Insulin resistance in adult rat offspring associated with maternal dietary fat and alcohol consumption

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Abstract

Maternal diet during pregnancy has been reported to alter the offspring’s ability to respond to a glucose challenge. The current studies report changes in basal and insulin-stimulated, in vitro glucose uptake in red (soleus) and white (extensor digitorum longus) muscle fiber types, as well as whole body insulin responsiveness of adult rat offspring associated with their mother’s dietary fat and alcohol content during pregnancy.

The offspring of Harlan-derived Sprague–Dawley female rats, dosed during pregnancy with ethanol (ETOH) via a liquid diet (35% of calories as ETOH) with either 12% or 35% of calories as fat, were compared with offspring from litters whose mothers were pair-fed an isocaloric amount of the liquid diet without ETOH. Maternal access to the liquid diets was terminated on day 20 of the pregnancies (sperm plug = day 0). The offspring were surrogate fostered within 48 h of birth to mothers which had consumed commercial chow throughout their pregnancy. Following weaning at 21 days of age, the offspring consumed only commercial rat chow and they were examined over the next 14 months for changes in glucose homeostasis as a consequence of in utero exposure to maternal dietary fat and/or alcohol.

The 35% maternal fat diet resulted in both in vivo and in vitro decreases in insulin sensitivity. Thus, compared with adults whose mother’s diet contained 12% fat, significant, in vitro muscle and in vivo whole body insulin resistance (measured by hyperinsulinemic–euglycemic clamping) was observed in adult rats whose mothers consumed 35% of dietary calories as fat. The addition of ethanol to the maternal 35% fat diet further reduced the offspring’s red muscle tissues in vitro response to insulin, but did not affect whole body insulin sensitivity. Muscle basal and insulin-stimulated receptor tyrosine kinase activity were significantly decreased (≈ 50%) by the 35% fat maternal diet but there was no compensatory increase in serum insulin or glucose levels.

Based upon both in vivo and in vitro data, these studies suggested that in utero exposure to 35% fat has a sustained effect on the adult offspring’s glucose uptake/insulin sensitivity and that the effect is paralleled, at least in part, by decreased insulin receptor tyrosine kinase activity. In utero ETOH exposure resulted in the loss of basal and insulin-stimulated, in vitro glucose uptake in red muscle fibers but maternal dietary ETOH glucose uptake had no detectable effect on either in vivo insulin sensitivity or muscle tyrosine kinase activity.

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Introduction

A large amount of literature (Hannah & Howard 1994, Rosholt et al. 1994, Barnard et al. 1995, Liu et al. 1995) describes the negative impact of a concurrent consumption of a high fat diet on glucose homeostasis and insulin responsiveness in both humans and animal models. Fewer studies have examined the effects of in utero exposure to high fat levels but at least one laboratory (Guo & Jen 1995) has examined the effect of maternal high fat feeding during pregnancy and lactation on the glucose/insulin ratio of the offspring at weaning. There have also been multiple reports (Villarroya & Mampel 1985, Vingan et al. 1986, Miller & Dow–Edwards 1988, Lopez–Tejero et al. 1989, Minuk et al. 1998) indicating that the fetal effects of alcohol exposure on glucose homeostasis may be sustained in the adult. Several laboratories have examined the effects of maternal alcohol consumption on the offspring’s glucose uptake/insulin response (Tee & Lee 1975, Villarroya & Mampel 1985, Lopez–Tejero et al. 1989, Minuk et al. 1998) by administering ethanol in the maternal drinking water. Using a liquid diet, Vingan et al. (1986) and Miller & Dow–Edwards (1988) have reported that fetal alcohol exposure causes permanent changes in adult brain glucose
metabolism, while another laboratory (Abel 2000) has shown that maternal ethanol (ETOH) plus high fat (lard) consumption had little effect on specific behaviors in the offspring.

Using a pregnant rat model with the mothers dosed via a liquid diet, the goal of the current studies was to examine changes occurring in basal and insulin-mediated glucose uptake in the adult progeny as a consequence of in utero alcohol and fat exposure. The studies utilized two levels of maternal dietary fat content, 12% and 35% of total calories. The study reports a decrease in the in vitro response to hyperinsulinemic–euglycemic clamping and a significant impairment of in vitro glucose uptake and insulin responsiveness in adult progeny as a consequence of in utero exposure to 35% maternal dietary fat. Maternal ETOH consumption was associated with a significant decrease in red and, to a degree, white muscle tissue basal and insulin–dependent glucose uptake, but maternal ETOH consumption had no detectable effect on whole body insulin responsiveness.

Western blotting of the muscle insulin receptor proteins showed no change in receptor protein levels but measurement of receptor-associated tyrosine kinase activity suggested that the mechanism underlying the dietary fat-induced changes in basal and insulin–dependent glucose uptake may involve the loss of receptor–associated tyrosine kinase activity as the offspring of mothers consuming the 35% fat diet during pregnancy had an approximately 50% decrease in both basal and insulin-stimulated tyrosine kinase activity.

Materials and Methods

Rat model

Virgin, Harlan-derived Sprague–Dawley female rats (150–175 g; Harlan, Indianapolis, IN, USA; n=75) were maintained on a 10 h light:14 h darkness cycle. The animals were fed commercial lab chow with a balanced liquid diet (DYETS, Inc., Bethlehem, PA, USA; diet No. 710260=12% of calories from fat or diet No. 710262=35% of calories from fat or diet No. 710260=12% of calories as fat) being available ad libitum (ad lib) for 5 days prior to mating. These diets are based upon the original Lieber & DeCarli (1994) diet for dosing rats with ethanol. At the initiation of pregnancy (sperm plug=day 0), the female animals were placed on the liquid diet containing either 12% or 35% calories as fat, as the only source of food and water. Each animal was further randomly assigned to one of three dietary groups: group 1 was ETOH-fed, group 2 was pair-fed an isocaloric amount of the liquid diet that the ETOH-fed animal had consumed in the previous 24 h and group 3 was ad lib-fed the liquid diet without ETOH as described by Lieber et al. (1989). All pregnancies were allowed to go to term with all mothers being returned to commercial rat chow (Wayne Lab Blox, Wayne Milling, NC, USA) on day 20 of their pregnancy. The neonates were culled to four males and four females per litter whenever possible and surrogate fostered to chow-fed mothers which had delivered within the previous 48 h. The surrogate-fostered pups were weaned at 21 days of age and fed commercial rat chow ad lib. All pregnant animals were handled regularly to minimize stress and they were weighed at 3-day intervals to maintain control of the pair-feedings. Both the 12% and 35% fat diets with and without ETOH were administered to two separate groups of pregnant rats such that the total number for each treatment group was at least six litters/study, i.e. the total number of pregnant animals receiving the liquid diet was 75. The total number of pups cross-fostered was ≥280 and the total number of pups studied for the current report was ≥160. The offspring were selected for study from the appropriate treatment litters by random draw.

All animal procedures were approved by the East Carolina University Animal Care and Use Committee. The East Carolina University animal research facilities and program are accredited by the American Association for Accreditation of Laboratory Animal Care.

Ethanol estimation

Blood alcohol levels (BAL) were assayed on days 0, 5, 10, 15, and 20 of the pregnancies by an NAD+-coupled spectrophotometric assay using alcohol dehydrogenase (bulletin No. 332-UV; Sigma, St Louis, MO, USA). Blood (200 µl) was drawn from a tail vein using a 23 gauge straight butterfly needle. Because previous experience had suggested that the drawing of blood from pregnant animals for the determination of BAL had a profound effect on maternal and fetal weights (Pennington et al. 1998), a separate group of concurrently pregnant animals were used for the BAL determinations, with the offspring of these mothers not being included in the results reported here.

Glucose uptake in isolated rat muscles

Basal and insulin-stimulated (10–7 M) glucose uptake as 2-deoxyglucose (2-DOG) were determined in the soleus and extensor digitorum longus (EDL) muscles of the adult rat offspring of each treatment group at ~85 days (range=75–95 days) and at ~140 days (range=135–145 days) of age using 3H-labeled 2-DOG. Unless noted to the contrary, animals used in this study and all other experiments were fasted overnight and the experimental procedures initiated as the animals came out of the dark portion of the light:darkness cycle (0700 h). Muscle tissue isolations and incubations were performed according to the methods of Dohm et al. (1988) except that the incubation media contained 0–47 mM [Ca2+] and the incubations were carried out at 30 °C rather that at 37 °C, as the muscles are more metabolically stable under these conditions (Dr G L Dohm, personal communication).
the suggested modifications, the uptake of 3-O-methylglucose was also examined for comparison with 2-DOG uptake. No significant difference in muscle uptake was found between 2-DOG and 3-O-methylglucose.

Serum glucose assay
Blood (≈ 0.5 ml) was collected from a tail vein using a 23 gauge straight butterfly needle. The samples were spun at 10,000 r.p.m. for 30 s in an Eppendorf microcentrifuge at room temperature. The serum supernatant was removed to a 0.65 ml centrifuge tube and immediately placed on wet ice. The samples were held at 0–4 °C until assayed using an ACE Clinical Chemistry System (Schiapparelli Biosystems Inc., Fairfield, NJ, USA).

Serum insulin assay
Serum samples obtained as described above were frozen in liquid nitrogen and held at −70 °C until assayed for insulin levels using a rat insulin-specific radioimmunoassay kit purchased from Linco Research (St Louis, MO, USA). The guinea pig-derived, anti-rat insulin antibody contained in this kit has low cross-reactivity with proinsulin and with 31,32 split proinsulin and thus gives a measure of ‘true’ insulin levels.

Surgical placement of catheters for euglycemic clamps
Following induction of anesthesia with ketamine/xylazine, each animal had a midline incision made in the shaved throat area, followed by blunt dissection to expose the animal’s right jugular vein. On the left side, the superior carotid was ligated and a small incision made in the artery. A PE-50 polyethylene catheter with a small bulb constructed for the weight of the rat and primed with heparinized, sterile saline was inserted into the carotid artery up to the aortic arch. The bulb of the catheter was placed against the superior ligature and an inferior ligature was tied around the vein and catheter. A comparable catheter was inserted into the right jugular vein using the same technique. Both catheters were externalized at the nape of the neck, between the scapulas.

Euglycemic clamps
Following placement of the catheters as described above, each animal was allowed to recover sufficiently to initiate weight gain (2–5 days) and was then subjected to a euglycemic clamp determination of insulin response according to the method of Kraegen et al. (1983). Insulin (Humulin; 4.2 µg/ml, Eli Lilly, Indianapolis, IN, USA) was administered from the Harvard Apparatus pump using a separate syringe with the flow rate being adjusted to maintain the initial (0 time) glucose value over the next 120 min.

Western blotting of insulin receptors
Using an Atto minigel electrophoresis apparatus (Crescent Chemical Co., Hauppauge, NY, USA), muscle tissue (50 µg/lane) was separated by SDS-PAGE and the proteins blotted to Immobilon P (Millipore Corp., Austin, TX, USA). A Santa Cruz Biotechnology (Santa Cruz, CA, USA) monoclonal antibody (catalogue No. SC-09, clone 29B4) to the insulin receptor β-subunit was used as the primary antibody. A 1:2500 dilution of a goat anti-mouse IgG protein conjugated to horseradish peroxidase (Sigma, catalogue No. A2304) was used as the second antibody.

Tyrosine kinase assay procedure
Tyrosine kinase was assayed in Nunc 96-well Maxisorp plates (Nalge Nunc, Naperville, IL, USA) coated with Santa Cruz Biotechnology 29B4 antibody against the human insulin receptor β-subunit. Rat muscle was homogenized by powdering in liquid nitrogen followed by polytronning on a medium setting in homogenization buffer (200 µg muscle/100 µl homogenization buffer). Approximately 750 µg supernate protein (75 µl) was added to each sample well and diluted with 25 µl buffer containing 50 mM Hepes (pH 7.5), 150 mM NaCl, 0.1% bovine serum albumin, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 2 µM leupeptin, 2 µM pepstatin, 12 mM benzamidine and 2 µM aprotinin. Samples of muscle tissue were incubated overnight at 4 °C with orbital mixing. Sample wells of the 96-well plate were washed five times in TBST (100 mM Tris, 0.9% NaCl, 0.01% Triton X-100, pH 7-7.5). Following pre-incubation with 30 µM cold ATP (Sigma), 4 µCi γ-32P ATP (10 mCi/ml; NEN Research Products, Boston, MA, USA), 3 mg/ml poly Glu-Tyr (4:1) obtained from Sigma was added to each sample well and incubated for 45 min at room temperature. Forty microliters of the reaction mix from each well were spotted onto individual strips of 3 mm Whatman filter paper and allowed to air dry. The strips were washed twice in 10% trichloroacetic acid, then in 10 mM sodium pyrophosphate solution for 20 min and allowed to air dry. The strips were counted for 32P activity in a Beckman LS 6500 Multipurpose scintillation counter.
Intra-assay coefficient of variance (CV) was 13·2% and interassay CV was 26·6%.

Statistical analyses of data

Group means and standard errors as well as post hoc testing of significant differences between means of the various treatment groups were calculated using the general linear model procedures of the SAS/PC statistical program (SAS Inc., Cary, NC, USA). Statistically significant differences between group means for each sex were determined using a $3 \times 2$ (dietary group $\times$ % fat) ANOVA with $P<0·05$ accepted as significant. Significant differences between individual groups were determined by the use of the least squared means test.

Results

Effect of maternal diet on neonate size and survival

When compared with mothers consuming the non-ETOH diet ad lib, maternal weight gain during pregnancy was impaired for those mothers consuming the ETOH diet and for the pair-fed mothers (Fig. 1). The fat content of the mothers’ diets had little effect on total maternal weight or on the relative maternal weight gain by pair-fed or ETOH-fed mothers (12% fat versus 35% fat, $P>0·10$).

Dosing of pregnant Sprague–Dawley rats with ETOH via the liquid diet resulted in average BALs between 100 and 150 mg/dl approximately 2 h into the dark portion of the light:darkness cycle, with the exception being BALs on day 20 of the pregnancies, which averaged <25 mg/dl, a decrease that paralleled the decrease in total diet consumption by all maternal treatment groups as parturition approached (data not shown). The length of gestation for the ETOH-treated mothers was 12–24 h longer compared with pair-fed and ad lib-fed mothers whose gestations averaged 21 days. There were no significant differences in litter size as a function of maternal dietary fat or ethanol content. However, there was a consistent decrease in the weights of the neonates of the ETOH-fed mothers (Table 1), a difference that was maintained throughout their adult lives (data not shown). ETOH-fed mothers also had more stillborn pups, and pups from ETOH-treated litters tended

![Figure 1](https://www.endocrinology.org/journals/fig1.jpg)  
**Figure 1** Maternal weight gain during pregnancy for mothers consuming a liquid diet with 35% of calories as fat. The diets consumed by the ad lib-fed, pair-fed and ETOH-fed mothers ($n=3$ for each treatment group) are described in the text. Day 25 data represent the maternal weights on postpartum day 3 and varied between 24 and 26 days following initiation of pregnancy. Both the 12% and 35% fat diets were administered to at least two separate groups of pregnant rats such that the total $n$ for each treatment group was $\geq 6$, i.e. the total number of pregnant animals receiving the liquid diet was 75. *Mean values that are significantly ($P<0·05$) different relative to the pair-fed and ETOH-fed mothers. Values given are the group means ± s.e.m.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Sex</th>
<th>Maternal diet</th>
<th>Ad lib-fed (g)</th>
<th>ETOH-fed (g)</th>
<th>Pair-fed (g)</th>
<th>Chow-fed (surrogates) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>Ad lib-fed</td>
<td>6·56 ± 0·22</td>
<td>4·90 ± 0·19*</td>
<td>5·76 ± 0·19</td>
<td>6·21 ± 0·08</td>
</tr>
<tr>
<td>1</td>
<td>Female</td>
<td>Ad lib-fed</td>
<td>5·84 ± 0·15</td>
<td>4·79 ± 0·18*</td>
<td>5·67 ± 0·20</td>
<td>6·05 ± 0·7</td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>ETOH-fed</td>
<td>21·8 ± 0·5</td>
<td>14·9 ± 0·4*</td>
<td>17·6 ± 0·8*</td>
<td>18·6 ± 0·8</td>
</tr>
<tr>
<td>10</td>
<td>Female</td>
<td>ETOH-fed</td>
<td>19·4 ± 0·5</td>
<td>13·1 ± 1·3*</td>
<td>17·7 ± 1·2</td>
<td>17·8 ± 1·2</td>
</tr>
<tr>
<td>20</td>
<td>Male</td>
<td>Pair-fed</td>
<td>46·3 ± 1·6</td>
<td>34·5 ± 0·4*</td>
<td>39·1 ± 1·6*</td>
<td>na</td>
</tr>
<tr>
<td>20</td>
<td>Female</td>
<td>Pair-fed</td>
<td>42·6 ± 0·7</td>
<td>32·4 ± 2·4*</td>
<td>39·7 ± 2·8</td>
<td>na</td>
</tr>
</tbody>
</table>

*Weight that is significantly ($P<0·05$) different from the offspring weight of ad lib-fed mothers of the same sex and age.  
na, data not available.
Table 2 Basal serum glucose and insulin levels. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Maternal diet (%) fat</th>
<th>Sex</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad lib-fed (12)</td>
<td>M</td>
<td>151±8±2±7</td>
<td>na</td>
</tr>
<tr>
<td>Ad lib-fed (35)</td>
<td>M</td>
<td>144±6±4±5</td>
<td>0.96±0.64†</td>
</tr>
<tr>
<td>Pair-fed (12)</td>
<td>M</td>
<td>188±3±11±7</td>
<td>0.79±0.20</td>
</tr>
<tr>
<td>Pair-fed (35)</td>
<td>M</td>
<td>168±1±13±4</td>
<td>0.63±0.04</td>
</tr>
<tr>
<td>ETOH-fed (12)</td>
<td>M</td>
<td>166±5±17±5</td>
<td>0.82±0.13</td>
</tr>
<tr>
<td>ETOH-fed (35)</td>
<td>M</td>
<td>153±1±6±7</td>
<td>0.74±0.06</td>
</tr>
<tr>
<td>Ad lib-fed (12)</td>
<td>F</td>
<td>156±8±26±7</td>
<td>0.35±0.12†</td>
</tr>
<tr>
<td>Ad lib-fed (35)</td>
<td>F</td>
<td>138±5±4±5</td>
<td>0.56±0.13†</td>
</tr>
<tr>
<td>Pair-fed (12)</td>
<td>F</td>
<td>144±3±10±2</td>
<td>1.36±0.63*$*</td>
</tr>
<tr>
<td>Pair-fed (35)</td>
<td>F</td>
<td>152±5±4±5</td>
<td>1.70±0.92*</td>
</tr>
<tr>
<td>ETOH-fed (12)</td>
<td>F</td>
<td>146±1±6±3</td>
<td>0.52±0.16†</td>
</tr>
<tr>
<td>ETOH-fed (35)</td>
<td>F</td>
<td>152±5±5±6</td>
<td>0.43±0.23†</td>
</tr>
</tbody>
</table>

Animals were between 180 and 360 days of age at the time of assay except for those values indicated by † where the animals were between 60 and 180 days when assayed.

*a <0·05 relative to ETOH- and ad lib-fed groups.

Effect of maternal diet on the offspring’s glucose and insulin levels and muscle glucose uptake

When compared with the offspring of pair-fed mothers, there was no significant difference in basal serum insulin or glucose levels between the groups except that female offspring of pair-fed mothers did have higher basal serum insulin levels (Table 2), suggesting that these animals maintained normal glycemia via hyperinsulinemia.

Basal and insulin-dependent glucose uptake by muscle tissue was significantly impaired in the offspring by maternal dietary fat and ETOH content (Table 3). The maternal diet containing 35% fat significantly impaired both basal (P<0·0001) and insulin-stimulated glucose uptake (P<0·0001) by the offspring. Maternal ETOH consumption also significantly impaired uptake (basal, P=0·0217; +insulin, P=0·0012). There were no significant interactions between % maternal dietary fat with ETOH, with respect to basal or insulin-dependent muscle glucose uptake. There were significant differences in basal and insulin-stimulated glucose uptake by sex of the offspring (basal, P<0·0001; +insulin, P<0·0001) but neither maternal dietary fat nor ETOH content interacted with this effect (data not shown). Thus, unless specifically noted, all data analyses were separated by sex.

The impairment of in vitro basal and insulin-stimulated glucose uptake in both soleus and EDL muscle tissues from male and female progeny was most robustly associated

Table 3 In vitro glucose uptake by soleus and EDL muscle fibers. Values represent mean glucose uptake (nmol/g tissue per min) ± S.E.M. with n ≥ 3 for each muscle in each treatment group and with each muscle sample being assayed in triplicate (ad lib data omitted for clarity)

<table>
<thead>
<tr>
<th>Maternal diet (%) fat</th>
<th>Male (soleus)</th>
<th>Female (soleus)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>+Insulin</td>
</tr>
<tr>
<td>Ad lib-fed (12)</td>
<td>14±1±2±0</td>
<td>81±7±11±2</td>
</tr>
<tr>
<td>Pair-fed (35)</td>
<td>6±1±0±4*</td>
<td>18±2±2±6*</td>
</tr>
<tr>
<td>ETOH-fed (12)</td>
<td>22±2±5±1*</td>
<td>46±3±7±8*</td>
</tr>
<tr>
<td>ETOH-fed (35)</td>
<td>5±9±1±4*</td>
<td>9±6±1±4*</td>
</tr>
<tr>
<td></td>
<td>Basal</td>
<td>+Insulin</td>
</tr>
<tr>
<td>Pair-fed (12)</td>
<td>43±9±9±1</td>
<td>65±7±6±9</td>
</tr>
<tr>
<td>Pair-fed (35)</td>
<td>7±1±1±7*</td>
<td>16±5±2±9*</td>
</tr>
<tr>
<td>ETOH-fed (12)</td>
<td>47±6±3±1</td>
<td>69±0±4±9</td>
</tr>
<tr>
<td>ETOH-fed (35)</td>
<td>8±7±2±0*</td>
<td>13±0±2±8*</td>
</tr>
</tbody>
</table>

Data are for 75- to 95-day-old male and female offspring of mothers consuming an ETOH-containing diet or an isocaloric, pair-fed diet with 12% or 35% of calories as fat.

*Value for an ETOH-fed diet group that is significantly (P<0·05) different from the comparable pair-fed diet group. +Value for a 35% fat group that is significantly (P<0·05) different from the comparable 12% fat group.
with 35% fat in the maternal diet (Table 3). Basal glucose uptake by muscles of animals whose mothers consumed 35% of their calories as fat was half or less of the uptake of offspring whose mothers consumed 12% dietary fat. Insulin-stimulated glucose uptake by soleus muscles was reduced four times in both the pair-fed and ETOH-fed male offspring whose mothers consumed the 35% fat diet as opposed to the comparable offspring treatment groups whose mothers’ diet contained 12% fat.

In addition to the inhibitory effect of the 35% maternal dietary fat content on their offspring’s muscle glucose uptake, the effect of maternal dietary ETOH on soleus muscle glucose uptake was also apparent in the offspring from both the 35% and 12% fat maternal dietary groups. In adult male rat offspring exposed to alcohol in utero, the insulin-dependent increase in glucose uptake was significantly decreased in the soleus muscles, regardless of the maternal dietary fat content (Fig. 2).

**Effect of maternal diet on offspring whole body glucose uptake**

To test the in vivo consequences of the apparent loss of in vitro insulin responsiveness, hyperinsulinemic–euglycemic clamps were performed on littermates of the animals in which the in vitro assays of glucose uptake were conducted. The impact of 35% fat in the maternal diet on the offspring’s whole body insulin response was apparent (Fig. 3). Across sexes and dietary ETOH level, the offspring of mothers consuming 35% dietary fat were significantly less sensitive to insulin as revealed by the clamp data (12% fat group removal rate = 206·1 ± 5·7 pM/g per min versus 179·0 ± 4·8 pM/g per min for the 35% fat group, *P* < 0·05). There was no additional effect of maternal dietary ETOH on the offspring’s in vivo insulin responsiveness (data not shown).

**Molecular changes in the offspring’s insulin signaling**

Measurement of basal and insulin-dependent, insulin receptor-associated tyrosine kinase activity in the muscle of the offspring indicated that 35% fat in the maternal diet resulted in a loss of both basal and insulin-stimulated tyrosine kinase activity in offspring muscle tissue (Fig. 4A) without significantly altering the total insulin receptor protein content (Fig. 4B). There was no additional effect on either parameter when ETOH was included in the maternal diet (data not shown).

**Discussion**

The experiments reported here employed a widely used rat model dosed via a liquid diet for the administration of ETOH to pregnant rats. The feeding paradigm included females given an isocaloric amount of the diet without ETOH and a group of mothers given ad lib access to the diet without ETOH. In addition, the model included two levels of maternal dietary fat (12% and 35% of total calories). The offspring from all pregnancies were surrogate fostered to non-treated mothers which had consumed only commercial rat chow and had delivered within 48 h of the treated mothers. Further, proven males used for breeding were arbitrarily placed with the females to randomize any paternal effects on the offspring. The
progeny of these pregnancies were studied over the next 14 months to determine the long-term effect(s) of maternal dietary fat and ETOH content on the offspring’s basal and insulin-stimulated glucose uptake.

While there were no significant differences in litter size as a function of maternal dietary fat or ethanol content, the offspring of ETOH-fed mothers were smaller and these mothers tended to have more pups die prior to surrogate fostering. Although approximately half of the ETOH-treated pups that died had suckled at least once, the fact that the ETOH-fed mothers had more pups die prior to surrogate fostering could suggest that the weaker (or metabolically more abnormal?) pups were being lost. However, no data are available to address this point.

It has been known for some time that alcohol administered via a liquid diet to the mother during pregnancy markedly decreases the weight of the pups at birth (for an example see Tee & Lee 1975). There have also been previous reports (Villarroya & Mampel 1985, Lopez-Tejero et al. 1989, Molina et al. 1994, Minuk et al. 1998) of altered whole body glucose homeostasis and insulin resistance in rat offspring as a consequence of maternal ETOH ingestion. Villarroya & Mampel (1985) administered ETOH (25%) to pregnant rats via their drinking water and also observed that the litter survival and body weight were decreased in the offspring of alcohol-treated mothers. Contrary to the current study, Villarroya & Mampel (1985) and others (Tee & Lee 1975) reported a decreased number of pups/litter at birth for the ETOH-fed mothers. Using the drinking water paradigm, Villarroya & Mampel (1985) found that newborn pups had elevated serum insulin levels but normal serum glucose values while 3-day-old pups had normal serum glucose levels associated with increased insulin response. Using a similar model, Lopez-Tejero et al. (1989) reported that...
maternal ETOH consumption caused hypoglycemia/hyperinsulinemia in the offspring throughout the neonatal period. At 30 days of age, the offspring of ETOH-fed mothers had increased glucose uptake and insulin sensitivity in response to a glucose challenge, while at 90 days these offspring had a normal glucose response and a hyperinsulinemic response to a glucose challenge.

Dow-Edwards and coworkers (Vingan et al. 1986, Miller & Dow-Edwards 1988, 1993), using a liquid diet, have examined the relationship between ETOH-induced changes in brain glucose metabolism and the behavioral deficits seen in children suffering from the fetal alcohol syndrome (FAS). Children with FAS are characterized by significant mental retardation and long-term behavioral problems. These studies found significant differences in the uptake of 2-DOG by adult offspring, depending upon the region of the brain examined.

A recent study (Minuk et al. 1998) examined insulin resistance in the offspring in the presence of atropine to determine the role of the autonomic nervous system in insulin resistance. These authors have identified a protein released by the liver in response to insulin (hepatic insulin-sensitizing substance; HISS) (Lautt et al. 2001) that mediates muscle insulin sensitivity. They have previously shown that secretion of this protein by the liver is dependent on acetylcholine/nitric oxide synthesis. They examined the offspring of ETOH-fed mothers and found that insulin resistance in these animals involved a decreased release of HISS. These specific molecular changes were not examined in the current study.

Overall, the literature supports the concept that some type of enduring change(s) occurs in the offspring glucose uptake/utilization as the result of the in vivo exposure to ETOH.

There are similarities between the literature cited and the current studies, e.g. decreased offspring weight, poorer litter survival and sustained changes in glucose uptake/insulin sensitivity but there are also significant differences, e.g. the current study found few changes in basal glucose or insulin levels and no change in the number of pups in the litters of ETOH-fed mothers at birth. These and other differences between the various studies could have several causes. For example, most of the studies cited above administered ETOH in the maternal drinking water and thus did not have the same control of critical differences in maternal caloric intake that the use of a liquid diet paradigm allows. In addition, the age at which the offspring were examined varied significantly between the various studies.

There have been numerous reports describing the detrimental effect of a high fat diet on concurrent glucose homeostasis (Hannah & Howard 1994, Rosholt et al. 1994, Barnard et al. 1995, Liu et al. 1995) but few laboratories have examined the long-term effects of maternal dietary fat content on glucose uptake by the offspring. One group (Guo & Jen 1995) has reported that maternal high fat feeding during pregnancy and lactation decreased glucose/insulin ratios of the offspring at weaning but the progeny were not examined at older ages.

The results reported here suggest that, compared with 12% maternal dietary fat content, 35% maternal dietary fat has profound, long-term consequences for the offspring. The data indicate that in vitro basal glucose uptake as well as in vitro uptake in the presence of super physiological levels (10^{-7}M) of exogenous insulin were significantly reduced in the red (soleus) and white (EDL) muscle fibers of offspring whose mothers consumed 35% fat. To test the possibility that the loss of insulin-dependent glucose uptake resulted from a maternal diet-induced difference in insulin levels or in the insulin receptors, the offspring’s serum insulin, glucose and muscle insulin receptor protein levels as well as basal and receptor-associated, insulin-stimulated tyrosine kinase activity were measured. There were no significant differences in basal serum glucose or insulin levels between the various groups except that female offspring of pair-fed mothers did have higher basal serum insulin levels, suggesting that these animals maintained normal glycemia via hyperinsulinemia. There was a tendency for the 12% fat maternal diet to produce slightly higher insulin receptor protein levels in the offspring. This increase paralleled the higher insulin-dependent glucose uptake in these offspring but the differences in receptor protein levels did not reach statistical significance. More importantly, offspring of mothers consuming the 35% fat diet had approximately 50% less receptor-associated, insulin-dependent tyrosine kinase activity compared with the offspring of mothers which consumed the 12% fat diet, a difference that was comparable to the differential in insulin-stimulated glucose uptake between these two groups. The lower basal glucose uptake seen in the progeny of mothers consuming 35% fat was also correlated with a decrease in basal receptor tyrosine kinase activity.

Decreased insulin-stimulated tyrosine kinase activity has been reported to be associated with insulin resistance (Goldfine et al. 1998, 1999) and with diabetes of pregnancy and gestational diabetes mellitus (Shao et al. 2000), suggesting that loss of the kinase activity could be associated with decreased basal as well as insulin-dependent glucose uptake. The inclusion of ETOH in either the 12% or 35% fat maternal diet caused no additional change in offspring tyrosine kinase activity, suggesting that the mechanism by which ETOH inhibited the offspring’s in vitro uptake of glucose may not be due to changes in receptor tyrosine kinase activity.

The possible association of in vitro changes with whole body insulin responsiveness was examined via hyperinsulinemic–euglycemic clamping. The impact of 35% maternal dietary fat on the offspring’s in vitro insulin response was paralleled by a significantly lower rate of glucose removal during euglycemic clamps in the offspring of the mothers which consumed 35% fat, but adding ETOH to the maternal diet failed to have any significant
effect on offspring glucose removal above that of dietary fat. Although maternal dietary ETOH resulted in a marked inhibition of in vitro basal and insulin-stimulated glucose uptake in red muscle fibers, the relatively smaller proportion of whole body red muscle to white muscle in the rat may explain the failure of maternal ETOH to significantly alter the whole body insulin response of the offspring.

It is unclear why changes in insulin receptor tyrosine kinase activity did not elicit some type of compensatory response, e.g. increased serum insulin levels. The failure to observe a compensatory increase in serum insulin could suggest that under fasting conditions the animals were able to maintain normal glycemia as the insulin/glucagon ratio fell. However, under either in vivo or in vitro experimental conditions where excess exogenous insulin was present, the animals with reduced tyrosine kinase activity were impaired in their ability to take up glucose.

Obviously, a large number of metabolic factors beyond those examined here impact upon whole body insulin response. Thus, the association between maternal dietary fat and ETOH content and altered glucose uptake must be examined as to the possible contribution of other factors to the observed changes. For instance, changing one component of the maternal dietary composition results in a compensatory change in other components, e.g. increasing the fat content of the maternal diet requires less carbohydrate to maintain an isocaloric mixture, the protein content of the diets being held relatively constant in the studies. These and other important nutritional factors must be further examined in order to determine the long-term impact of maternal diet during pregnancy on the offspring.

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