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Altered body composition and metabolism in the male offspring of high fat–fed rats

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Abstract

An intrauterine environment may play a role in predisposing a developing fetus to metabolic diseases during adulthood. We investigated the hypothesis that a maternal diet high in ω-6 polyunsaturated fat can modify the programming of an offspring's glucose tolerance, insulin sensitivity, body composition, lipid metabolism, and insulin signaling. High ω-6 polyunsaturated fat diets were fed to female rats 4 weeks before mating and throughout the gestation period. The offspring were maintained on chow diet. At 3 months of age, indirect calorimetry, oral glucose tolerance tests, and dual x-ray absorptiometry measurements were performed. Triglyceride content and β-hydroxyacyl coenzyme A dehydrogenase activity were determined in the liver and quadriceps muscle. Expression levels of key insulin signaling pathway proteins were measured in the liver and quadriceps muscle of the 3-month-old offspring. Offspring from the fat-fed dams had significantly increased proportions of both total body fat and abdominal fat. All offspring displayed normal insulin sensitivity and glucose tolerance, although the offspring from the fat-fed dams were significantly more hyperinsulinemic 15 minutes after an oral glucose challenge. Whole body fuel oxidation was not altered. The offspring of fat-fed dams had significantly elevated liver triglyceride content. Insulin signaling protein expression levels in the offspring of fat-fed dams were consistent with reduced hepatic insulin sensitivity but increased quadriceps insulin sensitivity. A maternal diet high in ω-6 polyunsaturated fat evokes programming within the metabolic processes of the offspring that may predispose the offspring to the development of metabolic diseases.

1. Introduction

Human epidemiological studies have revealed strong statistical relationships between poor fetal growth and the development of diseases associated with the metabolic syndrome [1-4]. The exact cause behind this relationship still remains unclear. Exposure to a suboptimal intrauterine environment may play an important role in predisposing a developing fetus to metabolic diseases during adulthood [2,4]. It is now widely recognized that many nutritional exposures throughout pregnancy and lactation can influence the programming of an offspring's metabolism. Animal studies investigating the link between early programming and adult metabolic disease have mainly concentrated on the effects of maternal nutrition such as calorie restriction [5] and protein restriction [6,7].

In today's Western society, the in utero environment is likely to be influenced by maternal nutritional insults such as excess fat consumption. There is little dispute regarding the deleterious effects of a high-fat diet—in human beings, excess fat consumption has been associated with increasing prevalence of cardiovascular disease [8], insulin resistance [9], and type 2 diabetes [10,11]. It is not only the quantity of fat but also the type of fat that influences the development of these diseases. Animal studies have shown that isocaloric diets that differ only in their fatty acid profile can provoke different effects on an animal's insulin sensitivity [12]. The

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more unsaturated the fat in the diet, the less harmful is that diet. Nevertheless, the class of unsaturated fat consumed does appear to be a factor in the development of disease. The ω-3 polyunsaturated fats are thought to be particularly beneficial to insulin action [13]. There is a significant negative impact on glucose homeostasis and insulin responsiveness induced by consumption of a diet high in ω-6 polyunsaturated fat [14].

An area of research that requires more detailed attention is the negative impact that maternal high fat feeding has on the offspring. Previous studies have demonstrated that the offspring of fat-fed dams have profound metabolic derangements such as increased amounts of body fat, increased liver weight, increased liver triglyceride content, higher blood glucose, and higher blood triglyceride levels [15] as well as vascular dysfunction [16]. Those studies mainly concentrated on the effects of a maternal diet high in saturated fat. Polyunsaturated fats are generally portrayed as being "good fats"; however, as explained above, diets high in ω-6 polyunsaturated fats do impose detrimental effects on insulin action in rodents [14]. The current study was designed to investigate the hypothesis that the consumption of a diet high in ω-6 polyunsaturated fat, before and throughout the gestation period, modifies the programming of the offspring's metabolic processes. We measured the glucose tolerance, insulin sensitivity, lipid metabolism, and the expression of key proteins in the insulin signaling pathway of the offspring of dams fed a diet high in ω-6 polyunsaturated fat.

2. Materials and methods

2.1. Materials

Unless stated otherwise, analytical-grade biochemicals were obtained from Sigma Chemicals (Sydney, Australia, for lipid metabolism experiments, and Dorset, UK, for Western blotting experiments). All primary antibodies used in Western blotting, except for anti–insulin receptor substrate (IRS) 1 and anti–glucose transporter protein 4 (GLUT4), were obtained from Santa Cruz Biotechnology (Santa Cruz, Calif). Anti-IRS1 antiserum was obtained from Upstate Biotechnology (Lake Placid, NY). Anti-GLUT4 antiserum was obtained from Chemicon International (Temecula, Calif). The secondary antibody used was the anti-rabbit immunoglobulin, peroxidase-linked species-specific whole antibody (from donkey), and was obtained from Amersham International Biotech UK Limited (Buckinghamshire, UK).

2.2. Study design

Sixteen virgin female and male outbred Wistar rats (obtained at 4 weeks of age from the Laboratory Animal Services, Sydney, Australia) were randomly assigned either a diet consisting of standard laboratory chow (12% fat, 23% protein, 65% carbohydrate) or a high ω-6 polyunsaturated fat diet (59% fat from safflower oil, 21% protein, 20% carbohydrate). All rats were fed ad libitum and had free access to water. The composition and source of the diets were as those described in Ref. [12]. All rats were housed in cages containing no more than 4 adult rats and were maintained at 22°C on a 12-hour light/dark cycle (light cycle from 6 AM to 6 PM) in the Department of Biochemistry Animal House, University of Sydney, Australia. All procedures involving animals were carried out according to the guidelines of, and with the prior approval of, the University of Sydney Animal Care and Ethics Committee.

After 4 weeks of feeding, 2 groups of 8 breeding pairs were established: chow-fed female and male rats and fat-fed female and male rats. Male rats were removed on the day the vaginal plug was expelled from the female rats. The respective diets were continued throughout the pregnancy; however, after the spontaneous delivery of offspring and hence during the lactation period, all mothers were fed the standard laboratory chow diet. At 21 days, all pups were weaned onto the same chow diet. It must be emphasized that only the mothers underwent dietary manipulation during pregnancy and that the male offspring were all fed standard laboratory chow. However, for simplicity, the offspring from the chow-fed mothers will be termed *CON* and those from the fat-fed mothers will be termed *FAT*.

Male offspring were studied at 3 months of age. Female offspring were not studied as it was thought that female sex hormones may play a protective role against the development of insulin resistance and associated disorders in the rat [17,18]. To perform a number of different experimental procedures on the offspring, the 3-month-old male offspring were randomly divided into 2 groups. Group 1 consisted of 16 CON offspring and 15 FAT offspring. Group 2 consisted of 7 CON offspring and 7 FAT offspring.

2.3. Group 1 procedures

Body composition, namely, the quantity of lean and fat tissue present, was measured using dual x-ray absorptiometry (DXA) in 10 CON offspring and 10 FAT offspring. Light anesthesia was induced by giving the rats an intraperitoneal injection of 15 mg/kg pentobarbitone sodium plus 25 mg/kg ketamine base. Dual x-ray absorptiometry measurements were performed using a narrow-angle ($<5^\circ$) fan-beam dual energy x-ray bone densitometer (Lunar Prodigy, GE Medical Systems, Diegem, Belgium). The proprietary "Small Animal" software, version 6.10, was used for both scanning and analysis. The anesthetized rats were placed in the prone position on the scan table of the densitometer. Limbs were stretched out and placed flat to allow for easier delineation of body regions during analysis. Starting at the head of the rat, the densitometer performed a rectilinear scan, passing the x-rays through the animal. A solid-state detector on the underside of the animal measured the attenuated x-ray beam and the information was transferred to a computer. This information was then analyzed to determine the total amount of lean tissue (g), fat tissue (g), and percentage body fat. Scans, each lasting

approximately 1 minute, were performed in duplicate. The precision of the DXA measurements was determined from the duplicate scans. Coefficients of variation for fat mass and lean mass were 4.0% and 0.6%, respectively.

Three days after the DXA measurements, the male offspring were subjected to an oral glucose tolerance test (OGTT). After an overnight fast, rats were orally gavaged with 3 g/kg body weight of a solution of 50% (w/v) glucose in 0.9% (w/v) saline. Blood samples (100 μL) were collected into 0.5 mL microfuge tubes containing 50 μL heparinized saline (10 mg/mL) at 0, 15, 30, 45, 60, 90, and 120 minutes postgavage. Blood samples were centrifuged (5 minutes at 12 000 rpm) and the plasma was collected and stored at −20°C for subsequent analysis.

Four days post-OGTT, the 3-month-old male offspring were anesthetized with an intraperitoneal injection of pentobarbitone sodium (30 mg/kg) in the morning after a normal overnight feed. The heart, quadriceps muscle, and liver were excised and freeze clamped in liquid nitrogen. All tissues were stored at −80°C until analysis.

2.4. Group 2 procedures

During a single 24-hour period (starting at 8 AM), 3-month-old offspring were subjected to indirect calorimetry measurements, similar to those previously described [19]. However, for this study, measurements were made using the Deltatrac open-circuit metabolic monitor (Deltatrac, Datex, Helsinki, Finland). For the first 7 hours of the measurement, rats only had free access to water to obtain a fasting respiratory quotient (RQ). Standard laboratory chow was then placed into the metabolic cage at 3 PM and indirect calorimetry measurements were continued until 8 AM the following morning. Upon completion of the 24-hour period, rats were returned to their standard cages.

Two days later, the rats were anesthetized with an intraperitoneal injection of pentobarbitone sodium (30 mg/kg) plus ketamine hydrochloride (50 mg/kg) and fitted with chronic carotid and jugular cannulas. Hyperinsulinemic-euglycemic clamp studies were conducted 5 days postsurgery in overnight-fasted, conscious rats as described previously [20,21]. Briefly, a continuous infusion of human insulin (Novo Biolabs, Bagsværd, Denmark) was given at a dose of 0.25 U/kg per hour so as to induce a state of hyperinsulinemia. The infusion rate of glucose (30% [w/v] solution made with sterile 0.9% [w/v] saline) was adjusted using a peristaltic pump (Watson-Marlow, Cornwall, UK) to reach and maintain euglycemia at 4.5 mmol/L. Thirty minutes after the establishment of euglycemia, the rats were killed by an intravenous infusion of pentobarbitone sodium (30 mg/kg).

2.5. Analytical methods

2.5.1. Glucose and insulin

Plasma samples collected during the OGTT were assayed for glucose and insulin concentrations. The concentration of glucose was determined using a glucose oxidase method. Plasma insulin concentration was determined using a double antibody radioimmunoassay using rat insulin standards (Novo Biolabs), iodine-125 insulin (Department of Endocrinology, University of Sydney), and antibodies (guinea pig anti-rat insulin antibody, goat anti–guinea pig IgG serum antibody, and guinea pig carrier IgG; Linco, St Louis, Mo).

2.5.2. Tissue triglycerides

Quadriceps muscle and liver triglycerides were extracted using a modification of the protocol previously described in Ref. [22]. Total tissue triglyceride concentrations were then quantified using a Triglyceride GPO-PAP assay kit (Roche Boehringer Mannheim, Castle Hill, NSW, Australia).

2.5.3. Tissue fatty acid oxidation

Quadriceps muscle and liver β-hydroxyacyl-Coenzyme A dehydrogenase (β-HAD) and citrate synthase (CS) activities were calculated to obtain a measure of the rate of fatty acid oxidation. Both these enzymes have previously been used as marker enzymes of fatty acid oxidation [23]. β-hydroxyacyl-Coenzyme A dehydrogenase activity was determined using a modification of the method previously described in Ref. [24]. Citrate synthase activity [25] was determined in the same homogenate that was used for determining β-HAD activity. To determine the fatty acid oxidation rate, both β-HAD and CS activities were initially calculated as units of enzyme activity per gram of wet tissue weight. The final rate of fatty oxidation was then expressed as a ratio of β-HAD activity to CS activity (units of β-HAD activity/unit of CS activity).

2.5.4. Insulin signaling proteins

The expression of key insulin signaling proteins was measured in the quadriceps muscle and liver. The tissues were homogenized in ice-cold lysis buffer (50 mmol/L HEPES, 150 mmol/L NaCl, 1 mmol/L Na3VO4, 10 mmol/L Na4P2O7, 30 mmol/L NaF, 10 mmol/L EDTA, 1% Triton X-100, pH 7.4) and then centrifuged at 13000rpm for 5 minutes at 4°C to remove insoluble material. Protein content of the extracted clear lysates was subsequently determined using a modification of the Lowry method [26]. Western blotting analysis was completed using a slightly modified version of that described in Ref. [27]. Briefly, whole cell lysates (20 μg protein) were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred onto nitrocellulose membranes and treated overnight. The specific proteins were detected using polyclonal primary antibodies diluted appropriately (1:500 anti–insulin receptor β subunit; 1:1000 anti-p85 [regulatory subunit of PI3-kinase]; 1:500 anti–protein kinase C ζ [PKCζ]; 1:800 anti-IRS1; 1:200 anti-IRS2; 1:4000 anti-GLUT4 antiserum) in phosphate-buffered saline containing 0.1% Tween 20 and 1% Marvel skimmed milk powder (Cadbury, Bournville, UK) and a horseradish peroxidase–coupled secondary antibody (diluted 1:1000 in the same buffer as the primary antibodies). The detected proteins were then made visible using enhanced chemiluminescence

reagents according to the manufacturer's instructions (Amersham International Biotech UK Limited) and were processed in a Xograph Compact X4 film processor (Xograph Imaging Systems Ltd, Gloucestershire, UK). The specific protein bands were then quantified using AlphaImager version 5.04 and AlphaEase version 3.3b software (Alpha Innotech Corp, San Leandro, Calif).

2.6. Statistical analysis

All results are expressed as mean ± SEM. The insulin signaling protein content detected in the tissues of the FAT offspring is expressed as a percentage of the detected CON protein content that was set to 100% ± SEM. Prior to statistical analysis, all data were checked for normal distribution and equal variance. Statistical analysis was performed by repeated-measures analysis of variance (ANOVA) using Statview (Abacus Concepts Inc, Berkeley, Calif) and Student *t* tests using EXCEL (Microsoft Office 2000), where appropriate. $P < .05$ was considered statistically significant. Where relevant, Bonferroni correction factor was applied to the significance level if multiple *t* tests were performed.

3. Results

3.1. Litter sizes and offspring body weight and body composition

Maternal high fat feeding resulted in a small reduction in litter size (fat-fed rats: 12 offspring ± 1 cf chow-fed rats: 15 offspring ± 1; $P < .05$). There were no differences in the birth weight (FAT: 6.5 ± 0.2 g cf CON: 6.9 ± 0.3 g) or the body weight at 3 months of age (FAT: 351.3 ± 7.9 g cf CON: 360.3 ± 11.1 g). Despite the lack of difference in body weight, significant differences were observed in the body composition of FAT offspring when compared with the CON offspring at 3 months of age (Table 1). Significant increases in percent body fat, both total and in the abdominal region, were observed in the FAT offspring. When body fat in grams was expressed as a ratio to lean mass in grams, the FAT offspring had significantly elevated ratios in both total body and in the abdominal region.

Table 1
Body composition of the 3-month-old offspring

	CON offspring (n = 10)	FAT offspring (n = 10)
Total body fat mass (g)	51.4 ± 2.4	55.2 ± 2.9
Total body lean mass (g)	315.6 ± 7.0	281.9 ± 7.9**
Total body fat mass/lean mass	0.16 ± 0.01	0.20 ± 0.01*
Total % body fat	13.6 ± 0.6	15.9 ± 0.6*
Abdominal fat mass (g)	12.5 ± 1.0	14.7 ± 1.2
Abdominal lean mass (g)	111.9 ± 4.0	93.4 ± 3.1**
Abdominal fat mass/lean mass	0.11 ± 0.01	0.16 ± 0.01**
Abdominal region % fat	9.9 ± 0.7	13.4 ± 0.9*

Results are expressed as means ± SEM.
* $P < .01$ cf CON offspring.
** $P < .005$ cf CON offspring.

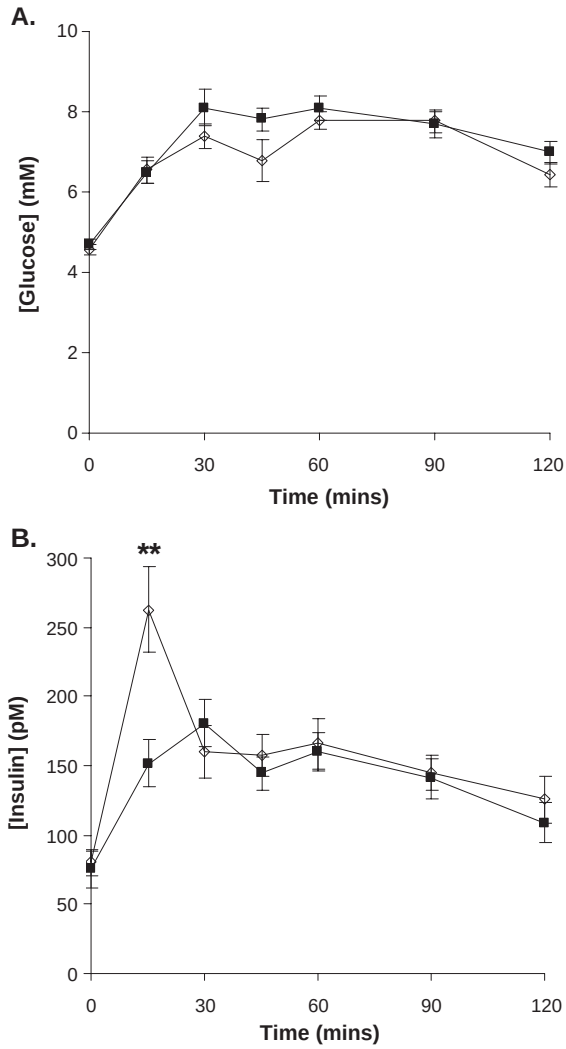

Fig. 1. Plasma glucose (A) and insulin (B) concentrations after an oral glucose challenge in the 3-month-old male offspring. After an overnight fast, the male offspring were gavaged with 3 g/kg body weight of a solution of 50% (w/v) glucose in 0.9% (w/v) saline. Plasma glucose and insulin concentrations were measured at the indicated times. Results are expressed as the mean ± SEM of data from 16 CON offspring (blocked square) and 15 FAT offspring (open diamond). Double asterisk indicates $P < .005$ cf CON offspring after a 15-minute insulin concentration (unpaired Student *t* test).

3.2. Oral glucose tolerance of the offspring

At 3 months of age, all offspring studied displayed normal glucose tolerance (Fig. 1A). The plasma glucose levels changed significantly during the OGTT in both the FAT and the CON offspring ($P < .001$; ANOVA). There was no difference observed in the total area under the glucose curve between the FAT offspring and CON offspring. Fig. 1B displays the plasma insulin levels in response to the oral glucose load. The plasma insulin levels changed significantly during the OGTT in both the FAT and the CON offspring ($P < .001$; ANOVA). This change in plasma insulin concentration over time was greater in the FAT offspring than in the CON offspring ($P < .01$). As can be

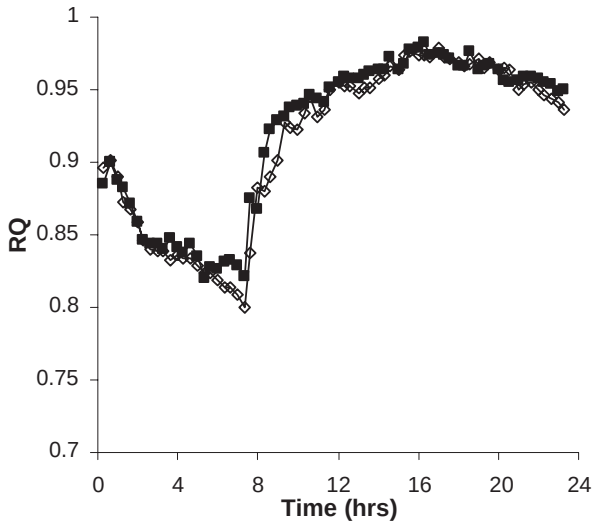

Fig. 2. Twenty-four–hour RQ pattern in the 3-month-old male offspring. Twenty-four–hour RQs were measured in 7 CON (blocked square) and 7 FAT (open diamond) 3-month-old male offspring using a Deltatrac open-circuit metabolic monitor. Offspring were fasted between 0 and 7 hours. Free access to food was then available after 7 hours until the end of the experiment. To obtain a fasted RQ value and a fed RQ for the 2 groups of offspring, an average of 9 data points was calculated. Fasted RQ values were calculated between 3.6 and 6.6 hours, whereas fed RQ values were calculated between 8.3 and 11.3 hours.

seen in Fig. 1B, the FAT offspring, although not showing fasting hyperinsulinemia, did secrete significantly more insulin 15 minutes after the oral glucose load ($P < .005$; unpaired Student t test). Despite this first-phase insulin hypersecretion, the FAT offspring had plasma insulin levels similar to those of the CON offspring by the end of the OGTT.

Table 2
Liver insulin signaling protein expression

	CON offspring (n = 16)	FAT offspring (n = 15)
Insulin receptor β subunit	100% ± 3.6%	53.3% ± 7.0%***
IRS1	100% ± 4.5%	82.9% ± 4.7%*
IRS2	100% ± 5.7%	93.0% ± 4.6%
p85 subunit of PI3-kinase	100% ± 3.4%	96.4% ± 2.6%
PKCζ	100% ± 2.8%	118.5% ± 4.1%**

FAT offspring protein expression is expressed as a percentage of the CON offspring protein expression (100% ± SEM).
* $P < .01$ cf CON offspring.
** $P < .005$ cf CON offspring.
*** $P < .001$ cf CON offspring.

3.3. Whole body insulin sensitivity of the offspring

Whole body insulin sensitivity was determined by the calculation of the required glucose infusion rate during the hyperinsulinemic-euglycemic clamp. No differences were observed between the FAT offspring and the CON offspring (FAT: 30.5 ± 0.9 mg glucose/min per kilogram body weight cf CON: 29.3 ± 1.3 mg glucose/min per kilogram body weight).

3.4. Whole body fuel oxidation of the offspring

Fig. 2 illustrates the pattern of whole body fuel oxidation in the 3-month-old offspring over a 24-hour period. It was observed that in both groups of offspring, the average RQ decreased during the period of fasting

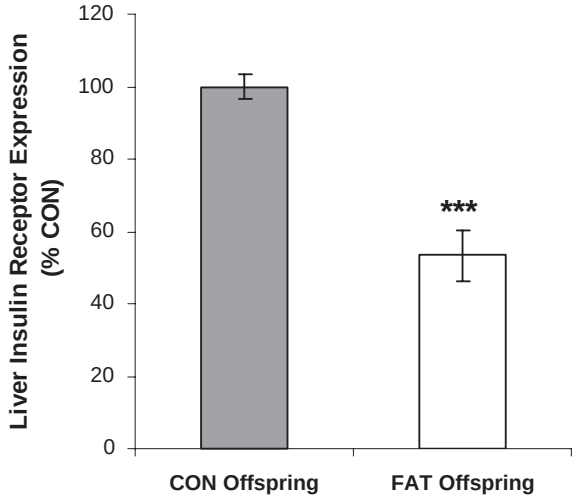

Fig. 3. Western blot analysis of the insulin receptor β subunit expression in liver lysates of the 3-month-old male offspring. Whole cell liver lysates (20 μg protein) were electrophoresed and transferred to nitrocellulose that was then reacted with an anti–insulin receptor antibody and expression was quantified as described in the Materials and methods section. Results are the means ± SEM of 16 separate CON samples and 15 separate FAT samples. The FAT offspring protein content is expressed as a percentage ± SEM of the detected CON offspring protein content (100% ± SEM). Triple asterisk indicates $P < .001$ cf CON offspring.

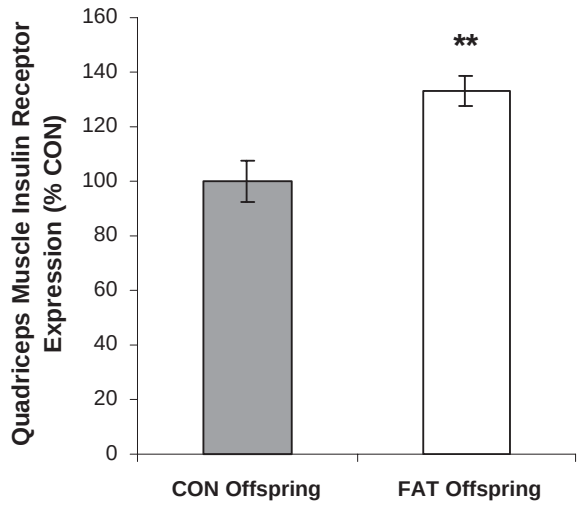

Fig. 4. Western blot analysis of the insulin receptor β subunit expression in quadriceps muscle lysates of the 3-month-old male offspring. Whole cell quadriceps muscle lysates (20 μg protein) were electrophoresed and transferred to nitrocellulose that was then reacted with an anti–insulin receptor antibody and expression was quantified as described in the Materials and methods section. Results are the means ± SEM of 16 separate CON samples and 15 separate FAT samples. The FAT offspring protein content is expressed as a percentage ± SEM of the detected CON offspring protein content (100% ± SEM). Double asterisk indicates $P < .005$ cf CON offspring.

(0-7 hours), reflecting a progressively higher proportion of fat oxidation with time. In response to a carbohydrate meal (after 7 hours), the average RQ increased, as expected, reflecting predominantly carbohydrate oxidation. To obtain a fasted RQ value and a fed RQ for the 2 groups of offspring, an average of 9 data points was calculated. Fasted RQ values were calculated between 3.6 and 6.6 hours, whereas fed RQ values were calculated between 8.3 and 11.3 hours. There was no statistical difference between the RQ of the FAT offspring and that of the CON offspring in either the fasted or the fed state.

3.5. Quadriceps muscle and liver lipid metabolism of the offspring

There was no difference observed in quadriceps muscle triglyceride content (FAT: 4.8 ± 0.3 μmol/g tissue cf CON: 4.2 ± 0.3 μmol/g tissue) or in the rate of fatty acid oxidation of the muscle, as reflected by β-HAD activity (FAT: 0.2 ± 0.0 U/U CS activity cf CON: 0.2 ± 0.0 U/U CS activity). The triglyceride content of the liver was significantly elevated in the FAT offspring (FAT: 5.9 ± 0.5 μmol/g tissue cf CON: 4.3 ± 0.5 μmol/g tissue; $P < .05$), with no apparent difference in the rate of fatty acid oxidation (FAT: 3.8 ± 0.1 U/U CS activity cf CON: 4.4 ± 0.3 U/U CS activity).

3.6. Tissue insulin signaling protein content of the offspring

A 46.7% ± 7.0% reduction ($P < .001$) in the protein expression of the insulin receptor β subunit was observed in the liver of the FAT offspring (Fig. 3). As shown in Table 2, liver IRS1 content was decreased ($P < .01$) and liver PKCζ expression was increased ($P < .005$) in the FAT offspring. When comparing the FAT offspring with the CON offspring, no statistical differences were observed in the hepatic expression of the p85 subunit of phosphatidylinositol-3-kinase (PI3-kinase) or IRS2 (Table 2).

There was a 33.3% ± 5.3% increase ($P < .005$) in the expression of insulin receptor β subunit protein within the quadriceps muscle of the FAT offspring (Fig. 4). Expression of the p85 was increased ($P < .05$) and the GLUT4 protein may have been increased ($P = .07$) in the quadriceps muscle of the FAT offspring. There were no differences in the expression of the other measured insulin signaling proteins in the quadriceps muscle of the FAT and CON offspring. These results are summarized in Table 3.

Table 3
Quadriceps muscle insulin signaling protein expression

	CON offspring (n = 16)	FAT offspring (n = 15)
Insulin receptor β subunit	100% ± 7.7%	133.3% ± 5.3%**
IRS1	100% ± 8.3%	93.4% ± 11.4%
IRS2	100% ± 5.9%	93.4% ± 5.7%
p85 subunit of PI3-kinase	100% ± 3.4%	110.0% ± 3.1%*
PKCζ	100% ± 10.9%	94.9% ± 6.4%
GLUT4	100% ± 8.0%	121.7% ± 8.4%

FAT offspring protein expression is expressed as a percentage of the CON offspring protein expression (100% ± SEM).

* $P < .05$ cf CON offspring.

** $P < .005$ cf CON offspring.

4. Discussion

The main aim of this study was to investigate the consequences of maternal high fat feeding on glucose and lipid metabolism in the offspring. The key components of the insulin signaling pathway were also assessed. Rats were fed a diet high in ω-6 polyunsaturated fat before and during the gestational period. The results of this study confirm and extend data demonstrating that adverse maternal nutrition may have permanent detrimental effects on the programming of postnatal metabolic processes. Potentially, this may increase the risk for the offspring to develop diseases during adulthood.

Maternal high fat feeding resulted in significantly smaller litter sizes. This finding has been reported previously in rats that were fed a mixed polyunsaturated fat diet before and during gestation [28]. It was suggested that lower numbers of corpora lutea and higher resorption numbers contributed to decreased litter sizes. The composition of dietary fatty acids can influence fertility. In particular, consumption of a diet high in ω-6 polyunsaturated fat can decrease the number of ova released [29], this may therefore be a reason why reduced litter sizes were observed in this present study.

Despite the smaller litter sizes, neither the birth weight of the FAT offspring nor their body weight at 3 months of age was significantly different from such of the CON offspring. Maternal high fat feeding did however affect the offspring's body composition. At 3 months of age, the FAT offspring had significantly increased proportions of total body fat and significantly increased proportions of body fat in the abdominal region. Weanlings of high-saturated fat–fed dams also have increased proportions of body fat [15,30]. The presence of elevated body fat, in particular, visceral fat, has emerged as a significant risk factor for the development of insulin resistance and associated metabolic disorders in both the human population [31,32] and in rodents [33,34].

No differences in whole body insulin sensitivity were apparent at 3 months of age. Enhanced first-phase insulin secretion in response to an oral glucose challenge was observed in these FAT offspring and has been documented previously in 3-month-old offspring of saturated fat–fed dams [28]. First-phase insulin secretion, which represents the secretion of ready-synthesized insulin via the fusion of granules with the plasma membrane, is often considered the most vulnerable phase of insulin secretion [35]. Alterations in first-phase insulin secretion may predict the development of obesity and insulin resistance [36-38]. The hypersecretion of insulin observed in the FAT offspring within 15 minutes after an oral glucose load, together with elevated proportions of total and abdominal body fat, may reflect an increased risk for the development of insulin resistance and associated metabolic diseases.

Liver triglyceride was significantly increased in the 3-month-old FAT offspring. This did not appear to be caused by altered fatty acid oxidation because there was no statistical difference in the activity of β-HAD between the FAT and CON offspring. Whole body fuel oxidation, as determined by indirect calorimetry, was also unaltered in the FAT offspring. The accumulation of liver triglyceride was therefore not the consequence of detectable defects in fat oxidation at either the whole body level or hepatic level.

Excess storage of triglyceride within the liver has been associated with high fat feeding and the occurrence of insulin resistance in animal models [16,39]. In this present study, the male offspring were not directly fed a high-fat diet but were exposed to maternal high fat feeding during their fetal life. The observation of significantly elevated accumulation of liver triglyceride suggests abnormal early metabolic programming caused by exposure to the maternal high-fat diet, resulting in predisposition to the development of metabolic diseases similar to that observed when high-fat diets are fed directly. The fat-fed dams in this present study were transferred from the high-fat diet to a standard laboratory chow diet immediately after having given birth in an attempt to control for early postnatal nutritional effects. However, it remains a possibility that some of the metabolic effects observed in the FAT offspring are mediated by the differences in milk composition between the lactating chow-fed and fat-fed dams. In future investigations, the analysis of the milk composition from the lactating rats is indeed warranted to ascertain whether the 2 groups of offspring are exposed to nutritional differences in early postnatal life.

Maternal high fat feeding also resulted in the offspring having modified expression of key proteins involved in the hepatic insulin signaling pathway. The reduced expression of the hepatic insulin receptor β subunit and IRS1 in the FAT offspring may be associated with a downregulation of liver insulin signaling and thus reduced insulin action. Elevated expression of hepatic PKCζ that could also be associated with such downregulation and increased PKCζ expression, not due to hyperglycemia, has been demonstrated in the liver of both diabetic human beings and in Zucker diabetic fatty rats [40]. It has been suggested that hepatic PKCζ may play a major regulatory role in a negative feedback mechanism induced by insulin [41]. Hepatic PKCζ mediates the serine/threonine phosphorylation of IRS1. Such phosphorylation degrades the IRS1 protein [42], which may result in the dissociation of IRS1 from the insulin receptor and hence in the inhibition of transduction of the insulin signal [41]. The observed reduction in IRS1 may therefore be due, at least in part, to increased PKCζ expression.

Taken together, the metabolic abnormalities observed in the liver of the offspring of high fat–fed dams reflect a phenotype that is commonly associated with reduced hepatic insulin sensitivity. However, from this study, it cannot be ascertained whether the altered expression levels of the hepatic insulin signaling proteins are the cause or the consequence of the observed elevated liver triglyceride levels or whether these phenotypic abnormalities occur simultaneously.

Lipid accumulation is one of the many skeletal muscle morphological abnormalities strongly associated with the degree of insulin resistance [12,43,44]. Triglyceride accumulation and fatty acid oxidation were not affected in the muscle of the 3-month-old male offspring of high fat–fed dams. Maternal high fat feeding resulted in alterations of the expression of key insulin signaling proteins in the quadriceps muscle of the 3-month-old FAT offspring. However, unlike the downregulation of the insulin signaling pathway observed in the liver, the pathway in the FAT offspring quadriceps muscle was found to be upregulated. Protein expression of the insulin receptor β subunit and that of the p85 were significantly increased. Glucose transporter protein 4 expression also tended to be increased; however, this did not reach statistical significance.

The apparent increased muscle insulin sensitivity may be a compensation for the hepatic insulin–resistant phenotype observed in the FAT offspring at 3 months of age. This may explain their normal glucose tolerance at this age. Hepatic insulin resistance precedes the development of muscle insulin resistance in other high fat–fed rat models [45,46].

The association between early programming and adult metabolic disease has proven a critical role for maternal nutrition. Maternal high fat feeding is one such insult that is of extreme importance. The results of this study strongly support the notion of adverse maternal nutrition predisposing the offspring to adult metabolic disease. The mechanistic basis of this programming is not well understood. Significant risk factors for the development of insulin resistance and associated metabolic diseases were evident in the young adult offspring of dams fed a diet enriched with ω-6 polyunsaturated fat. These metabolic abnormalities were similar to those observed in the offspring of dams fed a diet high in saturated fat. Further investigations are therefore warranted to establish the impact of aging on the exposed offspring.

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