Maternal Antioxidant Supplementation Prevents Adiposity in the Offspring of Western Diet–Fed Rats

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OBJECTIVE—Obesity in pregnancy significantly increases the risk of the offspring developing obesity after birth. The aims of this study were to test the hypothesis that maternal obesity increases oxidative stress during fetal development, and to determine whether administration of an antioxidant supplement to pregnant Western diet-fed rats would prevent the development of adiposity in the offspring.

RESEARCH DESIGN AND METHODS—Female Sprague Dawley rats were started on the designated diet at 4 weeks of age. Four groups of animals were studied: control chow (control); control + antioxidants (control+Aox); Western diet (Western); and Western diet + antioxidants (Western+Aox). The rats were mated at 12 to 14 weeks of age, and all pups were weaned onto control diet.

RESULTS—Offspring from dams fed the Western diet had significantly increased adiposity as early as 2 weeks of age as well as impaired glucose tolerance compared with offspring of dams fed a control diet. Inflammation and oxidative stress were increased in preimplantation embryos, fetuses, and newborns of Western diet-fed rats. Gene expression of proadipogenic and lipogenic genes was altered in fat tissue of rats at 2 weeks and 2 months of age. The addition of an antioxidant supplement decreased adiposity and normalized glucose tolerance.

CONCLUSIONS—Inflammation and oxidative stress appear to play a key role in the development of increased adiposity in the offspring of Western diet-fed pregnant dams. Restoration of the antioxidant balance during pregnancy in the Western diet-fed dam is associated with decreased adiposity in offspring. ***Diabetes*** **59:3058–3065, 2010**

Obesity is one of the most pervasive and burdensome public health problems in modern times. The steady increase in overweight reproductive-age women is correlated with increases in rates of childhood and infant obesity. A possible link between the abnormal intrauterine environment and abnormal growth and development of offspring must be considered (1). The period from conception to birth is a time of rapid growth, cellular replication and differentiation, and functional maturation of organ systems. These processes are very sensitive to alterations of the nutritional milieu, and the abnormal intrauterine metabolic milieu associated with obesity in pregnancy can have long lasting effects on the development of obesity and diabetes in offspring (2,3). Maternal obesity significantly increases fetal and neonatal adiposity in humans; thus, enhanced adipocyte development per se must play an important role in the genesis of obesity in the offspring (2).

It has been shown that obesity in the nonpregnant and pregnant state is associated with inflammation and oxidative stress (2–15). Obese individuals have higher plasma levels of 8-epi-prostaglandin F2α (PGF2α), an index of lipid peroxidation, and acute-phase proteins and proinflammatory cytokines such as tumor necrosis factor TNF-α and interleukin IL-6 (13–15). Recently, Hauguel-de Mouzon and colleagues (9,10) reported that expression of cytokines, inflammation-related genes, and genes linked to oxidative stress are markedly elevated in placenta of obese women. These studies demonstrate that not only does adipose tissue release inflammatory molecules, but that the placenta also contributes to the inflammatory/oxidant state and the stimuli favoring fetal fat accretion derived from maternal or placental sources. Thus, maternal obesity in pregnancy creates a very abnormal milieu in which the embryo and fetus develop. Further, a normal redox state is critical for embryonic stem cell differentiation (16). However, it is not known whether the offspring of obese mothers have an increased oxidant load or whether increased oxidative stress is linked to the development of obesity. The hypothesis that oxidative stress is causally linked to the development of obesity in offspring can be tested by determining whether antioxidants prevent increased adiposity in the offspring of obese mothers.

The beneficial effects of antioxidant vitamins supplementation are attributed to their ability to scavenge free radicals, control nitric oxide synthesis or release, inhibit reactive oxygen species generation, and upregulate antioxidant enzyme activities that metabolize these molecules (17). Vitamins A, C, and E are nonenzymatic antioxidants that have properties of free radical scavengers. Vitamin C administration has been shown to reduce the adiposity induced by the intake of a high-fat diet in rats (18,19). Vitamin E has a particularly important role in preventing the oxidation of LDLs and thus has been the recent subject of investigation for use in cardiovascular disease. The antioxidant properties of zinc and selenium have also been demonstrated. Zinc directly inhibits the formation of O^{2-} by inhibiting the NADPH oxidase complex that catalyzes its formation, and indirectly, by inducing the production of metallothionein, a free radical scavenger. Selenium, in the form of selenoproteins (most notably selenocysteine), directly catalyzes the reduction of H_2O_2 and various other peroxides.

Studies performed to evaluate the effectiveness of anti-

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TABLE 1
Diet composition per weight of chow and per kcal

	Control	Control +Aox	Western	Western +Aox
Protein (% per 100 g)	15.9	15.9	21.6	21.9
Protein (% per kcal)	20.1	20.1	19.9	19.9
Carbohydrate (% per 100 g)	45	45	50.2	50.2
Carbohydrate (% per kcal)	56.7	56.7	46.3	46.3
Fat (% per 100 g)	8.2	8.2	16.2	16.2
Fat (% per kcal)	23.1	23.1	33.7	33.7
Total vitamin A (IU/kg)	4,600	23,000	8,900*	32,000**
Total vitamin E (IU/kg)	86	260	118*	360**
Total vitamin C (g/kg)	0	5.6	0	5.6
Total selenium (mg/kg)	0.165	0.5	0.225*	0.675**

*Indicates that this vitamin or mineral is present in the same absolute amount as the control on an energy basis (per kcal). **Indicates that this mineral is present in the same absolute amount as in the control+Aox chow per kcal.

oxidant supplementation in obese human adults have had mixed results. Of the many studies, only two have shown any positive effects (20,21). However, since adiposity significantly increases early in life, it is likely that there is a critical window of vulnerability early in development such that interventions given at this stage may have greater success in preventing the development of obesity.

Several investigators have used animal models of high-fat or Western style diet-induced obesity (a diet that has increased fat and carbohydrate content) and have shown that maternal over-nutrition induces increased adiposity and permanent changes in metabolism in offspring (20–33). The aims of this study were to test the hypothesis that a Western-style diet fed during pregnancy increases oxidative stress, thereby potentiating adipogenesis in the offspring, and to determine whether administration of an antioxidant supplement to pregnant Western diet-fed rats would prevent the development of increased adiposity in offspring.

RESEARCH DESIGN AND METHODS

Female Sprague Dawley rats were started on the designated diet (Table 1) at the time of weaning at 4 weeks of age. Four groups of animals were studied: control chow (control); control + antioxidants (control+Aox); Western diet (Western); and Western diet + antioxidants (Western+Aox). All diets were custom made by Harlan Teklad (please see data in the supplementary appendix (available online at http://diabetes.diabetesjournals.org/cgi/content/full/db10-0301/DC1 for details of the diets). The control and Western diets had the same micronutrient composition and differed only in the macronutrient and caloric content. The antioxidant supplement did not alter the caloric content of the chow. The Western diet had ~300% fat (as saturated fat), 150% carbohydrates (mainly as simple carbohydrates), and 95% protein compared with the control diet (Table 1). Offspring were weaned onto standard rat chow. The amounts of the antioxidant supplements in the Aox groups are shown in Table 1.

The female rats were bred with Sprague Dawley male rats between 12–16 weeks of age and were allowed to deliver spontaneously. Thus, female rats were 4 weeks old when they were started on the diets, 10–12 weeks old when they were bred, and 13–15 weeks old at the end of pregnancy. For studies in the offspring, at weaning, all diets were changed back to a control diet and this control diet was continued throughout life. All litters were culled to 8 pups. Studies were performed only in male rats.

For experiments in blastocysts, female rats were treated with 30 IU of pregnant mare serum gonadotrophin (PMSG) intraperitoneally. Ovulation was induced with 50 IU intraperitoneally of human chorionic gonadotrophin (hCG) 48 h later. Female rats were then caged overnight with a proven male. Between 10–12 embryos were flushed from the oviduct from pregnant rat donors killed 5 days after mating (blastocyst stage). Although there tended to be a lower number of blastocysts in the offspring of the obese dams, this was not statistically significant. The antioxidant supplement did not affect embryonic viability. Reduced (GSH) and oxidized (GSSG) glutathione levels were measured in groups of 10 to 16 blastocysts by high-performance liquid chromatography separation (C-18 reversed-phase column) combined with fluorescence detection after derivatization with O-phthaldehyde.

For fetal studies, pregnant rats were killed on gestational day 18. The number of pups in each litter was recorded. Trunk blood was collected in heparinized tubes and centrifuged, and the plasma was stored at −80°C for hormone and substrate analyses. Fetal blood from all of the fetuses in the same litter was pooled, and their plasma was stored as indicated above.

All animal protocols were submitted to and approved by The Children's Hospital of Philadelphia Animal Care Committee.

Glucose tolerance tests. Studies were done in offspring at 2 months of age. Animals were fasted for 18 h before study. At 0 min, blood was obtained from the dorsal tail vein for measures of blood glucose and plasma insulin, and then an intraperitoneal bolus of glucose (2 mg glucose per gram of body weight) was given and serial blood glucose measurements were done using the Hemacue glucose analyzer (Angelholm, Sweden).

Metabolic measurements. The following were measured in plasma in the fasted state (for pregnant animals, measures were done at day 21 gestation) using commercially available kits: free fatty acids (Zen-Bio, Research Triangle Park, NC), leptin (IBL-America, Minneapolis, MN). thiobarbituric acid reactive substances ([TBARS] Cayman Chemical, Ann Arbor, MI), glutathione peroxidase ([GSH] Biovision, Mountain View, CA), and C-reactive protein (CRP) (IBL). Plasma insulin was measured by radioimmunoassay (Penn Diabetes Core at the University of Pennsylvania).

To determine the quantity of reactive oxygen species (ROS) produced by blastocysts, the relative intensity of ROS production was measured using 2′,7′-dichlorodihydrofluorescein diacetate (DCHFDA; Sigma). The nonfluorescent dye generates a fluorescence signal after reacting with ROS. Embryos were harvested, incubated in InVitroCare medium (KSOM/AA medium containing 0.2 mmol/l glucose, 0.2 mmol/l pyruvate, and 10 mmol/l lactate) 5% CO_2 in 95% air, 37°C, and then incubated for an additional hour in medium containing 10 μmol/l DCHFDA, and then washed in fresh InVitroCare medium before being placed on a glass slide and covered with a cover slip. Fluorescence was determined in the culture medium using a fluorescence plate reader with excitation wavelength at 505 nm and emission wavelength at 540 nm.

Real-time PCR. Total RNA was isolated from starting material (stored at −80°C) using one of three commercially available kits: RNAqueous Micro kit (Ambion) for blastocysts; RNA easy lipid kit (Quiagen) for adipose tissue; and RNA easy tissue kit (Quiagen) for placenta. Complimentary DNA was synthesized using the ThermoScript RT-PCR system (Invitrogen). Real-time PCR was performed on a LightCycler using the FastStart DNA MasterSYBER Green I (Roche Diagnostics) according to the protocol provided by the manufacturer. All real-time PCR data were normalized to the housekeeping gene β actin. All primers (TaqMan) were obtained from Applied Biosystems (Carlsbad, CA).

Fat mass. Body fat was measured using dual emission X-ray absorptiometry (PIXImus DEXA, General Electric, Madison, WI) and reported as the ratios of fat mass and body weight. The DEXA scanner was specialized for small animals. The instrument settings used were as follows: a scan speed of 40 mm/s, a resolution of 1.0 × 1.0 mm, and automatic/manual histogram width estimation. The coefficient of variation, as assessed by three repeated measurements (with repositioning of the rat between each measurement), was less than 5%. Measures were done on day 21 of pregnancy ($n = 5$ animals each group), and at 2 weeks, 2 months, and 6 months of age in the offspring (a total of 7 animals from different litters from each group).

Statistical analysis. The significance of differences among groups was examined using a two-way ANOVA analysis and Tukey-Kramer for post hoc analysis. All values are presented as means ± SE. A P value of < 0.05 was considered significant. All data were analyzed using Prism data analysis software.

RESULTS

Body weights at the initiation of the study (at 4 weeks of age) were not different (Fig. 1); however, at the time of breeding, dams fed the Western and the Western+Aox diets were significantly heavier than the two control groups (Fig. 1) and had a significantly higher rate of weight gain and fat mass before pregnancy compared with those fed a control diet (Table 2). Addition of the antioxidant supplement to the Western diet did not significantly affect weight gain or body composition (Table 2). The daily energy intake was increased in dams fed the Western diets; however, there was no difference in food consump-

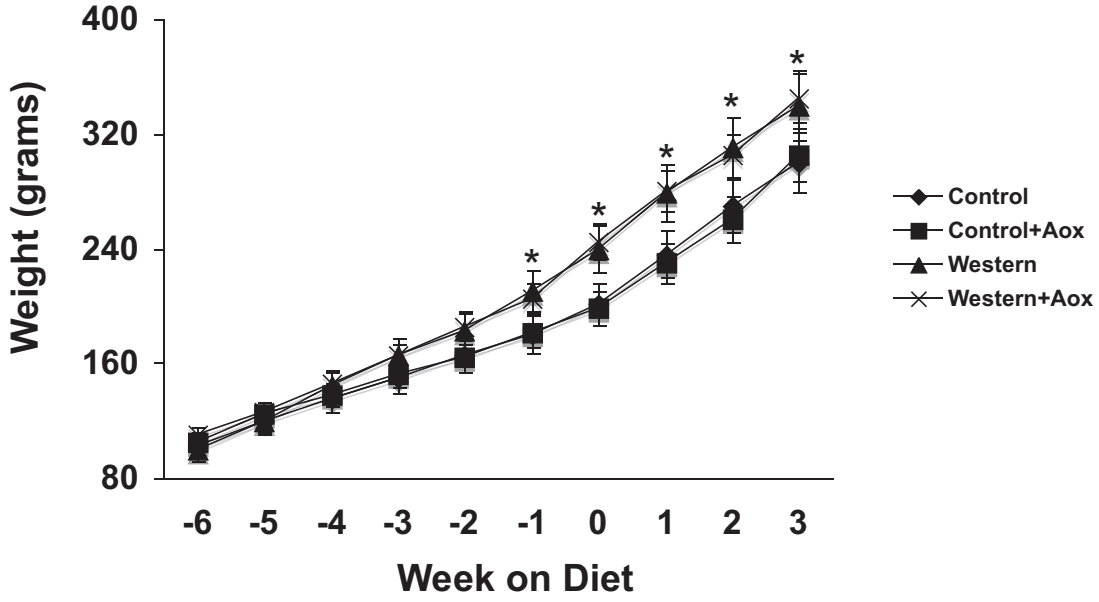

FIG. 1. Weights of female dams in the four study groups. Animals were started on the diets at weaning at 4 weeks of age. The negative numbers refer to weeks before pregnancy, 0 is at breeding, and the positive numbers refer to weeks during pregnancy; $n = 10$ dams in each group. *$P < 0.05$ Western and Western+Aox versus control diet.

tion between the four groups, which averaged 5g/100 g body weight daily.

Glucose levels did not differ between groups. However, insulin concentrations were significantly higher in the Western diet-fed dams and were decreased by the antioxidant supplement (Table 2). As expected, leptin levels were also higher in the Western diet-fed dams compared with control and control+Aox-fed dams (Table 2).

Weights and fat content of offspring. Birthweights of the pups did not differ among the four groups and averaged 5.09 ± 0.05; 5.11 ± 0.05; 5.11 ± 0.04; and 5.12 ± 0.04 (controls, control+Aox, Western, Western+Aox, respectively; $n = 5$ litters from each group). There were no differences in litter size among the four groups at birth. By 2 weeks of age, offspring of Western diet-fed dams had increased fat mass, which was ameliorated in the offspring of the Western diet dams given the antioxidant supplement (Fig. 2*A*). There was no effect of the antioxidant supplement on birth weight or fat mass in the control chow group (Fig. 2*A*). At 2 months of age, total and central fat mass of the offspring of the Western diet-fed dams remained significantly increased compared with offspring of control diet-fed dams (Fig. 2*B*). In contrast, total fat content was significantly reduced in the adult Western+Aox offspring compared with the Western-diet group and did not differ from the control groups.

Metabolic parameters. In the fetus (day 18 gestation), there were no significant differences in plasma levels of glucose, insulin, free fatty acids, or leptin among the four groups. However, at birth and at 2 weeks of age, free fatty acids, insulin, and leptin, but not glucose levels, were significantly increased in the offspring of Western diet-fed dams (Table 3). At 2 months of age, insulin and leptin levels remained elevated (Table 3) and glucose tolerance tests demonstrated mildly impaired glucose tolerance in the offspring of Western diet-fed dams compared with controls (Fig. 3). The degree to which insulin resistance or ß-cell dysfunction impair glucose tolerance and whether this worsens with age remain to be determined. Of note, in a slightly different model of obesity in pregnancy, offspring do develop ß-cell dysfunction later in life (34).

The Western+Aox animals demonstrated marked improvement in glucose homeostasis (Table 3 and Fig. 3). Together, these data show that the addition of an antioxidant supplement during pregnancy is associated with decreased adiposity in the offspring and its associated complications of glucose intolerance in the offspring of Western diet-fed dams.

Oxidative stress. Maternal obesity induces a marked inflammatory response (9,10), which in turn causes mitochondrial dysfunction resulting in increased production of reactive oxygen species (35,36). To determine whether

TABLE 2
Endocrine-metabolic parameters in normal and Western diet-fed pregnant rats at day 21 gestation

	Control	Control+Aox	Western	Western+Aox
Leptin (ng/ml)	1.00 ± 0.64	2.15 ± 0.42	4.97 ± 0.42*	2.11 ± 0.38
FFA (μEq/ml)	925 ± 128	854 ± 75	1,298 ± 101*	877 ± 235
Blood glucose (mg/dl)	129 ± 14	122 ± 15	134 ± 15	127 ± 10
Insulin (ng/ml)	0.51 ± 0.02	0.53 ± 0.03	0.82 ± 0.03*	0.60 ± 0.03
Total fat mass (% body mass)	14.2 ± 2.5	15.8 ± 3.8	20.4 ± 1.8*	20.7 ± 2.2*
Food consumption (g/100g body weight/day)#	5.1 ± 1.2	5.3 ± 0.4	4.9 ± 1.5	4.8 ± 1.6

Data are means ± SE. *$P < 0.05$ vs. control diet. #Measurement of food consumption was started on day 1 of pregnancy. FFA, free fatty acid.

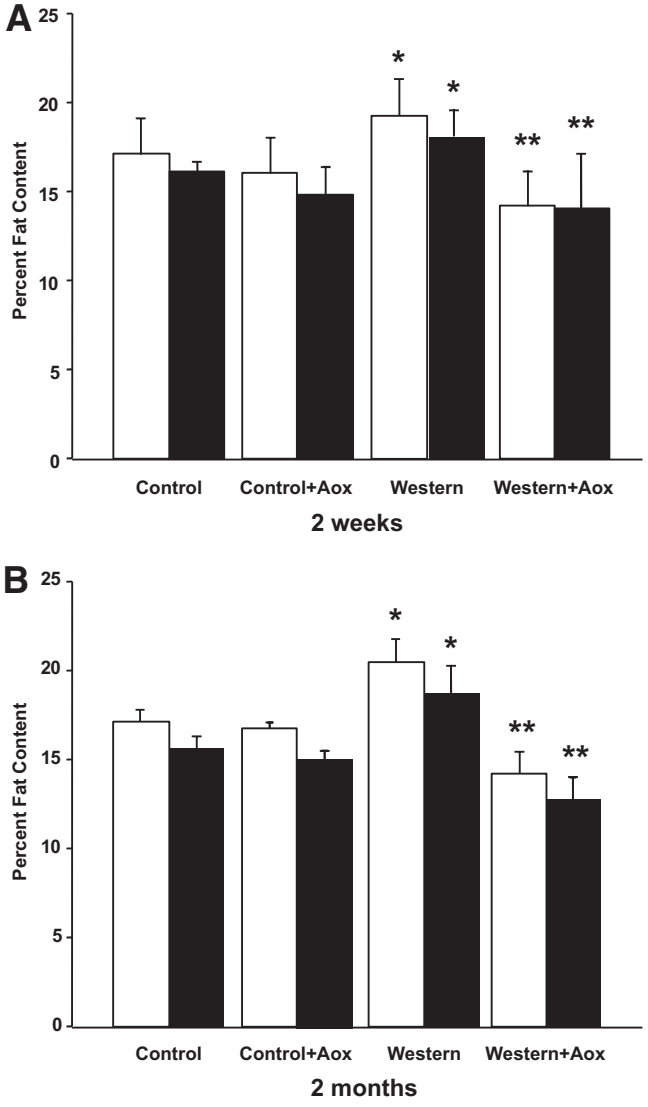

FIG. 2. ***A*****: Maternal antioxidant supplement normalizes body fat in 2-week-old offspring of Western diet-fed rats. At 2 weeks of age, total and visceral fat were measured by Dexa scanning. *White bar* represents total fat and *black bar* represents visceral fat. Data shown are ratio of fat mass and body weight (percentage of total and visceral fat ± SEM), $n = 5$ animals in each group; *$P < 0.05$ Western diet versus control, control+Aox, and Western+Aox; **$P < 0.05$ Western+Aox versus Western diet.** ***B*****: Maternal antioxidant supplement normalizes body fat in 2 month-old offspring of Western diet-fed rats. At 2 months of age, total and visceral fat were measured by Dexa scanning. *White bar* represents total fat and *black bar* represents visceral fat. Data shown are percentages of total and visceral fat ± SEM; $n = 5$ animals each group, *$P < 0.05$ Western diet versus Control, Control+Aox, and Western+Aox; **$P < 0.05$ versus Western+Aox versus Western diet.**

and when exposure to a Western diet induces oxidative stress in the offspring during development, we measured indexes of oxidative stress in preimplantation embryos, fetuses, and newborns.

GSH and GSSG levels were measured in blastocysts from dams of all four groups (experiments were repeated in three separate litters of each group). GSH levels were modestly decreased in embryos from Western diet-fed dams compared with controls ($P < 0.05$ vs. controls) (Fig. 4*A* and *B*). Antioxidant supplementation normalized GSH and GSSG content in Western diet blastocysts, but had no effect on controls (Fig. 4*A* and *B*).

Decreased levels of GSH suggested that exposure to a Western diet during pregnancy induced oxidative stress in the embryo. Therefore, we measured ROS levels in blastocysts from all four groups. As expected, ROS levels as determined by DCHFDA fluorescence detection were significantly higher in blastocysts of Western diet-fed dams compared with controls, controls+Aox, and Western+Aox (Fig. 4*C*).

Similarly, measures of oxidative stress were significantly elevated in fetal and newborn offspring of Western diet-fed dams. Serum levels of the inflammatory marker, C reactive protein (Fig. 5*A*), and TBARS (Fig. 5*B*) were significantly elevated, whereas serum levels of GSH were significantly reduced (Fig. 5*C*). Antioxidant supplementation reduced these measures of inflammation and oxidative stress in the Western+Aox offspring to levels that were significantly different from Western diet-fed offspring (Fig. 5*A*–*C*).

Adipogenesis. There is a rapid and dramatic expansion of the adipose lineage that occurs during the first month of postnatal life in the rodent, and our data demonstrate that exposure to a Western diet during development accentuates this process. We found that mRNA levels of Pref1, Wisp2, and PPARγ were markedly elevated in fat tissues from 2-week-old and 2-month-old offspring of Western diet-fed dams compared with controls (Fig. 6*A*). Pref1 and Wisp2 maintain the adipocyte precursor cell in a committed but undifferentiated state. Interestingly, expression of BEST5, a gene that promotes differentiation of mesenchymal stem cells into bone (37), was markedly reduced in fat tissue of offspring of obese dams (Fig. 6*A*). Thus, exposure to a Western style diet during development increases expression of genes that promote expansion of adipocyte precursor pools and lipid storage in fat tissue.

Expression of genes regulating lipogenesis, including SREBP1c, Acyl CoA Synthase 1, fatty acid synthase (FAS), and fatty acid translocase was also significantly increased in fat tissue from offspring of Western diet-fed dams compared with controls (Fig. 6*B*). Thus, Western diet induced maternal adiposity not only expands the adipocyte precursor pool, but also promotes lipid storage in fat tissue of their offspring. Most importantly, antioxidant supplementation before and during pregnancy nearly normalized gene expression in Western diet offspring (Fig. 6*A* and *B*). Thus, our data suggest that one of the underlying mechanisms of increased adiposity in offspring of obese dams is related to oxidative stress promoting adipogenesis and lipid storage.

DISCUSSION

There are a number of critical periods during development that appear to influence the later development of obesity. It is likely that the risk of developing obesity in the offspring of an obese mother is caused by a continuum of exposure from the prepregnant state (possibly affecting oocyte quality) to the exposure of the offspring during lactation. In the present study we have demonstrated that a Western-style diet before and during pregnancy and lactation results in increased fat mass and glucose intolerance in offspring. These results are in agreement with several studies showing that offspring of dams fed a high-fat diet or a Western-style diet (high in fat and carbohydrate) have increased body fat and glucose intolerance in the offspring (22–33,38–40).

Of major importance is our finding that exposure to a Western-style diet before and during pregnancy alters the

TABLE 3
Endocrine-metabolic parameters in offspring of normal and Western diet-fed pregnant rats at birth

	Control	Control+AOX	Western	Western+AOX
Fetal d (18) (n = 5 litters)				
Leptin (ng/ml)	2.2 ± 0.2	1.9 ± 0.2	2.1 ± 0.1	2.3 ± 0.2
FFA (μEq/ml)	35.3 ± 2.8	27.9 ± 3.1	33.2 ± 4.6	30.2 ± 4.9
Blood glucose (mg/dl)	54.4 ± 6.7	57.9 ± 5.9	61.6 ± 7.7	58.14 ± 6.3
Insulin (ng/ml)	1.49 ± 0.07	1.51 ± 0.08	1.58 ± 0.09	1.55 ± 0.08
Birth (n = 5 litters)				
Leptin (ng/ml)	2.8 ± 0.3	2.9 ± 0.2	3.8 ± 0.1*	3.0 ± 0.3
FFA (μEq/ml)	64.8 ± 6.9	57.9 ± 5.1	105.7 ± 9.9*	76.6 ± 7.5
Blood glucose (mg/dl)	88.2 ± 8.8	77.4 ± 9.4	81.0 ± 8.3	86.6 ± 9.4
Insulin (ng/ml)	0.47 ± 0.06	0.49 ± 0.06	0.68 ± 0.07*	0.47 ± 0.07
2 weeks (n = 5 litters)				
Leptin (ng/ml)	4.2 ± 0.6	3.9 ± 0.7	7.8 ± 0.9*	4.1 ± 0.8
FFA (μEq/ml)	38.5 ± 4.2	41 ± 4.3	52.5 ± 5.5*	43 ± 5.6
Blood glucose (mg/dl)	93.6 ± 10.4	97.2 ± 11.5	104.4 ± 9.7	95.4 ± 9.5
Insulin (ng/ml)	0.81 ± 0.05	0.84 ± 0.04	1.9 ± 0.02*	0.92 ± 0.01
2 months (n = 8)				
Leptin (ng/ml)	14.7 ± 1.5	13.2 ± 2.5	25 ± 1.9*	14.5 ± 1.9
FFA (μEq/ml)	15.7 ± 1.3	12.4 ± 1.4	13.4 ± 1.6	12.9 ± 1.5
Blood glucose (mg/dl)	118.8 ± 10.8	117 ± 11.9	120.6 ± 14.0	122.0 ± 14.4
Insulin (ng/ml)	1.4 ± 0.06	1.8 ± 0.04	2.9 ± 0.09*	1.9 ± 0.05

Data are means ± SE. *$P < 0.05$ vs. control diet. FFA, free fatty acid.

redox state as early as preimplantation development, leading to mild oxidative stress. This altered state persists throughout gestation and early life—critical stages for adipogenesis. Our finding that administration of an antioxidant supplement given to the dam reverses oxidative stress and completely prevents the development of adiposity and glucose intolerance in the offspring suggests that oxidative stress plays an important role in the development of obesity.

In support of the link between oxidative stress and the development of adiposity are two recent studies that showed that ROS are involved in the regulation of fat development (40,41). Preventing the accumulation of $P66^{SHC}$ generated free radicals decreases fat mass and promotes resistance to diet-induced obesity (41).

A number of studies in humans have demonstrated that obesity is associated with an inflammatory state, which in turn induces oxidative stress (4,9–11,42,43). Recently, several studies reported that expression of cytokines, inflammation-related genes, and genes linked to oxidative stress are markedly elevated in the placenta and serum of obese women (2,8–13,44,45). These investigators hypothesize that not only does adipose tissue release inflammatory molecules, but that the placenta also contributes to the inflammatory/oxidant state and the stimuli favoring fetal fat accretion derived from maternal or placental sources. Thus, exposure to a Western-style diet in pregnancy creates a very abnormal milieu in which the embryo and fetus develop. We postulate that before placentation, maternal adipose tissue is the primary source for inflammatory molecules and oxidants. Once the placenta develops, the fetus is further exposed to oxidative stress, creating a vicious cycle. It is likely that exposure of newborn offspring to a Western-style diet during lactation further potentiates this process.

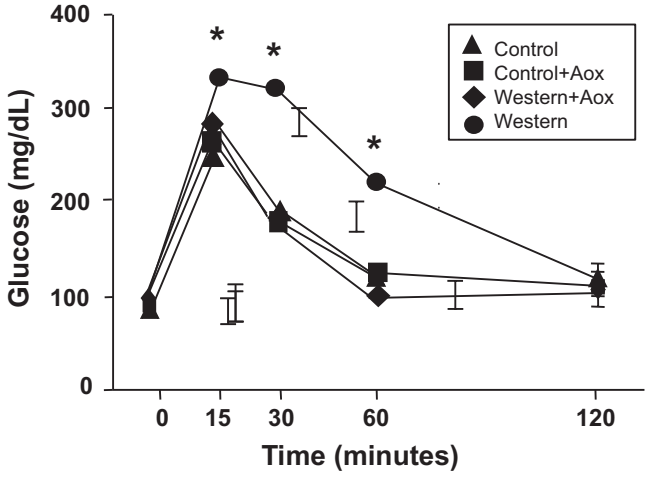

FIG. 3. Maternal antioxidant supplement improves glucose tolerance in 2-month-old offspring of Western diet-fed rats. At 2 months of age, offspring were given 2 g glucose/kg intraperitoneally and glucose was measured 15, 30, 60, and 120 min after injection. Data shown are ± SEM; n = 5 for each group; *$P < 0.05$ Western diet versus control, control+Aox, and Western+Aox.

Offspring of Western diet-fed dams exhibit increased fat mass very early in life, suggesting that adipocyte development per se plays an important role in the genesis of obesity in the offspring. Further, fetuses and newborns of obese women have increased adiposity (44). This is not to say that increased maternal adiposity does not program appetite or energy expenditure later in life—it likely does (46,47). However, our data implicate an important role for the potentiation of adipogenesis in early life as a causal mechanism for the later development of obesity.

Adipocyte precursor cells isolated from fat express high levels of mesenchymal stem cell markers such as Pref-1, Wisp2, extracellular matrix genes, and antiangiogenic factors (48,49). In our studies, we have found that exposure to a Western-style diet in utero and during lactation significantly increases expression of similar genes in the fat tissues of young offspring. Many of these genes maintain the adipocyte precursor cell in a committed but undifferentiated state. Our finding that altered expression of these genes persists in the fat tissues of older offspring of Western diet-fed dams suggests that the adipocyte precur-

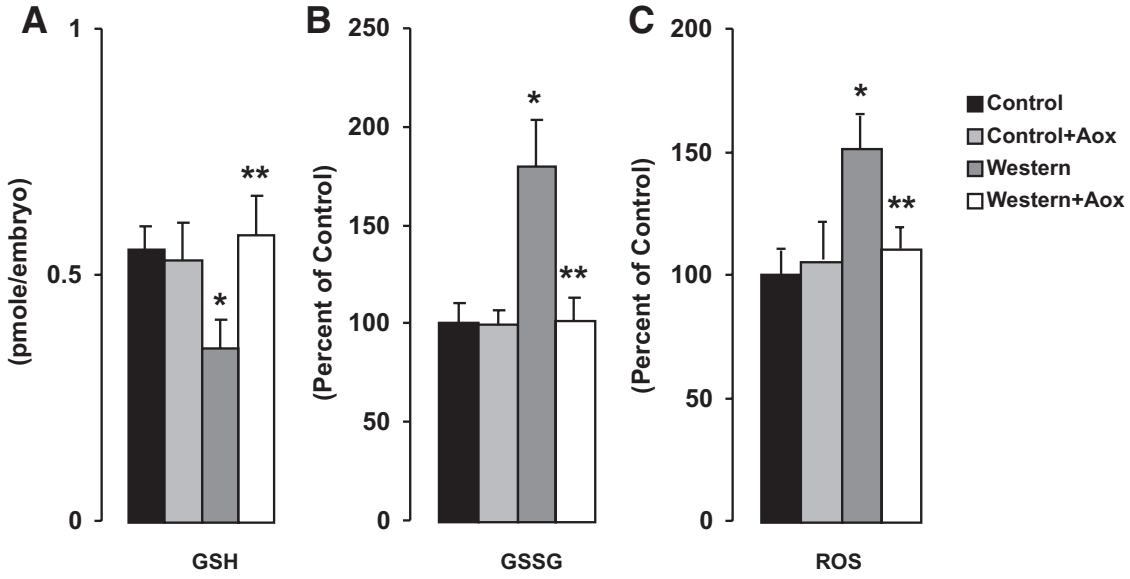

FIG. 4. Altered redox state in preimplantation embryos of Western diet-fed rats. Preimplantation embryos were harvested from pregnant rats, and GSH (*A*), GSSG (*B*), and ROS (*C*) levels were measured as described in METHODS. Data shown are ± SEM, $n = 3$ litters for each group, *$P < 0.05$ Western diet versus control, Aox+control, and Western+Aox; **$P < 0.05$ versus Western+Aox versus Western diet.

sor pool continues to expand albeit at a much slower rate, which may be one explanation for the progressive increase in fat mass in the offspring.

Our results suggest that the mechanisms underlying enhanced adipogenesis in the offspring are related to oxidative stress. Exposure to increased levels of reactive oxygen species has been shown to facilitate adipocyte differentiation in vitro (50). Our data suggest that oxidative stress enhances adipocyte differentiation in vivo in addition to increasing the adipocyte precursor pool.

Although it is well established that obesity is associated with increased oxidative stress, it is also possible that exposure of the pregnant dam to a high-fat diet per se (independent of obesity) results in oxidative stress in the offspring. Further, it is also possible that increased levels of free fatty acid independent of obesity could

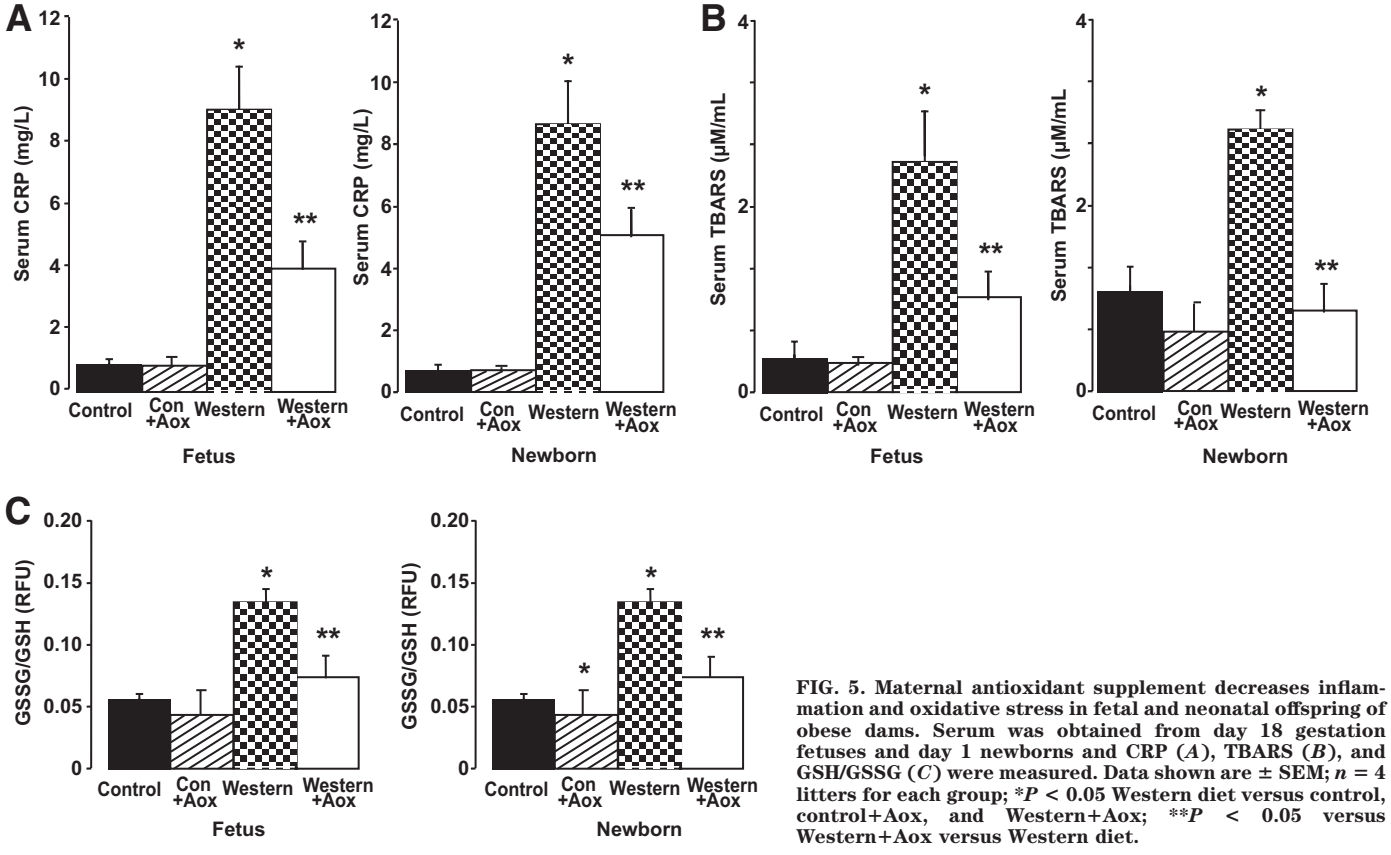

FIG. 5. Maternal antioxidant supplement decreases inflammation and oxidative stress in fetal and neonatal offspring of obese dams. Serum was obtained from day 18 gestation fetuses and day 1 newborns and CRP (*A*), TBARS (*B*), and GSH/GSSG (*C*) were measured. Data shown are ± SEM; $n = 4$ litters for each group; *$P < 0.05$ Western diet versus control, control+Aox, and Western+Aox; **$P < 0.05$ versus Western+Aox versus Western diet.

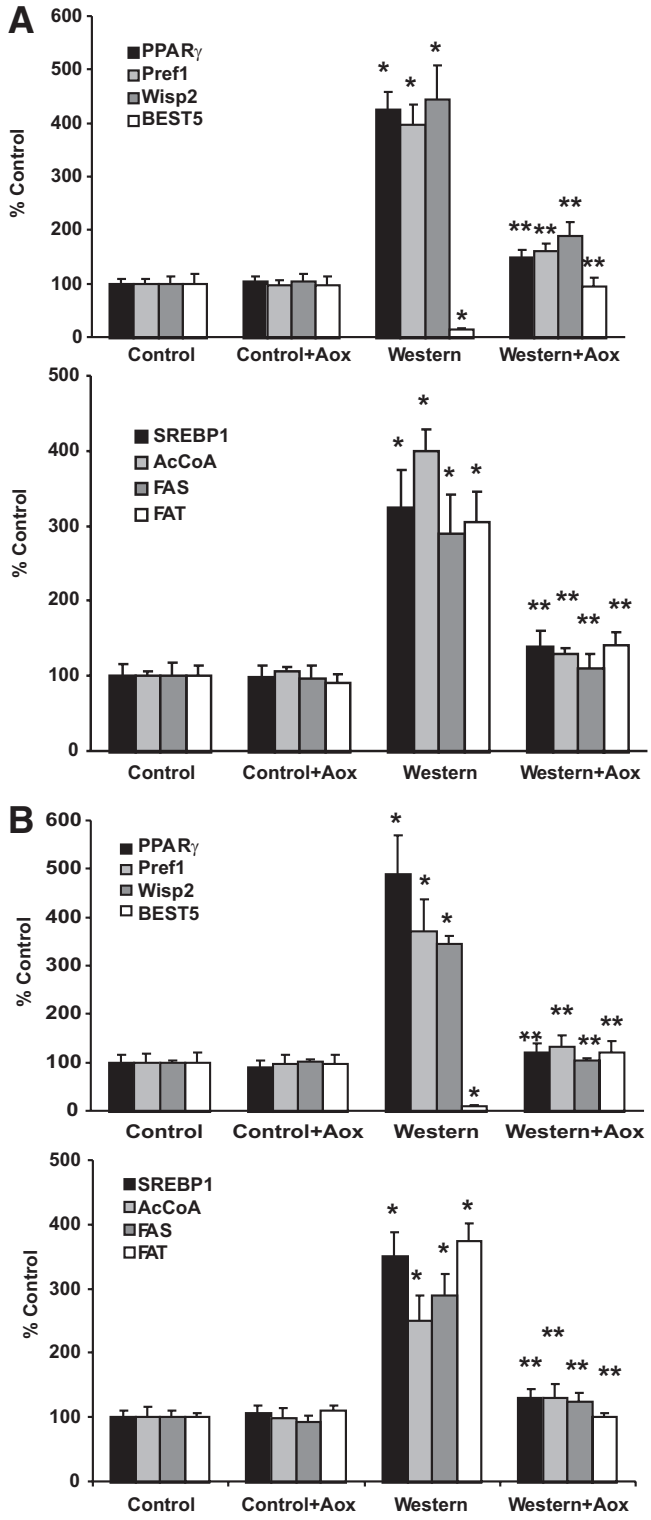

FIG. 6. Maternal antioxidant supplement normalizes gene expression in fat tissue from offspring of obese dams. Visceral fat tissue was harvested from 2-week-old (*A*) and 2-month-old (*B*) offspring and mRNA isolated as described in METHODS. Data shown are ± SEM; $n = 4$ litters for each group; *$P < 0.05$ Western diet versus control, control+Aox, and Western+Aox; **$P < 0.05$ versus Western+Aox versus Western diet.

result in changes in gene expression in the offspring. It is of note that the antioxidant supplement decreased free fatty acid levels in the Western diet-fed dams.

As obesity begins to affect large numbers of women of reproductive age, the role of the adipocyte as a metabolically active participant in fetal programming has come to the forefront. Although it is known that obesity is associated with inflammation, this study suggests that inflammation plays a role in intergenerational obesity and implicates oxidative stress as a central factor in fetal programming of obesity in the offspring.

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S.S. researched data and contributed to writing of the manuscript. R.A.S. contributed to writing of the manuscript.

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