Fetal Ethanol Exposure Causes Hepatic Insulin Sensitizing Substance-dependent Insulin Resistance

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ABSTRACT

Fetal alcohol syndrome (FAS) and type 2 diabetes occur with high incidence in the same populations and both are associated with autonomic neuropathies. The authors have recently shown that hepatic parasympathetic neuropathy results in insulin resistance of skeletal muscle. The authors tested the hypothesis that fetal ethanol exposure (FEE) results in hepatic insulin sensitizing substance (HISS) -dependent insulin resistance (HDIR) in the adult offspring of rats. The parasympathetic nerves normally provide a permissive signal to the liver, allowing insulin to cause the release of HISS, which stimulates glucose uptake in skeletal muscle. Blockade of the neural signal prevents HISS release, and a state of HDIR ensues, whereby the response to insulin is reduced by 50 to 60% in the fed state. The rapid insulin sensitivity test (RIST), a transient euglycemic clamp, was used. The RIST index is the amount (mg) of glucose per kg of body weight required to maintain euglycemia following a bolus of insulin (50 mU/kg). Adult male offspring of dams who received ethanol through the maternal drinking water, throughout gestation and nursing, showed ethanol dose-related HDIR. The control RIST index of 195.3±5.5 mg glucose/kg body weight decreased progressively with maternal consumption of 5 to 20% vol/vol ethanol to 122.5±13.9 mg glucose/kg body weight in the 20% ethanol group. HISS-independent insulin action and the glucose disposal effect of insulin-like growth factor (IGF) -1

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RÉSUMÉ

On retrouve souvent le syndrome d'alcoolisation fœtal (SAF) et le diabète de type 2 au sein des mêmes populations et les deux sont associés à des neuropathies autonomes. Les auteurs ont récemment démontré qu'une neuropathie parasympathique hépatique aboutit à une insulinorésistance du muscle squelettique. Les auteurs ont vérifié l'hypothèse voulant que l'exposition du fœtus à l'éthanol (EFÉ) aboutisse à une insulinorésistance dépendante d'une substance sensibilisant l'insuline hépatique (HISS), ou HDIR, chez les descendants adultes de rats. Les nerfs parasympathiques donnent normalement un signal permissif au foie, permettant à l'insuline de déclencher la libération de la HISS, laquelle stimule la captation de glucose dans le muscle squelettique. Le blocage du signal neural empêche la libération de la HISS et un état de HDIR s'ensuit, ce qui fait que la réponse à l'insuline est réduite de 50 à 60 % lorsque les animaux ont mangé. Le test rapide de sensibilité à l'insuline (TRSI), un clamp euglycémique passager, a été utilisé. L'indice du TRSI est la quantité (mg) de glucose par kg de poids corporel nécessaire pour maintenir l'euglycémie après l'administration d'un bolus d'insuline (50 mU/kg). Les descendants mâles adultes de mères qui avaient consommé l'éthanol ajouté à leur eau pendant la gestation et l'allaitement ont présenté une HDIR liée à la dose d'éthanol. L'indice du TRSI des témoins a baissé progressivement par suite de la consommation maternelle de 5 à 20 % vol/vol d'éthanol de 195,3 ± 5,5 mg glucose/kg de poids corporel à 122,5 ± 13,9 mg glucose/kg de poids corporel dans le groupe qui avait consommé de l'éthanol à 20 %. L'action de l'insuline indépendante de la HISS et l'effet d'élimination du glucose du facteur de croissance semblable à l'insuline (FCI)-1 n'ont pas changé. L'EFÉ a entraîné une insulinorésistance entièrement attribuable à la suppression liée à la dose d'éthanol de l'action de la HISS (HDIR) chez les descendants mâles adultes. La possibilité d'un lien semblable chez l'humain devra être évaluée.

were unaltered. FEE lead to insulin resistance completely attributable to ethanol dose-related suppression of HISS action (HDIR) in the adult male offspring. The possibility of the same relationship in humans requires evaluation.

INTRODUCTION

Insulin action to increase glucose uptake at skeletal muscle has been shown to be dependent upon the insulin-mediated release of a hepatic insulin sensitizing substance (HISS) from the liver (1). After its release from the liver, HISS enters the circulation and enhances glucose uptake at skeletal muscle, accounting for 50 to 60% of the stimulation of whole-body glucose disposal in response to a bolus of insulin (5 to 100 mU/kg) in the fed state. In addition, the hepatic parasympathetic nerves (2-5), through hepatic nitric oxide (NO) production (6), play a permissive role in the hepatic release of HISS. Pharmacological or surgical interruption of the hepatic parasympathetic, NO-dependent release of HISS by hepatic muscarinic receptor antagonism (2,7), hepatic nitric oxide synthase (NOS) antagonism (6,7) or liver denervation (4,5,7) resulted in HISS-dependent insulin resistance (HDIR) without affecting the HISS-independent component of insulin action. HDIR produced by surgical denervation of the liver was reversed by intraportal, but not intravenous (IV), administration of acetylcholine in rats (3) and dogs (8). Furthermore, administration of an NO donor, 3-morpholinosydnonimine (SIN-1), completely reversed HDIR produced by denervation of the liver or hepatic NOS antagonism in rats (6) and rabbits (9). The hepatic release of HISS is physiologically regulated by the prandial state. HISS release is at its highest after feeding and declines progressively with duration of fasting (7). Insulin action is reduced by fasting by 55% in rats (7). Thus, in order for insulin to have its full effect in the fed state, functional hepatic parasympathetic nerves must be able to cause HISS release. In the absence of HISS release, the response to insulin is similar in the fed and fasted conditions: a state of HDIR (7).

There is a high prevalence of fetal ethanol effects (10) and type 2 diabetes (11) in socioeconomically disadvantaged groups in many areas of the world. Fetal ethanol exposure (FEE) can lead to abnormalities of the central nervous system (CNS), which may manifest as learning and memory deficits, lowered intelligence quotient (IQ), attention deficit, mental retardation and microencephaly (12).

Ethanol exposure during embryogenesis results in changes in fetal cerebral metabolism (13,14). Maternal ethanol exposure reduces rat fetal cerebral uptake of glucose and oxygen (13). These alterations in cerebral metabolism can contribute to CNS defects resulting from FEE. Balduini and colleagues (15) have shown that administration of ethanol to developing rats during the brain growth spurt (postnatal exposure up to 15 days) leads to selective decreases in muscarinic receptor-induced proliferation of glial cells that may lead to microencephaly. Reduction of brain weight occurred only in those animals with muscarinic abnormalities, suggesting that muscarinic receptors may be a target for the neurotoxic effects of ethanol during this stage of brain development.

In addition, adenosine, an endogenous neuromodulator and neuroprotective agent, has been suggested to be a mediator of the effects of ethanol (16). Recently, it has been shown that FEE reduced adenosine A_1 receptor mRNA and protein density in the cortex and cerebellum, suggesting that a disruption in the adenosine system could be a cause of ethanol-induced central neurotoxicity (17).

Human and animal studies have described many endocrine and metabolic systems that are affected by FEE (18-20). Chronic ethanol exposure in the rat during pregnancy produces a high insulin release in response to a glucose load in newborns and up to 3 days after birth (21), and in 30-day-old adult rats (22). Castells and colleagues (23) have also shown abnormal oral glucose tolerance tests (OGTTs) in children with fetal alcohol syndrome (FAS).

Based on these observations, the authors hypothesized that FEE can lead to hepatic parasympathetic neuropathy that may result in HDIR. To test the hypothesis that FEE leads to HDIR, a range of doses of ethanol (5, 10, 15 and 20%) was provided through the drinking water to female rats prior to and throughout the pregnancy and up to the time of weaning. The rationale for exposure of the pups to ethanol up to the weaning stage was based on reports that ethanol exposure has the greatest effect during the rapid brain growth phase that occurs in utero in humans but after birth in rats (22). After weaning, the offspring received no further exposure to ethanol. Insulin sensitivity was evaluated using the rapid insulin sensitivity test (RIST) (24) in adult offspring from the ages of 41 to 75 days. Atropine, a muscarinic receptor antagonist, was administered to block HISS release and produce a full state of HDIR. Therefore, atropine served as a tool to differentiate the HISS-dependent and the HISS-independent components of insulin action.

The authors have shown previously that insulin and insulin-like growth factor (IGF) -1 have similar effects on glucose disposal as assessed by the RIST protocol (25). However, 50 to 60% of insulin action was shown to be through the hepatic release of HISS; the IGF-1 action was shown to not be dependent upon the release of HISS (25). Insulin resistance typical of that seen in type 2 diabetes (26,27) is not associated with resistance to the glucose disposal action of IGF-1. If the insulin resistance associated with FEE is due to HDIR, it should not result in an altered response to IGF-1. The authors hypothesized that FEE causes impaired HISS release that results in insulin, but not IGF-1, resistance. To test this hypothesis, the authors measured IGF-1 sensitivity using the

RIST protocol with IGF-1 in some of the males in the 0, 5 and 15% ethanol groups.

In utero exposure to ethanol resulted in dose-dependent HDIR in young adult offspring without affecting the HISS-independent component of insulin action or the glucose disposal effect of IGF-1. This is consistent with the hypothesis that FEE causes insulin resistance in the adult offspring secondary to impairment of HISS release.

MATERIALS AND METHODS

FEE model

Female Sprague-Dawley rats (216.1±5.4 g, n=23) underwent a training period to accustom them to the taste of ethanol in water. The dams were divided into 5 groups: 0 (no ethanol in the drinking water), 5, 10, 15 and 20% ethanol in the drinking water. Water and food (standard laboratory chow) intake were monitored for 4 days prior to the introduction of ethanol 5% vol/vol as the sole source of liquid intake. After 2 days, or until food and water consumption returned to normal levels or stabilized, ethanol content was increased to 10% in the second group of rats. The same procedure was followed for administration of concentrations of 15 and 20% ethanol in the third and fourth groups. When food and water consumption were stabilized, the male rat was introduced to the female and the date of conception was noted. Control (0%) dams were treated in the same manner, but ethanol was not included in the drinking water.

At birth, the litter composition, mortality and birth weights were determined. To minimize nutritional deficiencies, all litters were culled to 12 and the pups were nursed by the dam. The nursing dam continued to receive ethanol through the drinking water and, as the pups became mobile, the water bottle was raised to a level to prevent the pups from reaching the water. After weaning, the pups were raised in a normal manner until the time of testing for insulin sensitivity at age 41 to 75 days.

Animals were treated according to the guidelines of the Canadian Council on Animal Care, and all protocols were approved by the Ethics Committee on Animal Care at the University of Manitoba, Winnipeg, Manitoba, Canada.

Surgical procedures

The rats were fasted overnight (8 hours) and were fed standard laboratory rat food for 2 hours before the start of any surgical procedures to ensure that testing occurred in the postprandial state.

The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg, Somnotol®, MTC Pharmaceuticals, Mississauga, Ontario, Canada). Neither the HISS-dependent nor the HISS-independent insulin action is affected by pentobarbital (28). The temperature was maintained at 37.5±0.5°C by means of a temperature-controlled surgical table and a lamp over the table. The body temperature was monitored with a rectal probe thermometer (Fisher Scientific, Pittsburg, Pennsylvania, United States [US]). The rats were heparinized with 100 IU/kg of heparin.

A femoral arterial-venous shunt was established, as described previously (24). Arterial blood samples were taken from the shunt via puncture of the silicone sleeve. IV atropine, insulin and IGF-1 were administered through a puncture in the sleeve of the shunt. Arterial blood pressure was monitored via the arterial-venous shunt by briefly clamping the silicon sleeve on the venous side of the shunt. To maintain anesthesia throughout the experiment, the left jugular vein was cannulated with a catheter (polyethylene tubing, PE-50) to allow a continuous infusion of pentobarbital solution (1.0 mL/100 g body weight/hour, 1.08 mg/mL). Another catheter (polyethylene tubing, PE-50) preloaded with a D-glucose solution (100 mg/mL) was inserted (with a 23-gauge needle at the delivery end) into a silicone sleeve of the anesthetic catheter. The D-glucose catheter was connected to a continuously variable infusion pump (Harvard Apparatus, Holliston, Massachusetts, US). Spontaneous respiration was allowed through a tracheal tube (polyethylene tubing, PE-240).

Determination of insulin sensitivity

The RIST

The operating procedure for determination of insulin sensitivity using the RIST has previously been detailed elsewhere (24). Briefly, the animals were allowed to stabilize for at least

Table 1. Dams' body weights

Stage	*Ethanol exposure (% vol/vol ethanol in drinking water)*				
	0 (n=8)	5 (n=3)	10 (n=2)	15 (n=7)	20 (n=3)
Prebreeding (g)	281.4±10.5	316.7±29.4	230–256	261.1±17.4	278.8±23.2
Prebirth (g)	417.9±12.2	441.3±33.7	300–355	374.9±17.4	379.3±37.6
Preweaning (g)	344.3±6.9	361.3±14.8	239–343	261.2±13.4*	245.2±22.9*

Data are mean±SE; the range was used for the 10% ethanol group
*p<0.05 vs. dams in 0 and 5% ethanol groups

SE = standard error

30 minutes after the surgical preparation, prior to the first arterial glucose sampling. Arterial blood samples were then taken every 5 minutes by puncture from the shunt, and glucose concentrations were immediately analyzed by the oxidase method with a YSI 2300 STAT Plus™ Glucose & Lactate Analyzer (Yellow Springs Instruments Inc., Yellow Springs, Ohio, US) until a stable basal glucose concentration was established. A sample for determination of insulin level was taken at this stable point. IV insulin was then infused via the shunt at a dose of 50 mU/kg over a 5-minute period (in 0.5-mL saline at 0.1 mL/minute). To avoid hypoglycemia, the glucose infusion (5 mg/kg/minute) was started 1 minute after initiation of the insulin infusion. On the basis of the arterial glucose concentrations measured at 2-minute intervals, the infusion rate of the glucose pump was adjusted to maintain euglycemia throughout the full period (30 to 35 minutes) of insulin action. The RIST index is the amount of glucose infused (mg/kg body weight) after insulin administration; representing the magnitude of insulin sensitivity.

RIST in control and after atropine

A control RIST was performed on the adult male rats of the 0 (n=24), 5 (n=9), 10 (n=6), 15 (n=27) and 20% (n=12) ethanol groups. After the control RIST, IV atropine (1.0 mg/kg) was administered over 5 minutes to block HISS release. Basal glucose concentration was determined and another RIST was performed to quantitate the HISS-independent component of insulin action.

Determination of IGF-1 sensitivity

In some of the rats from the 0 (n=4), 5 (n=6) and 15% (n=7) ethanol groups, IGF-1 sensitivity was measured using the RIST with IGF-1 (200 μg/kg) infused for 5 minutes instead of insulin. The authors have previously shown that IGF-1 (200 μg/kg) and insulin (50 mU/kg or 2.27 μg/kg) have similar glucose disposal effects both in the magnitude and the pharmacodynamics of the response (25). Administration of atropine inhibited the release of HISS and produced HDIR; however, it did not affect IGF-1 sensitivity (25).

Drugs

The human insulin was purchased from Eli Lilly and Company (Indianapolis, Indiana, US). The 95% ethanol, atropine and D-glucose were purchased from Sigma-Aldrich Corporation (St. Louis, Missouri, US). Recombinant human insulin-like growth factor (rhIGF) -1 was donated by Genentech, Inc. (San Francisco, California, US). All chemicals were dissolved in saline. The rat insulin enzyme-linked immunosorbent assay (ELISA) kit was purchased from American Laboratory Products Company, Ltd. (Windham, New Hampshire, US).

Data analysis

Data were analyzed using 1-way analysis of variance (ANOVA) followed by a Tukey-Kramer multiple comparison test in each group or paired or unpaired t tests, when applicable. The ethanol dose-dependency was analyzed using the analysis of covariance (ANCOVA) followed by a linear contrast test. The mortality rate dose-dependency was analyzed using the Exact Mantel-Haenszel Trend Test. The analyzed data were expressed as mean±standard error (SE) throughout. Differences were accepted as statistically significant at $p<0.05$.

RESULTS

Dams

The dams in all ethanol groups had similar body weights before the breeding and just before giving birth (Table 1). However, the preweaning body weights of the dams in the 15 and 20% ethanol groups were significantly lower than the body weights of the dams in the 0 and 5% ethanol groups (Table 1).

Table 2. Dams' daily fluid consumption

Stage	*Ethanol exposure (% vol/vol ethanol in drinking water)*				
	0 (n=8)	5 (n=3)	10 (n=2)	15 (n=7)	20 (n=3)
Prebreeding (mL/100 g)	10.2±0.5	11.4±1.5	8.6–13.9	11.0±0.9	12.8±1.2
Gestation (mL/100 g)	9.2±0.4	11.4±0.5	8.4–8.5	6.7±0.3‡	8.4±2.1
Preweaning (mL/100 g)	25.4±2.3*†	20.7±1.4*†	12.5–17.8	14.0±0.9*†	12.7±0.7

Data are mean±SE; the range was used for the 10% ethanol group
*$p<0.05$ vs. prebreeding
†$p<0.01$ vs. gestation
‡$p<0.001$ vs. prebreeding

SE = standard error

The average fluid consumption did not change significantly during gestation in any groups, except for a significant decrease in the 15% ethanol group (Table 2). The average fluid consumption significantly increased during the preweaning period compared to the prebreeding period in all except the 20% ethanol group (Table 2). Exposure to ethanol during nursing causes an ethanol dose-dependent decrease in fluid intake.

Litter demographics

There were no significant differences between the mean number of pups delivered by the dams, the mean litter weights and the mean pup weights in any of the ethanol groups (Table 3). Prenatal exposure to ethanol caused a dose-dependent increase in mortality in the pups before weaning ($p<0.05$, Table 3). The number of days to wean was similar in all groups, except that the pups in the 20% ethanol group took significantly longer to wean (Table 3).

Insulin sensitivity

The male offspring from all groups were removed for experiments at age 41 to 75 days. There was no significant difference in age between any of the groups, except that the 20% ethanol group was significantly older than the 5% group (Table 4). The pups had similar body weights in all groups, except that the pups in the 20% group were significantly lighter than those in the 0% group (Table 4). Basal insulin levels were analyzed in some of the males of the 0 (n=13), 15 (n=8) and 20% (n=5) ethanol groups, and there were no significant differences in the insulin levels between the 3 groups (6.7±2.0, 4.0±1.1 and 6.4±1.8 ηg/mL, respectively). No correlation between the basal glucose and the basal insulin levels was observed in the same 3 groups.

The RIST index used to express insulin or IGF-1 sensitivity is the total amount of glucose (mg/kg) infused over 30 to 35 minutes in order to maintain euglycemia at the baseline level after insulin (50 mU/kg) or IGF-1 (200 µg/kg) administration over 5 minutes.

Control RIST indices

There were no significant differences between the control RIST indices in the 0, 5 and 10% ethanol groups; however, the control RIST indices of the 15 and 20% groups were significantly lower than the control RIST index of the 0% group ($p<0.001$) (Figure 1). There was a significant ethanol dose-dependent decrease in insulin sensitivity in adult offspring ($p<0.0001$).

No correlations were observed between the control RIST indices and the ages, body weights, mean arterial pressures, basal glucose concentrations, basal insulin concentrations or glucose:insulin ratios of the 0, 15 and 20% ethanol groups.

RIST indices after atropine administration

After administration of IV atropine (1.0 mg/kg), the control RIST index was significantly reduced in all groups ($p<0.01$) (Figure 1). The males in the 15 and 20% ethanol groups were insulin resistant, as shown by a reduced control RIST index. The HISS-independent component of insulin action (post-atropine) was not affected. The insulin resistance was accounted for by an ethanol dose-dependent reduction in HISS-dependent glucose disposal ($p<0.001$) (Figure 1 insert).

IGF-1 sensitivity

IGF-1 sensitivity was measured in some of the male adult offspring of the 0 (n=4), 5 (n=5) and 15% (n=7) ethanol groups. There was no significant difference in the RIST index with insulin (50 mU/kg or 2.27 µg/kg) between the 0 and 5% groups and the 5 and 15% groups, but there was a significant difference in the RIST index with insulin between the 0 and 15% groups (Figure 2). The IGF-1 (200 µg/kg) RIST index was similar in all groups. The IGF-1 RIST indices of all the groups were compared to the IGF-1 (200 µg/kg) RIST index (266.8±26.2 mg glucose/kg body weight) in a previously published study (28). No significant differences were observed between the published RIST index and the RIST index of each of the groups. The RIST indices with

Table 3. Litter demographics

Demographics	Dams' ethanol exposure (% vol/vol ethanol in drinking water)				
	0 (n=104)	5 (n=45)	10 (n=26)	15 (n=89)	20 (n=28)
Mean number of pups	13.0±1.6	15.0±1.2	13.0±0.0	12.7±0.8	9.3±2.7
Mean litter weight (g)	103.6±11.1	114.0±8.2	105.0±7.1	103.7±4.8	62.2±21.8
Mean pup weight (g)	8.2±0.6	7.7±0.7	8.1±0.5	8.3±0.6	6.4±0.7
Mean days to wean	20.1±0.6	17.7±0.4	19.0±0.0	19.0±0.6	24.0±1.4*
Pup mortality rate (%)	1.0	4.4	0	0	21.4*

Data are mean±SE
*$p<0.05$ vs. other groups

SE = standard error

insulin and with IGF-1 were similar in the 0% ethanol group, confirming the bio-equivalence of the doses. There was, however, a significant difference between the RIST index with insulin and the RIST index with IGF-1 in the 5 and 15% ethanol groups (Figure 2). In utero exposure to 15% ethanol caused insulin, but not IGF-1, resistance in adult rats. Although insulin resistance could not be detected in the 5% group using a 1-way ANOVA comparison of RIST indices with insulin, the significant difference in the paired responses to IGF-1 and insulin revealed impaired insulin action even at the 5% ethanol dose.

DISCUSSION

Exposure to ethanol through the maternal water supply led to dose-dependent HDIR in male adult rat offspring. The data are consistent with the hypothesis that FEE leads to hepatic parasympathetic dysfunction and a subsequent reduced ability of insulin to cause HISS release. It remains possible that the defect reported represents blockade of HISS action in peripheral tissues; however, HDIR occurred without affecting the HISS-independent component of insulin action or the glucose disposal action of IGF-1.

Technical issues

In the current model of FEE, different concentrations of ethanol (5, 10, 15 and 20%) were provided in the dams' drinking water. The dams were administered ethanol before breeding, throughout pregnancy and until the pups were weaned.

The RIST was used to measure insulin sensitivity in the male pups of all the ethanol-exposed dams when they were young adults. The RIST has been described previously (24) and has been used extensively to quantitate mechanisms of physiological and pathological regulation of HISS-dependent and HISS-independent insulin action (7). Although the chemical identity of HISS is not yet clear, the use of the RIST allows for detailed pharmacodynamic demonstration of HISS action (7). Pentobarbital anesthesia, used throughout, does not alter either the HISS-dependent or HISS-independent components of insulin action in normal male rats (28).

The selection of an adequate control group is fraught with difficulties. Pair feeding is not appropriate since the nutritional complication is more related to processing and metabolism, rather than the consumption of food. Liquid pairing would require depriving the control group of considerable water at different time points. Even full pairing of food and water would not deal with the effects of ethanol on the processing and metabolism of both food and water. The authors, therefore, elected to use an ad libitum food and water control with full acknowledgement that this design cannot determine the mechanism of FEE-induced HDIR as a direct or indirect toxic action of ethanol. In this regard, the model is similar to the human use of alcohol and fetal ethanol-induced effects.

HDIR

The RIST indices showed significant ethanol dose-dependent reduction that was entirely accounted for by reduction in the HISS-dependent component of insulin action with no tendency for reduction in the HISS-independent, direct insulin action (Figure 1).

Atropine has been shown to block the HISS-dependent component of insulin action (2,7). Administration of IV atropine produced significant additional HDIR in all of the groups by producing a maximal decrease in the HISS-dependent component of insulin action (Figure 1). This indicates that even the most severely affected groups retained some portion of the HISS-dependent component, which was further blocked by atropine.

Nutritional factors

FEE has been associated with malnutrition of the mother, reflected by low pre-pregnancy weight or poor maternal weight gain during pregnancy (29-31). Weinberg (32) indicated that alcohol consumption may alter metabolism, transport, utilization, activation and storage of almost every essential

Table 4. Condition of pups

Parameter	Dams' ethanol exposure (% vol/vol ethanol in drinking water)				
	0 (n=24)	5 (n=9)	10 (n=6)	15 (n=27)	20 (n=12)
Age (days)	57.4±2.0	52.0±0.9	54.0±0.9	59.5±1.8	64.8±1.9*
Body weight (g)	322.2±14.2	276.1±13.0	262.3±7.1	300.2±12.9	275.8±4.5†
Mean arterial pressure (mm Hg)	97.4±3.7	95.8±6.2	94.2±5.2	98.7±3.1	105.2±5.3
Basal glucose (mmol/L)	6.7±0.2	7.3±0.4	6.5±0.3	6.5±0.1	6.5±0.2

Data are mean±SE
*$p<0.01$ vs. 5% group
†$p<0.05$ vs. 0% group

SE = standard error

nutrient. Alcohol has been shown to cause gastrointestinal dysfunction, such as inhibition of nutrient absorption from the gut, inhibition of placental transport of nutrients essential for fetal growth, and impairment of energy-dependent mechanisms in nutrient utilization (33-36). Furthermore, chronic alcohol consumption decreases blood flow to the placenta (37-40), reduces placental glucose and reduces the availability and transport of other nutrients (41) to the fetus. Thus, some of the toxic effects of FEE may have been through nutritional interference.

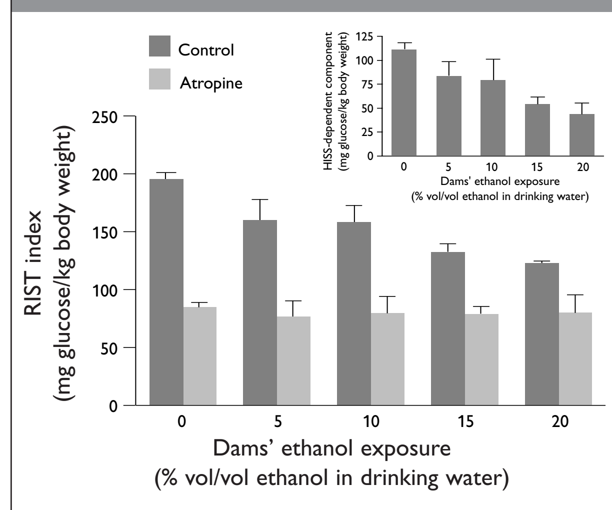
All of the dams' body weights just before giving birth were similar in all groups. However, the preweaning weights of the dams in the 15 and 20% ethanol groups were significantly lower than those of the dams in the 0 and 5% groups. In addition, the average fluid consumption was not significantly increased during gestation in any of the dams; however, it was significantly decreased in the 15% group. From birth to the time of weaning, fluid intake was almost tripled in the dams in the 0% group, but this increased fluid consumption decreased in an ethanol dose-dependent manner and was not significantly increased in the dams in the 20% group. Thus, exposure to ethanol during nursing caused a dose-dependent decrease in fluid intake. This indicates that during nursing the ethanol-treated groups of dams may have been dehydrated. The possible undernourishment and dehydration of the dams during gestation or nursing could have had severe effects on their pups. However, the pups exposed to 5 and 10% ethanol did not exhibit any significant reduction in insulin sensitivity, but those exposed to 15 and 20% ethanol were insulin resistant. Others (42) have shown that pair-fed control dams (0% ethanol) were underweight, but that their pups were not affected by the undernourishment of their mother. However, the ethanol-exposed dams in this study (42) were underweight and their pups were severely affected by FEE. The dams in the present study all had similar prepartum weights.

FEE has been shown to result in decreased litter size, survival and weight (43); however, in the present study, there were no significant differences between litter size and pup weight in any of the groups. Ethanol caused a dose-dependent increase in mortality in the pups from birth to weaning. The pups that were most affected by FEE appear to have died before they were tested for insulin sensitivity. It is possible that the pups with the highest degree of HDIR did not survive, thus the degree of insulin resistance induced by FEE may have been more severe than reported here.

The FEE in the present study clearly caused insulin resistance secondary to impairment of the hepatic release of HISS in response to insulin. Whether this neuropathy was caused solely by the toxic effects of ethanol or whether malnutrition and dehydration secondary to ethanol ingestion had additive roles cannot be determined based on the results of the present study.

This study does not establish when the impairment of the release of HISS leading to insulin resistance occurred with FEE. Alterations in glucose metabolism have been shown in fetuses (44) and neonates (42), and OGTTs have shown elevated glucose and insulin levels in rats at the ages of 30 and 90 days, indicating that insulin responsiveness was reduced at both time points (22). The observation of abnormalities in FEE fetuses, neonates and adult offspring that were nursed by surrogate dams not exposed to ethanol strongly suggests that the damage occurred in utero (22). The human fetus is more sensitive to FEE in the third trimester, during which the rapid burst of brain growth occurs; however, the rapid burst of brain growth in rats occurs after birth for up to 15 days (15). The rats in the present study were exposed to ethanol throughout the entire gestation and nursing periods in order to more clearly mimic the human situation. Further studies are required to elucidate the period of susceptibility to FEE.

Figure 1. RIST indices in controls and after IV atropine (1.0 mg/kg) administration in the adult male offspring of the 0 (n=24), 5 (n=9), 10 (n=6), 15 (n=27) and 20% (n=12) prenatal ethanol-exposed groups



The insert shows the HISS-dependent component of insulin action calculated by subtraction of the HISS-independent component of insulin action (post-atropine) from the control response consisting of a HISS-dependent and HISS-independent component of insulin action. FEE caused a significant ethanol dose-dependent decrease in insulin sensitivity through significant dose-dependent decrease in the HISS-dependent component of insulin action. The HISS-independent (post-atropine) component was not affected.

Values are mean±SE

FEE = fetal ethanol exposure
HISS = hepatic insulin sensitizing substance
IV = intravenous
RIST = rapid insulin sensitivity test
SE = standard error

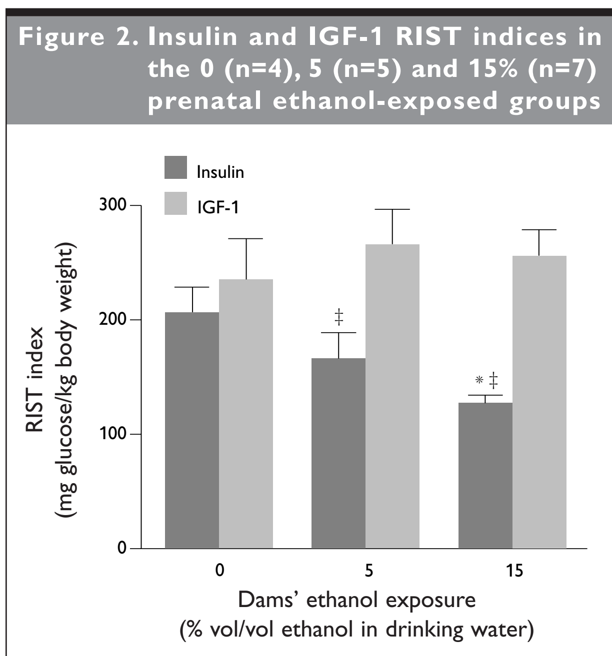
IGF-1 sensitivity

The authors have previously demonstrated that insulin and IGF-1 have similar effects on glucose disposal, as assessed by the RIST (25). However, it was determined that insulin, but not IGF-1, action occurred via the hepatic release of HISS (25). The glucose disposal action of IGF-1 was not altered by atropine or fasting, although both stimuli produced HDIR (25). Based on these observations, the authors hypothesized that if FEE causes hepatic parasympathetic neuropathy resulting in insulin resistance solely through inhibition of HISS release, then IGF-1 action should not be affected by FEE.

IGF-1 sensitivity was tested using the RIST in some of the male adult offspring in the 0, 5 and 15% ethanol groups. The 0% group showed similar insulin and IGF-1 sensitivity (Figure 2) at doses previously shown to be bio-equivalent in glucose disposal in normal rats (25). Comparison of the response to IGF-1 and insulin in the same animal proved to be a very sensitive indication of HDIR. The unpaired comparison of insulin action in the 0 and 5% ethanol groups showed no statistically significant insulin resistance, whereas the paired comparison of the bio-equivalent IGF-1 and insulin responses revealed a reduced insulin action even with the lowest ethanol exposure. Comparison of the responses to 50 mU/kg of insulin and 200 μg/kg of IGF-1 appears to be a useful tool to detect HDIR. The unaltered response to IGF-1 is consistent with the hypothesis that the insulin resistance produced by FEE is secondary to lack of HISS action and that the direct effect of both insulin (i.e. post-atropine, HISS-independent insulin action) and IGF-1 are not altered.

In conclusion, early developmental (prenatal to weaning) exposure to ethanol produced insulin resistance in the adult male rat offspring through ethanol dose-related inhibition of the HISS-dependent component of insulin action. The HISS-independent component of insulin action was not affected by FEE. The high prevalence of type 2 diabetes in many underprivileged areas of the world may be, in part, explained by the fact that prenatal exposure to alcohol inhibits the hepatic release of HISS, leading to HDIR.

Figure 2. Insulin and IGF-1 RIST indices in the 0 (n=4), 5 (n=5) and 15% (n=7) prenatal ethanol-exposed groups



Prenatal exposure to 15% ethanol produced insulin, but not IGF-1, resistance. The insulin RIST indices of the 5 and 15% groups were significantly lower than their paired IGF-1 RIST indices.

Values are mean±SE
*$p<0.05$ compared to the insulin RIST in the 0% group
‡$p<0.01$ compared to the paired IGF-1 RIST

IGF = insulin-like growth factor
RIST = rapid insulin sensitivity test
SE = standard error

ACKNOWLEDGEMENTS

This study was funded by an IHRT grant from Canadian Institutes of Health Research and a grant from the Canadian Diabetes Association in honour of the late John E. Leech. Dr. P. Sadri was supported by an Industrial Research Fellowship awarded by the National Science and Engineering Research Council of Canada. Dr. S. Takayama was supported by a training fellowship from the Government of Japan.

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