IH compared to intermittent air exposure on the hypercapnic response in the fructose fed rats (data not shown). Thus, the data of the 10 rats were combined. Ventilatory responsiveness to acute hypercapnia (Figure 3) decreased during fructose administration (P=0.0304), which then returned to baseline values during washout in the 10 animals. Frequency responsiveness to hypercapnia (Table 3) tended to decrease with fructose feeding and further during washout (P =0.0331). In contrast, the body weight corrected Vₜ responsiveness to hypercapnia was not altered by fructose administration, but increased significantly during washout from 40.6 ± 6.5% to 74.8 ± 5.7% (P=0.009).
To determine the underlying mechanisms responsible for changes in ventilatory responsiveness, the raw data of the frequency of breathing, body weight corrected tidal volume and body weight corrected ventilation in air and in response to hypercapnia are shown in Table 4. During exposure to air, body weight corrected tidal volume and consequently minute ventilation decreased when fructose was removed. While rats received fructose and were exposed to hypercapnia, tidal volume and minute ventilation were decreased relative to baseline values. During exposure to hypercapnia there was a significant decrease in frequency of breathing (P=0.004), tidal volume (P=0.01) and minute ventilation (P=0.0008) after fructose was removed. Thus, since tidal volume in air dropped to a greater extent than the drop during exposure to hypercapnia, the tidal volume responsiveness (Table 3) actually increased.

Data from 6 control rats who did not receive fructose showed hypercapnic ventilatory responsiveness that was similar at baseline, after intermittent air, after IH and during washout (Figure 5A). These values were comparable for data in the fructose intervention at baseline and during washout, reinforcing the fact that fructose feeding was responsible for the decrease in hypercapnic responsiveness.

Ventilatory responsiveness to acute hypoxia was different between rats, exposed to IH and rats exposed to intermittent air during fructose feeding (Figure 4). While the ventilatory responsiveness of the IH group showed no statistically significant effect of either fructose feeding or washout, the group exposed to fructose and intermittent air showed a tendency toward decreased ventilatory responsiveness to acute hypoxia (P=0.0621) and an increased response to acute hypoxia during washout (70 ± 11% to 128
± 20%, P=0.0067, Table 5 and Figure 4). When the tidal volume and frequency responses of the 2 groups were analyzed, they showed that a different strategy in response to acute hypoxia was utilized in each group. Thus, in the IH exposed rats, frequency of breathing responses were significantly decreased with fructose feeding, and did not rebound during washout (one way repeated ANOVA, P=0.0115). In contrast, the intermittent air exposed rats did not exhibit significant differences between baseline, fructose feeding and washout on the frequency responsiveness to acute hypoxia.

Weight corrected \( V_T \) responsiveness exhibited a significant effect of treatment in the group of rats exposed to intermittent air (P=0.0059). Tidal volume responsiveness decreased (P=0.0548) during fructose feeding and significantly rebounded during washout, P=0.0012 (53.7 ±13.1 % (baseline), 15.2 ± 4.8 % (fructose) and 58.1 ± 6.4% (washout)). In contrast, there was no significant effect on \( V_T \) responses of fructose feeding or washout in the group exposed to IH.

To determine the underlying mechanisms in ventilatory responsiveness to hypoxia in the 2 groups reported above, the raw data are presented in Table 6. During washout and air exposure the intermittent air group showed a significant decrease in frequency of breathing (P=0.03), not observed in the IH group. In contrast, during exposure to acute hypoxia, the IH group, but not the intermittent air group, showed a significant overall decrease in frequency (F4, 8=8.20, P=0.0115) that was evident when fructose was given and during removal of fructose. With fructose feeding, only the IH group deceased body weight corrected ventilation during acute hypoxic exposure, due primarily to a decrease of breathing frequency. During washout both groups displayed significant decreases in
body weight corrected tidal volume and minute ventilation when exposed to air and acute hypoxia.

In the 6 control rats not exposed to fructose feeding, the hypoxic responsiveness at baseline, after intermittent air, after IH and during washout is shown in Figure 5B. After intermittent air exposure hypoxic responsiveness was 125.3 ± 27.6% and after IH 101.5 ± 20.0% (P>0.36). These values are also comparable to baseline values shown in Table 4. However, following washout after IH, the ventilatory response of the 6 control rats to acute hypoxia was 158.3 ± 23.9%. In contrast, during washout in rats that received fructose and IH, ventilatory responsiveness to hypoxia was only 88 ± 9 % (Figure 4). Thus, fructose feeding depressed the ventilatory responsiveness to acute hypoxia and did not return to baseline values in the IH group.

Therefore, fructose feeding decreased ventilatory responses to both hypoxia and to hypercapnia. However, IH relative to intermittent air exposure had different effects on the acute ventilatory responses of rats exposed to acute hypoxia during fructose feeding and washout.

**Discussion**

Fructose feeding in this study induced an increase in systolic blood pressure and heart rate, which returned to baseline values once fructose was removed from the rats’ drinking water. Exposure of fructose fed rats to IH resulted in an increase in plasma insulin levels that returned to values observed in the intermittent air group during fructose feeding and washout. Tidal volume, frequency, and minute ventilation decreased during washout, as did CO₂ production. Since the decrease in CO₂ production was greater than in ventilation, the ventilatory equivalent increased during washout. Ventilatory
responsiveness to acute hypercapnia was depressed in the entire group, without exhibiting differences due to IH or air exposure, contrasting to the effects of IH on responsiveness to acute hypoxia. These ventilatory changes were not seen in exposing rats to either intermittent air or hypoxia without fructose. Each of these findings will be discussed below.

**Fructose Feeding and Cardiovascular Function**

Administration of fructose to rats either in solid food or in drinking water results in the elevations in systolic blood pressure (SBP) and heart rate (HR) that are both dependent upon the concentration of the fructose and the length of time the fructose is given (4, 8, 19). Dai and McNeill (4) evaluated the effects of 5, 10 and 20% fructose in drinking water relative to control on the variables listed above over a 12-week period. They reported that the optimal concentration of 10% fructose increased SBP by about 30 mm Hg after 2 weeks of treatment and returned to baseline values after a 2 week washout period. In the present study SBP showed significant, but smaller increases of about 10 to 15 mm Hg. The difference between the present study and several published reports is that to evaluate HR and SBP most investigators use the tail cuff method that requires restraint and heating of rats. This technique in conjunction with fructose feeding may further activate the autonomic nervous system and confound the blood pressure measurements reported by several investigators (4, 18, 19). Only one other study (8) reported findings comparable to ours. These investigators accustomed their animals to the equipment prior to commencing the study, suggesting that the increase in HR reflects an increased sympathetic stimulation noted with fructose feeding (9) as seen with increased urinary
excretion of epinephrine and norepinephrine. Increased blood pressure with fructose feeding is also maintained by stimulation of the renin-angiotensin system. For example Shinozaki and colleagues (18) showed that fructose feeding elevated angiotensin II levels in rats. Elevated SBP in fructose fed rats can also be prevented by administration of an angiotensin AT1 receptor blocker, olmesartan during fructose feeding (18). Thus, the elevations of both HR and SBP during fructose administration in our study may have been caused by several factors that were reversed during washout.

**Fructose Feeding, Glucose, Insulin Levels and IH**

Several studies have shown that fructose feeding contributes to elevated levels of cholesterol, triglycerides, and to the development of insulin resistance, which may result in elevated levels of insulin with or without increases in plasma glucose levels (4, 22, 23). In the present study the combination of IH and fructose resulted in marked elevations of plasma insulin levels that during washout became comparable to those in the intermittent air group. The intake of fructose in this group compared to the intermittent air group was not different (data not presented). In a recent study Polotsky and coworkers (14) reported that exposing genetically obese insulin resistant mice (ob/ob) to IH elevated fasting plasma insulin levels further. Control mice exposed to IH did not increase plasma insulin levels. Prior infusion of leptin into ob/ob-hypoxic exposed mice prevented the increased insulin resistance. Moreover, the only gene that was up-regulated in white adipose tissue in lean IH exposed mice was leptin. Whether fructose feeding and IH cause the same effect needs to be investigated.
A potential mechanism for the increased levels of insulin in the IH exposed group may be increased levels of tumor necrosis factor alpha that are elevated during fructose feeding (24). Common pathways that both hypoxia and insulin resistance can act through include p38 mitogen-activated protein kinase and hypoxia inducible factor 1α (HIF) (10, 17). Moreover HIF can be further increased by angiotensin II (17). Thus, the interaction between IH and elevated levels of insulin on cardiopulmonary function needs to be investigated at the molecular level.

**Fructose Feeding Affects Control of Breathing**

This is the first study that evaluated the effects of fructose feeding on control of breathing. Ventilation during air exposure was not affected while the animals were given fructose, but decreased below baseline values during washout. One mechanism that may explain this observation is the decrease in CO₂ production during washout; however this drop was greater than that of ventilation. Consequently, the ventilatory equivalent increased during washout. Factors responsible for these findings may be due to alterations in central modulators of breathing. Fructose feeding has been shown to have profound effects on brain tryptophan, serotonin (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA). Thibault (23) analyzed tryptophan, 5-HT and 5-HIAA levels in the hypothalamus, thalamus, raphe nuclei and brain stem of rats fed a diet high in fructose relative to a control diet. She noted that tryptophan levels were increased in all brain regions. 5-HT and 5-HIAA levels were increased in the hypothalamus, but markedly decreased in the thalamus, raphe nuclei and brain stem with fructose feeding. The effects of washout were not evaluated in that study. Since low serotonin level can
depress ventilation (12), this neurotransmitter system may be involved in the results obtained in the present study. Evaluating serotonin levels and its metabolites, as well as receptor levels in brain stem regions associated with control of breathing during and after fructose feeding may help determine if this stipulation is correct.

In several animal models of insulin insensitivity such as in the ob/ob mouse (20), the obese Zucker rat (6) and the BIO 14.6 hamster (16) ventilatory responses to hypercapnia and hypoxia are abnormally low, similar to the response of fructose fed rats in the present study. However, the underlying mechanisms for the depression of breathing in all these models vary. For example, administration of leptin to the ob/ob mouse improves ventilation (20). When BIO 14.6 hamsters received thyroid hormone supplementation, control of breathing normalized (16). In the present study ventilatory responsiveness to hypercapnia returned to baseline values after washout, while tidal volume responsiveness actually increased and frequency responsiveness remained at the same level as during fructose feeding. This suggests that regulation of tidal volume and frequency in response to hypercapnia is altered not only by fructose feeding, but also washout. Moreover this pattern was not seen while rats were breathing air or in control rats exposed either to intermittent air or IH. Thus, clearly fructose feeding affects hypercapnic responsiveness. The mechanisms responsible for this finding are not known, but may involve alterations of neurotransmitter levels such as 5 HT (23), leptin (14), and possibly cytokines such as TNF alpha (15) acting at the level of respiratory muscles (26).

The ventilatory responsiveness of rats to acute hypoxia was modified by both fructose feeding and concomitant exposure to IH. Evidence for this is 3 fold: 1. The
intermittent air exposure group’s responsiveness to hypoxia did not exhibit the same tidal volume and frequency pattern as did the group exposed to IH and 2. Rats not given fructose, but exposed to either intermittent air or hypoxia demonstrated responses comparable to baseline values in the fructose intervention. 3. The combination of fructose and IH had a more long lasting effect than the intermittent air and fructose, since the IH group showed a tendency (P= 0.052) to maintain a decrease in minute ventilation responsiveness during washout, whereas the values for the group exposed to intermittent air increased from the fructose values and were similar to baseline values. These divergent findings suggest that the underlying mechanisms responsible for the results are different.

The results of this study may have several clinical implications. First, excess dietary fructose may contribute to development of insulin insensitivity (11) that contributes to the development of hypertension (1) and to depression of ventilation in response to hypoxia and hypercapnia as seen in patients with sleep apnea (3,13). Second, the combination of a predisposition or a subclinical state of insulin insensitivity and IH, as seen in sleep apnea may contribute to the development of further insulin insensitivity and ultimately type 2 diabetes. A recent study by Harsch and colleagues (7) found that constant positive airway pressure treatment of sleep apnea patients who also had insulin insensitivity resulted in an improvement in insulin sensitivity without any change in body mass index. Moreover, patients who were not obese showed greater improvements than did obese patients. By understanding the underlying mechanisms responsible for the contribution of insulin insensitivity to cardiopulmonary dysfunction and the exacerbating
role of IH, treatment of patients who manifest either one or both of these abnormalities may be helped.

In summary, fructose feeding increases heart rate and SBP, but decreases ventilation and ventilatory responses to hypoxia and hypercapnia. Moreover, the ventilatory responses to acute hypoxia are different depending upon concurrent exposures to IH that also increase insulin levels compared to rats exposed to fructose and intermittent air.
Acknowledgements
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References


**Figure legends**

1. Schematic of the fructose study and control study. The experimental conditions, measurements made and numbers of rats undergoing each treatment are shown.

2. Heart rate (beats/min) and Systolic blood pressure (SBP) in 4 rats at baseline, during fructose feeding and after washout. The asterisks denote significant differences (P<0.05) between treatments. Values are means ± SEM. Note the increases in heart rate and SBP during fructose feeding that returns to baseline values after washout.

3. Percent hypercapnic responsiveness in 10 rats at baseline, during fructose feeding and after washout. This variable was calculated by taking the ventilation in response to hypercapnia, subtracting the air value preceding the hypercapnic exposure from it, dividing the difference by the air value and multiplying the resultant by 100. Letters A and B denote significant differences (P<0.05) between treatments while AB is not different from either A or B. The washout value had a P value of 0.055. Values are means ± SEM.

4. Percent hypoxic responsiveness in rats at baseline, during fructose feeding (while 5 were exposed to intermittent air and 5 to intermittent hypoxia) and after washout. The percent hypoxic responsiveness was calculated by taking the ventilation in response to hypoxia, subtracting the air value preceding the hypoxic exposure from it, dividing the difference by the air value and multiplying the resultant by 100. The # sign indicates a significant decrease in ventilation during fructose exposure in the intermittent air-exposed group. The ANOVA for minute ventilation responsiveness in the intermittent hypoxic group was P=0.0527. Values are means ± SEM.

5. Hypercapnic (A) and hypoxic responsiveness (B) as defined in legends from figures 3 and 4 of 6 control rats not given fructose at baseline, after intermittent air exposure, after
intermittent hypoxic exposure and during washout. The asterisk in 5B indicts that during washout, hypoxic responsiveness was significantly greater than at other periods. Values are means ± SEM.
Table 1

Body Weights Glucose and Insulin Levels of Rats Exposed Intermittently to Air or Hypoxia during Fructose

<table>
<thead>
<tr>
<th></th>
<th>Hypoxia Exposed</th>
<th>Air Exposed</th>
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<tbody>
<tr>
<td></td>
<td>Fructose</td>
<td>Washout</td>
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<tr>
<td>Body Weight</td>
<td>478 ± 12</td>
<td>483 ± 4</td>
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<tr>
<td>Glucose</td>
<td>5.66 ± 0.21</td>
<td>5.42 ± 0.27</td>
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<tr>
<td>Insulin</td>
<td>26.2 ± 6.40*</td>
<td>8.47 ± 1.30</td>
</tr>
</tbody>
</table>

Body weight (grams) and glucose levels (mmol/L) have 5 animals per group. Insulin values were obtained in 4 animals per group. Values are means ± SEM. The asterisk denotes that the insulin values (ng/ml) in the fructose and hypoxia exposed group is higher (P<0.001) than in the other 3 groups. Note that insulin levels were elevated in the group exposed intermittently during fructose administration relative to rats expose to intermittent air. Removal of hypoxia and fructose resulted in insulin levels comparable to that of air exposed rats.
Table 2  
Effects of Fructose Feeding on Ventilatory Variables in air, CO₂ production and VE/VCO₂

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Fructose Feeding</th>
<th>Washout</th>
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<tbody>
<tr>
<td>VE/BW</td>
<td>137 ± 5.6</td>
<td>146 ± 9.7</td>
<td>86 ± 6.5**</td>
</tr>
<tr>
<td>VT/BW</td>
<td>1.4 ± 0.07</td>
<td>1.4 ± 0.06</td>
<td>0.9 ± 0.05**</td>
</tr>
<tr>
<td>F</td>
<td>98 ± 4</td>
<td>99 ± 5</td>
<td>91 ± 4 *</td>
</tr>
<tr>
<td>VCO₂/BW</td>
<td>91 ± 0.6</td>
<td>92 ± 0.5</td>
<td>45 ± 0.04**</td>
</tr>
<tr>
<td>VE/VCO₂</td>
<td>15.3 ± 0.76</td>
<td>15.7 ± 0.84</td>
<td>19.7 ± 1.0**</td>
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</tbody>
</table>

There are 10 animals per group. Values are means ± SEM. Variables are body weight corrected (BW X1000) VE = minute ventilation (in ml/min/gram), VT = tidal volume (ml/breath) and VCO₂ = CO₂ production (ml/min). The F stands for frequency of breathing. The last variable is the ratio of ventilation to CO₂ production. One asterisk denotes a significant difference between fructose feeding and washout of P<0.05, while two asterisks denote significant differences of P<0.01.
Table 3
Effects of Fructose Feeding on Frequency and Tidal Volume Responsiveness to Acute Hypercapnia

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Fructose</th>
<th>Washout</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>56.3 ± 5.1%</td>
<td>45.0 ± 4.7%</td>
<td>36.8 ± 5.9%</td>
<td>P=0.0331</td>
</tr>
<tr>
<td>Tidal Volume</td>
<td>45.1 ± 7.6%</td>
<td>40.6 ± 6.5%</td>
<td>74.8 ± 5.7%*</td>
<td>P=0.0026</td>
</tr>
</tbody>
</table>

Frequency of breathing and tidal volume responsiveness of 10 rats exposed to acute hypercapnia. The ANOVA refers to a one way repeated ANOVA for treatment. The asterisks indicates a significant increase in tidal volume responsiveness comparing Fructose and Washout (P=0.009).
Table 4

Effects of Fructose Feeding on Frequency of Breathing, Tidal Volume, and Minute Ventilation in Air and in Hypercapnia

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Fructose</th>
<th>Washout</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (Air)</td>
<td>94 ± 4</td>
<td>101 ± 4</td>
<td>98 ± 5</td>
</tr>
<tr>
<td>Frequency (CO₂)</td>
<td>149 ± 5</td>
<td>145 ± 3</td>
<td>132 ± 2 **</td>
</tr>
<tr>
<td>( V_T/\text{BW} ) (Air)</td>
<td>1.4 ± 0.07</td>
<td>1.3 ± 0.05</td>
<td>0.86 ± 0.04 **</td>
</tr>
<tr>
<td>( V_T/\text{BW} ) (CO₂)</td>
<td>2.0 ± 0.10</td>
<td>1.7 ± 0.05 *</td>
<td>1.5 ± 0.07 **</td>
</tr>
<tr>
<td>( V_E/\text{BW} ) (Air)</td>
<td>132 ± 10.7</td>
<td>126 ± 6.0</td>
<td>84 ± 6.4 *</td>
</tr>
<tr>
<td>( V_E/\text{BW} ) (CO₂)</td>
<td>302 ± 16.2</td>
<td>253 ± 8.1 **</td>
<td>197 ± 10.0 **</td>
</tr>
</tbody>
</table>

There are 10 animals per group. Values are means ± SEM. Variables are body weight corrected (BW X1000) \( V_E \) = minute ventilation (in ml/min/gram) and \( V_T \) = tidal volume (ml/breath). Frequency of breathing is in breaths per minute. One asterisk denotes a significant difference between fructose feeding or washout and baseline of \( P<0.05 \), while two asterisks denote significant differences of \( P<0.01 \).
### Table 5

Ventilatory Responsiveness to Acute Hypoxia of Rats Intermittently Exposed to Hypoxia or Air

<table>
<thead>
<tr>
<th></th>
<th>Intermittent Hypoxia</th>
<th></th>
<th></th>
<th>Intermittent Air</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Fructose</td>
<td>Washout</td>
<td>Baseline</td>
<td>Fructose</td>
<td>Washout</td>
</tr>
<tr>
<td>Frequency</td>
<td>144 ± 12 %</td>
<td>124 ± 8 %</td>
<td>121 ± 7 %§</td>
<td>137 ± 6 %</td>
<td>146 ± 6%</td>
<td>129 ± 4 %</td>
</tr>
<tr>
<td>Tidal Volume</td>
<td>58 ± 9 %</td>
<td>46 ± 5 %</td>
<td>44 ± 6%</td>
<td>54 ± 13 %</td>
<td>15 ± 5 %</td>
<td>58 ± 6 %*§</td>
</tr>
</tbody>
</table>

Frequency and tidal volume responsiveness to hypoxia in rats exposed either to intermittent hypoxia or air during fructose feeding. Baseline is prior to fructose feeding or to exposures of intermittent hypoxia or air. Washout refers to removal of intermittent exposures and fructose. The asterisk indicates that the tidal volume responsiveness of rats exposed to intermittent air increases significantly during washout relative to fructose feeding (P=0.0012). The § symbol denotes a significant one way ANOVA effect of treatment for frequency responsiveness of the intermittent hypoxia exposed group and tidal volume responsiveness of the intermittent air exposed group.
Table 6
Effects of Fructose Feeding on Frequency of Breathing, Tidal Volume, and Minute Ventilation in Air and in Hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Intermittent Hypoxia (n=5)</th>
<th>Intermittent Air (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Fructose</td>
</tr>
<tr>
<td>F (air)</td>
<td>99 ± 3</td>
<td>98 ± 9</td>
</tr>
<tr>
<td>F (hypoxia)</td>
<td>144 ± 12</td>
<td>124 ± 8*</td>
</tr>
<tr>
<td>V_T/BW (air)</td>
<td>1.4 ± 0.10</td>
<td>1.4 ± 0.05</td>
</tr>
<tr>
<td>V_T/BW (hypoxia)</td>
<td>2.1 ± 0.11</td>
<td>2.0 ± 0.04</td>
</tr>
<tr>
<td>V_E/BW (air)</td>
<td>136 ± 11</td>
<td>135 ± 15</td>
</tr>
<tr>
<td>V_E/BW (hypoxia)</td>
<td>308 ± 33</td>
<td>241 ± 12*</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Variables are body weight corrected (BW X1000) $V_E =$ minute ventilation (in ml/min/gram) and $V_T =$ tidal volume (ml/breath). Frequency of breathing is in breaths per minute. One asterisk denotes a significant difference between fructose feeding or washout and baseline of $P<0.05$, while two asterisks denote significant differences of $P<0.01$. 
Figure 1

**Fructose Feeding**

Baseline VE & metabolism (n=10)

- Telemetry implantation in 4 rats
- 10% fructose in drinking water

- Intermittent hypoxia (n=5, 2 with telemetry)
- VE & metabolism, followed by insulin and glucose measurement
- Remove fructose feeding
- Washout (n=10)
- VE & metabolism, followed by insulin and glucose measurement

**Control**

Baseline VE & metabolism (n=6)

- Intermittent air (n=6)
- VE & metabolism
- Intermittent hypoxia (n=6)
- VE & metabolism
- Washout (n=6)
- VE & metabolism
Figure 2.
Figure 3 Hypercapnic Responsiveness

Baseline | Fructose | Washout

Percent

A

B

AB

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Hypoxic Responsiveness

Figure 4
Figure 5A.

Figure 5B.