Pregnant Diabetic Rats Fed the Antioxidant Butylated Hydroxytoluene Show Decreased Occurrence of Malformations in Offspring

Ulf J. Eriksson and C. Martin Simán

The increased incidence of congenital malformations in diabetic pregnancy may be associated with an excess of free oxygen radicals in the embryo. We have previously blocked the dysmorphogenesis of rat embryos exposed to high glucose and β-hydroxybutyrate concentrations in vitro by increasing the antioxidant capacity of the conceptus. In the present study, we attempted to diminish the teratogenic process in vivo in a rat model of diabetic pregnancy. Thus, pregnant diabetic and normal rats were fed either a standard diet or a diet enriched with 1% of the antioxidant butylated hydroxytoluene (BHT). The fetuses of the diabetic rats were smaller than the fetuses of the normal rats (body weight 2.70 g vs. 3.68 g) when the mothers were fed a standard diet. The BHT diet increased the fetal weight in the offspring of diabetic rats (3.17 g), with no change in fetuses of the normal rats (3.65 g). The placentas of diabetic rats were heavier than the placentas of normal rats; this difference was not present in the BHT-fed rats. The BHT treatment had no effect on the rate of resorptions, which was increased in the diabetic rats compared with the normal rats. In contrast, the increased rate of congenital malformations in the offspring of diabetic rats (19%), compared with that in the normal rats (0%), was markedly decreased by the BHT diet (2.3%). No malformations were found in the normal rats treated with BHT. These data support the notion that an excess of free oxygen radicals in the embryo contributes to the teratogenic process of diabetic pregnancy and, thus, suggest an area for future preventive therapeutic treatment. *Diabetes* 45:1497–1502, 1996

From the Department of Medical Cell Biology, University of Uppsala, Uppsala, Sweden.

Address correspondence and reprint requests to Dr. Ulf J. Eriksson, Department of Medical Cell Biology, University of Uppsala, Biomedicum, P.O. Box 571, S-751 23 Uppsala, Sweden. E-mail: ulf.eriksson@medcellbiol.uu.se.

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ANOVA, analysis of variance; BHT, butylated hydroxytoluene; HPLC, high-pressure liquid chromotography; MD, manifestly diabetic rats fed a control diet; MDB, manifestly diabetic rats fed a BHT-supplemented diet; MDE, manifestly diabetic rats fed an ether-treated diet; N, normal rats fed a control diet; NB, normal rats fed a BHT-supplemented diet; NE, normal rats fed an ether-treated diet.

Maternal diabetes during early pregnancy is associated with a considerable risk for malformations in the offspring. Good metabolic control of the maternal disease is considered to diminish the risk of fetal dysmorphogenesis markedly. However, despite current intensive metabolic care of the pregnant diabetic mother, the malformation rate of the offspring of diabetic mothers is still twice that of the normal population (1–5). The mechanisms for diabetes-induced embryonic maldevelopment remain largely unknown, although a number of hypotheses have been suggested during the last decade based on clinical and experimental findings (6,7).

Several experimental studies have attempted to identify causative teratological mechanisms in diabetic pregnancy. Investigations using the whole embryo culture system have shown that the addition of the scavenging enzymes superoxide dismutase, catalase, or glutathione peroxidase can normalize the development of embryos exposed to a high glucose milieu (8). These results indicate that free oxygen radicals are involved in the teratogenic process. In subsequent studies, the mitochondria have been suggested as a possible source of the oxygen radicals (9,10). Alternatively, a decreased cellular antioxidative status may constitute the basis for a relative excess of free oxygen radicals in the embryo exposed to a diabetic environment (11).

The compound butylated hydroxytoluene (BHT) is an antioxidant that is commonly used as a preservative in lipid-containing products, such as stains and cosmetics, and as an additive to various foods. It has been demonstrated to have several positive therapeutic effects. BHT administration to animals has been shown to prevent sugar-induced cataracts (12,13), to block cholesterol-induced atherosclerosis (14), and to diminish tumorigenesis in rats that have been exposed to carcinogenic compounds (15–17). Based on these findings, we aimed to study whether BHT administration to pregnant diabetic animals could have a protective effect on the development of their offspring. In the present study, BHT was fed to normal and diabetic rats by adding it to pelleted food at a concentration of 1%, and the offspring were compared with the offspring from normal and diabetic rats that were fed a control diet. After the interruption of gestation on day 20 of pregnancy, the effects on the mother and offspring were analyzed by determining the maternal

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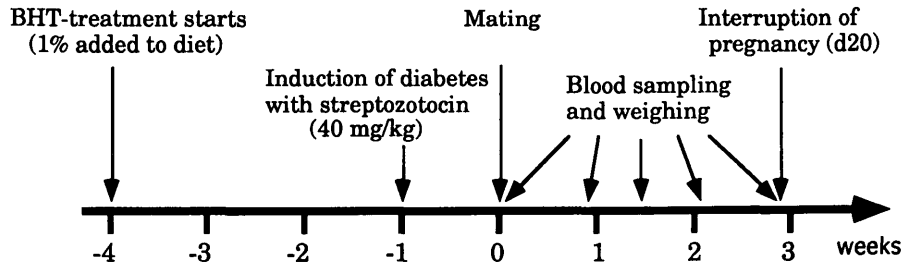

FIG. 1. Schematic outline of the experimental design. Note that the treatment with a BHT-supplemented diet began 3 weeks before inducing diabetes with streptozotocin and that mating took place at least 1 week after the injection of the diabetogenic drug, thereby ensuring that a manifestly diabetic state was present at the time of conception.

liver weight and the concentration of BHT and α-tocopherol and by evaluating the fetal outcome.

RESEARCH DESIGN AND METHODS

Animals. Virgin female Sprague-Dawley rats of a local malformation-prone strain were used (18). They were subjected to a 12-h light/12-h dark schedule and had free access to tap water and laboratory diet (see below). Half of the rats were made manifestly diabetic with an intravenous injection of 40 mg/kg streptozotocin 1–2 weeks before mating with nondiabetic males of the same strain. The remaining female rats served as normal nondiabetic controls and were not given any injection. The presence of a manifestly diabetic state in a rat was defined as a serum glucose concentration >20 mmol/l (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA) 1 week after the streptozotocin injection (Fig. 1).

Animal diets. Three different types of diets were used, all based on a commercial pelleted rat diet (R 36, Lactamin AB, Stockholm, Sweden). The diet treatment started 4 weeks before mating and continued throughout pregnancy (Fig. 1). Two groups of female rats, one normal group and one manifestly diabetic group, were given regular pellets; these rats were denoted N and MD, respectively. Two groups of female rats, one normal and one diabetic, were given pelleted food that had been enriched with 1% butylated hydroxytoluene (BHT; 2,6-di-tert-butyl-4-methylphenol; Merck, Schuchardt, Munich, Germany); they were denoted NB and MDB, respectively. The 1% BHT enrichment was performed by dissolving 7 g crystallized BHT in ~70 ml ether (Merck, Darmstadt, Germany) and soaking 700 g R 36 in the ether solution until total evaporation of the ether solution had occurred (about 12 h). Two additional groups of female normal and diabetic rats were used. These animals were given R 36 rat diet that had been soaked in ether without BHT, in accordance with the procedure outlined above; they were denoted NE and MDE, respectively.

Induction and course of pregnancy. The rats were mated overnight, and the morning when sperm were found in the vaginal smear was denoted gestational day 0. Throughout pregnancy (i.e., on gestational days 0, 6, 10, 16, and 20), the animals were weighed and had their serum glucose levels determined.

Evaluation of the outcome of pregnancy. At a gestational age of 20 days, the pregnant rats were killed by cervical dislocation, the fetuses and placentas were quickly dissected out, and the viable fetuses were weighed and examined for gross malformations, as previously outlined (19). Briefly, the nonviable fetuses were termed "resorbed," regardless of their size and state of development. The viable fetuses showing an abnormally short mandible (the majority of the malformations), the absence of tail, or other gross defects (e.g., omphalocele and cranial malformation) were denoted "malformed." The maternal livers were also dissected out, weighed, and stored at −70°C after rapid freezing in liquid nitrogen.

Analysis of BHT. The concentration of BHT in the maternal liver samples was analyzed with the aid of a high-pressure liquid chromatography (HPLC) system, essentially as described by Terao et al. (20). Briefly, 1 g of the frozen liver samples was homogenized in 6 ml methanol with a Sorvall Omnimixer for 2 min, and 3 ml of this homogenate was incubated with 3 ml chloroform for 1 h in a preweighed glass tube. The remaining homogenate was saved for the estimation of α-tocopherol (see below). Thereafter, 2 ml of 1.5 mmol/l $CaCl_2$ was added, and the sample was centrifuged for 10 min. The chloroform phase was carefully dried with N_2, and the total lipid content was weighed using a Mettler AE 163 balance. The extracted lipids were dissolved in acetone, incubated for 1 h on ice, and centrifuged for 3 min. The supernatant was transferred to a new tube, dried with N_2, and finally dissolved in 0.5 ml acetonitrile, thereby yielding a sample ready for injection in the HPLC. Reverse-phase HPLC was then performed with a Shimadzu LC-10 AD liquid chromatography system, using a Spherisorb C^{18} ODS (4.6 × 250 mm) 5-μm column and a 5-μm C^{18} ODS guard column (4.6 × 10 mm; Phase Separation Ltd., U.K.). The effluent was monitored with a Shimadzu SPD-10A spectrophotometer at wavelength of 280 nm. The sample was injected through a 100-μl loop injector. The column was eluted with a 10-min linear gradient of acetonitrile-water (80/20 vol/vol) to 100% acetonitrile at a solvent flow rate of 1 ml/min. After 10 min, the flow rate was held constant for 2 min followed by acetonitrile-water (80:20 vol/vol) for 12 min. BHT eluted at approximately 9.4 min.

Analysis of α-tocopherol. To estimate the content of α-tocopherol, 200 μl of the liver homogenate in methanol was initially diluted with an aliquot of 300 μl methanol. The sample was manually shaken for 3 min with hexane in a Teflon stopped glass tube. After centrifugation, 100 μl of the hexane phase was analyzed by normal-phase HPLC by injection into a 5-μm Spherisorb amino column (4.6 × 250 mm) with a flow rate of 1 ml/min. The eluent was isocratic isooctane/tert-butyl-methyl-ether/methanol (75/25/0.5 vol/vol/vol). The effluent was monitored with a Shimadzu RF-10A fluorometer at an excitation wavelength of 295 nm and an emission of 327 nm. α-tocopherol eluted at approximately 4.5 min.

Statistical analysis. Significant differences between and within groups were estimated by analysis of variance (ANOVA) (21). The applied test was Fischer protected least square difference (PLSD) ad modum Winer (22) at the 95% significance level and with nonparametric one-way analysis of variance (ANOVA) for proportions and number of resorptions and malformations. The statistical analyses were made with the program StatView (version 4.02) or SAS (version 6.10) for Macintosh.

RESULTS

Induction and course of pregnancy. All groups gained weight during pregnancy. The normal groups (N, NB, and NE) showed greater body weight increase than the manifestly diabetic groups (MD, MDB, and MDE) (Fig. 2). The NB rats increased less than the N and the NE rats, whereas all the diabetic rats (MD, MDB, and MDE) showed a similar growth pattern with only a marginal weight increase during pregnancy, which gave the result that all the normal rats had higher body weight than all the diabetic animals on gestational day 20 ($P < 0.05$, determined by ANOVA; Fig. 2). The serum glucose concentrations decreased marginally in the normal rats between day 0 and day 20 (significantly in the normal control-diet group, whereas the NB and the NE rats showed similar trends, bordering on significance), all of which had lower concentrations than the diabetic rats at all time points (Fig. 3). In contrast to the normal rats, the serum glucose levels of the diabetic rats increased (MD) or remained the same (MDB and MDE) between gestational day 0 and 20 (Fig. 3).

Maternal status at termination of pregnancy. The livers of the pregnant rats in the diabetic groups tended to be heavier on gestational day 20 than the livers of the corresponding pregnant nondiabetic rats (Table 1, first column). The BHT-supplemented diet yielded increased liver weight in both the NB and MDB rats, compared with that of the N and MD rats (Table 1). This increase in liver weight was also apparent when it was expressed as a percentage of the total body weight (Table 1, second col-

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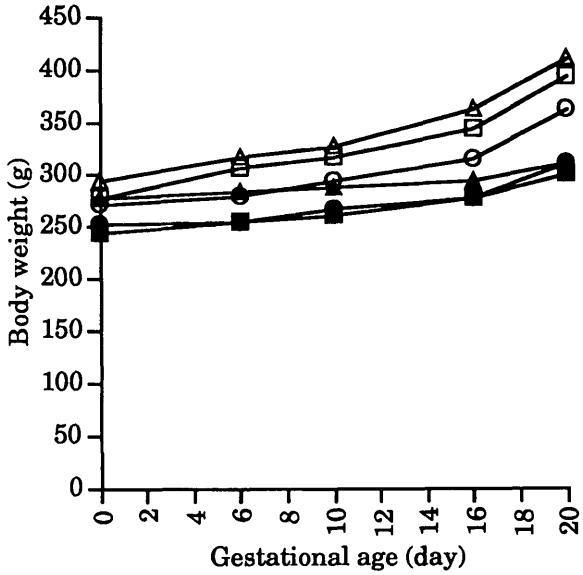

FIG. 2. Maternal body weights in the different groups during pregnancy. The pregnant rats were either normal (N) or manifestly diabetic (MD), some of which were given BHT- (NB and MDB) or ether-treated (NE and MDE) pellets. —□—, N; —○—, NB; —△—, NE; —■—, MD; —●—, MDB; —▲—, MDE.

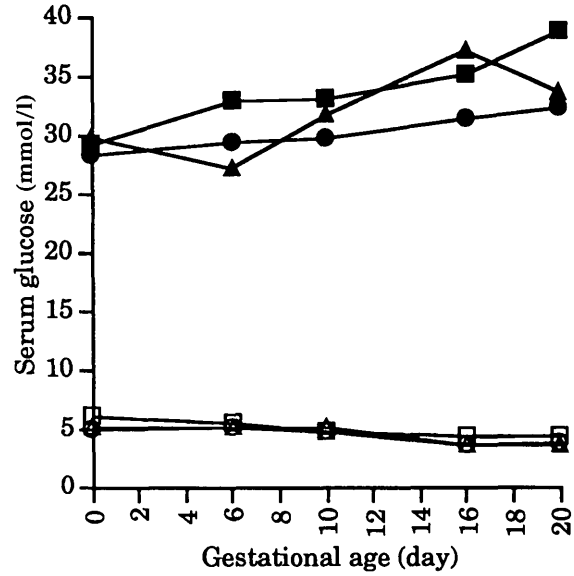

FIG. 3. Maternal serum glucose concentrations in the different groups during pregnancy. The pregnant rats were either normal (N) or manifestly diabetic (MD), some of which were given BHT- (NB and MDB) or ether-treated (NE and MDE) pellets. —□—, N; —○—, NB; —△—, NE; —■—, MD; —●—, MDB; —▲—, MDE.

umn). In contrast, the increased liver weight in MDE rats (MDE compared with MD) did not remain when the liver weight was expressed as a percentage of the body weight in the different groups. Measurement of total lipid concentration in the livers showed no difference within the groups of normal and diabetic rats with the exception of the MDB rats, which had higher lipid content than the MD rats (Table 1, column 3).

Measurement of BHT in the livers of the NB and MDB rats confirmed that the rats had accumulated BHT from the diet. The livers of the diabetic rats contained three times more BHT per lipids than the livers of the normal rats given the same BHT-enriched diet (Table 1, column 4).

The α-tocopherol concentrations in the livers of the MD and MDE rats showed a tendency to be numerically higher than those in the N and NE rats, respectively, but no statistical significances emerged. BHT supplemented diet, on the other hand, reduced the α-tocopherol concentration in the liver lipid fraction by ~70% in both the NB and MDB group (Table 1, column 5).

Fetal outcome. The fetal and placental weights were similar in all the normal fetuses (N, NB, and NE). The body weight was generally decreased in the fetuses of diabetic rats compared with fetuses of normal animals. The weight of the offspring of diabetic animals on BHT-supplemented diet was increased compared with the weight of the fetuses of untreated diabetic rats, although not completely normalized (Table 2). The MD placentas were heavier than the N placentas, whereas both the MDB and MDE placenta did not differ from the corresponding normal placentas (Table 2).

The implantation rate varied between the groups. A trend toward increased number of implantations was found in the ether-treated groups (NE and MDE, Table 3). The rate of resorptions was increased in the offspring of the diabetic rats, compared with the offspring of the normal rats, and BHT had no effect within the two groups (Table 3). (The N, NB, and NE rats showed similar rates of resorptions, as did the MD, MDB, and MDE rats.) The rate of malformations was significantly increased in the

TABLE 1
Weight and lipid concentrations of maternal liver on gestational day 20 in normal and manifestly diabetic rats with a control diet (N and MD), a BHT-supplemented diet (NB and MDB), or an ether-treated diet (NE and MDE)

Group	*n*	Liver weight (g)	Liver/body ratio (%)	Total lipid (mg/g)	BHT (μg/g lipid)	α-tocopherol (mg/g lipid)
N	7	11.8 ± 0.4	3.1 ± 0.1	41 ± 3	—	1.07 ± 0.08
NB	10	17.0 ± 0.7*†	4.6 ± 0.2*	41 ± 2	21 ± 7	0.32 ± 0.03*†
NE	4	13.4 ± 0.6	3.3 ± 0.2†‡	44 ± 3	—	0.83 ± 0.02†
MD	9	14.4 ± 0.5*	5.1 ± 0.3*	39 ± 2	—	1.29 ± 0.13
MDB	13	18.5 ± 0.5*†	6.4 ± 0.3*†‡	46 ± 2*†‡	61 ± 11‡	0.36 ± 0.05*†
MDE	4	16.5 ± 0.6*†	5.4 ± 0.4*	38 ± 2	—	1.38 ± 0.09*

Data are means ± SE. The concentrations of BHT in the NB and MDB rats and α-tocopherol concentrations in all groups are given. Differences between means were assessed by ANOVA. *$P < 0.05$ vs. the N group; †$P < 0.05$ vs. the MD group; ‡$P < 0.05$ vs. the NB group.

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TABLE 2
Weight of fetuses and placentas at gestational day 20 in normal and manifestly diabetic rats with a control diet (N and MD), a BHT-supplemented diet (NB and MDB), or an ether-treated diet (NE and MDE)

Group	Number of litters	Mean fetal weight (g)	Mean placental weight (g)
N	13	3.68 ± 0.05	0.57 ± 0.03
NB	17	3.65 ± 0.06	0.61 ± 0.03
NE	6	3.52 ± 0.11	0.61 ± 0.04
MD	14	2.70 ± 0.06*‡	0.69 ± 0.03*‡
MDB	19	3.17 ± 0.10*†‡	0.63 ± 0.02
MDE	4	2.49 ± 0.11*†‡	0.59 ± 0.03

Data are means ± SE. Differences between means were assessed by ANOVA. *$P < 0.05$ vs. the N group; †$P < 0.05$ vs. the MD group; ‡$P < 0.05$ vs. the NB group.

offspring of the diabetic rats compared with the offspring of the normal animals (Table 3). The addition of BHT to the diet decreased the malformation rate to normal levels in the MDB group. No malformations were found in the normal rats treated with BHT (Table 3).

DISCUSSION

Supplementing the diet of pregnant diabetic rats with the antioxidant BHT markedly diminished the occurrence of skeletal malformations in the offspring and, in addition, restored fetal body weight toward normal levels. The administration of an antiteratogenic agent to diabetic pregnant animals has been performed previously in a few studies. The most prominent such agent, insulin, has been given to diabetic pregnant animals and improved fetal outcome (19,23,24). Among other (noninsulin) agents previously tested, the addition of arachidonic acid distinguishes itself as the treatment with the most pronounced alleviation of embryonic dysmorphogenesis in experimental diabetic pregnancy (25). Recent reports have also suggested that vitamin E treatment may be beneficial to the embryonic development in diabetic pregnancy (26,27).

The in vivo finding that treatment with BHT normalizes fetal development and morphogenesis in the offspring of diabetic rats is in agreement with the results of previous in vitro studies with cultured embryos showing that exogenously administered antioxidants can protect from glucose- and ketone-induced malformations (9,10,11,28). Also, transgenic mice with an increased activity of the radical-scavenging enzyme superoxide dismutase are partly protected against the teratogenic effects of a diabetic environment both in vitro (29) and in vivo (30). Altogether, these results indicate that there is a basic component in the teratogenic process that can be altered by free oxygen scavengers and that this process is present in in vitro and in vivo models of diabetic pregnancy.

Treatment with a BHT-enriched diet did not alter the rate of resorptions in the offspring of the diabetic animals, although it did decrease the rate of congenital malformations. This suggests that the diabetes-induced cellular processes behind the resorptions and the skeletal malformations are distinctly different. In addition, the exact antiteratogenic mechanism of the BHT treatment is not known. The possibility that the administration of the compound normalized the maternal diabetic state does not gain support, because the diabetic rats showed similar serum glucose concentrations and similar weight gain during pregnancy, regardless of whether they were on the BHT diet. Thus, it appears likely that the major effect of BHT was exerted in the conceptus.

The susceptible period for the induction of congenital malformation due to diabetes has been suggested to be present early in pregnancy. In humans, the induction probably occurs before the 7th week (31), and in rats the susceptible period has been identified from gestational day 6 to day 10 (32,33). During this period of early organogenesis, the mandible is formed in the rat embryo. In the rat strain used in the present study, the underdevelopment of the mandible, micrognathia, has been shown to be a diabetes-specific malformation, because it only occurs in the offspring of diabetic mothers (18). In the formation of the mandible, the migration of a specific cell population, the neural crest cells, from the cranial aspects of the neural tube to the first visceral arch is of paramount

TABLE 3
Fetal outcome on gestational day 20 in normal and manifestly diabetic rats with a control diet (N and MD), a BHT-supplemented diet (NB and MDB), or an ether-treated diet (NE and MDE)

	Number of litters	Number of implantations	Number of resorptions	Number of malformations	Resorptions of implantations per litter (%)	Malformations of viable fetuses per litter (%)	Implantations per litter	Resorptions per litter	Malformations per litter
n	13	149	9	0	6 ± 3	0	11.5 ± 0.4	0.7 ± 0.4	0
NB	17	181	16	0	10 ± 3†	0†	10.6 ± 0.6	0.9 ± 0.3†	0†
NE	6	76	11	2	14 ± 8	5.5 ± 5†	12.7 ± 1.4	1.8 ± 1.1	0.3 ± 0.3†
MD	14	136	30	22	23 ± 5*	19 ± 6*	9.7 ± 0.7*	2.1 ± 0.5	1.6 ± 0.5*
MDB	19	199	57	4	28 ± 5*	2.3 ± 1†	10.5 ± 0.4	2.6 ± 0.6*	0.2 ± 0.1†
MDE	4	53	17	9	32 ± 13*	21 ± 9*	13.2 ± 1.3†	4.2 ± 1.7*	2.2 ± 1.0*

Data are means ± SE. Number of implantations, resorptions, and malformations are given as an absolute number, mean proportion per litter, and mean number per litter. Statistical evaluation was performed with a nonparametric one-way ANOVA (SAS). *$P < 0.05$ vs. the N group; †$P < 0.05$ vs. the MD group.

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importance (34). In vitro studies have shown that the migratory capacity of neural crest cells from the rat strain used in this study are decreased by high glucose in the medium. This effect can be reversed by addition of antioxidants to the culture medium (28). In this context, it is of interest to note that neural crest cells may have a low free oxygen radical scavenging capacity (35). In addition, the composition of the extracellular matrix, which assists the neural crest cell migration, is altered in embryos cultured in high glucose (36). Therefore, the neural crest cells and their migration may be the target for the free oxygen radicals when inducing malformations. To normalize embryonic development during this early period of diabetic pregnancy, treatment with antioxidants may be a substantial adjunct to conventional diabetes treatment.

The putative teratogenicity of the antioxidant chosen in the present study, BHT, has been debated (37,38,39). However, it was recently shown in a multigenerational study in mice that BHT does not have any adverse effect on either reproductive or neurobehavioral parameters (40). BHT is regularly added to lipid-containing foodstuff, and several investigations have been searching for possible adverse effects of the compound. Thus, there are reports of high BHT doses promoting carcinogenesis when given in conjunction with a tumorigenic agent (41,42) and to induce acute hepatotoxicity in rats (43). We found increased liver weight in the pregnant rats of the BHT-treated groups a phenomenon also previously observed (44,45) and likely to reflect the hepatotoxic capacity of the compound. Furthermore, α-tocopherol was decreased in the maternal liver of both the BHT-treated groups. These effects might be explained by a bioactivation of BHT to the pro-oxidative compound BHT-quinone-methides, a process which is catalyzed by the cytochrome P-450 enzyme system (46). Additionally, cytochrome P-450 expression is covariant with the location of degeneration in the liver of BHT-treated rats (47). Thus, the decreased hepatic vitamin E concentration in the two BHT groups in this study is probably explained by formation of the BHT metabolite in the maternal liver.

No adverse effect of BHT on fetal development was found with regard to fetal and placental weight, resorption rate, or gross malformation rate in the present study. On the other hand, BHT treatment normalized fetal weight to some extent in the offspring of diabetic rats and showed a strong protective activity against diabetes-induced malformations. Of special note is that the dose used in the present study, 1%, is ~50 times greater than the dose used in human food supplementation (48).

Although all BHT-treated animals were given the same dose of BHT, the diabetic rats accumulated nearly three times more BHT in their livers then the controls. This is probably partly explained by a higher food consumption in the diabetic rats (49) and the lipophilic property of BHT. During the prediabetic period, most of the dietary BHT is likely to accumulate in adipose tissue (20). When the rats were made diabetic with streptozotocin, the fat stores were consumed and the BHT was subsequently released back to the circulation and redistributed in the animal and a large proportion was accumulated in the liver (45).

In conclusion, the results from this study support the view that an imbalance of free oxygen radicals is involved in the dysmorphogenesis of diabetic pregnancy. The results also suggest that maternal antioxidant therapy may protect the development of the offspring, although the large doses needed for the protective effect defines new areas for future research.

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REFERENCES

1. Kucera J: Rate and type of congenital anomalies among offspring of diabetic women. *J Reprod Med* 7:61–70, 1971
2. Mills JL: Malformations in infants of diabetic mothers. *Teratology* 25:385–394, 1982
3. Mølsted-Pedersen L, Kühl C: Obstetrical management in diabetic pregnancy: the Copenhagen experience. *Diabetologia* 29:13–16, 1986
4. Hanson U, Persson B, Thunell S: Relationship between haemoglobin A1c in early type 1 (insulin-dependent) diabetic pregnancy and the occurrence of spontaneous abortion and fetal malformation in Sweden. *Diabetologia* 33:100–104, 1990
5. Kitzmiller JL, Gavin LA, Gin GD, Jovanovic-Peterson L, Main EK, Zigrang WD: Preconception care of diabetes: glycemic control prevents congenital anomalies. *JAMA* 265:731–736, 1991
6. Baker L, Piddington R: Diabetic embryopathy: a selective review of recent trends. *J Diabetic Complications* 7:204–212, 1993
7. Eriksson UJ, Borg LAH, Forsberg H, Simán CM, Suzuki N, Yang X: Can fetal loss be prevented? The biochemical basis of diabetic embryopathy. *Diabetes Rev* 4:49–69, 1996
8. Eriksson UJ, Borg LAH: Protection by free oxygen radical scavenging enzymes against glucose-induced embryonic malformations in vitro. *Diabetologia* 34:325–331, 1991
9. Eriksson UJ, Borg LAH: Diabetes and embryonic malformations: role of substrate-induced free-oxygen radical production for dysmorphogenesis in cultured rat embryos. *Diabetes* 42:411–419, 1993
10. Yang X, Borg LAH, Eriksson UJ: Altered mitochondrial morphology of rat embryos in diabetic pregnancy. *Anat Rec* 241:255–267, 1995
11. Trocino RA, Akazawa S, Ishibashi M, Matsumoto K, Matsuo H, Yamamoto H, Goto S, Urata Y, Kondo T, Nagataki S: Significance of glutathione depletion and oxidative stress in early embryogenesis in glucose-induced rat embryo culture. *Diabetes* 44:992–998, 1995
12. Linklater HA, Dzialoszynski T, McLeod HL, Sanford SE Jr: Modelling cortical cataract VIII: effects of butylated hydroxytoluene (BHT) in reducing protein leakage from lenses in diabetic rats. *Exp Eye Res* 43:305–313, 1986
13. Ansari NH, Srivastava SK: Allopurinol promotes and butylated hydroxy toluene prevents sugar-induced cataractogenesis. *Biochem Biophys Res Commun* 168:939–943, 1990
14. Björkhem I, Henriksson-Freyschuss A, Breuer O, Diczfalusy U, Berglund L, Henriksson P: The antioxidant butylated hydroxytoluene protects against atherosclerosis. *Arterioscler Thromb* 11:15–22, 1991
15. Tatsua M, Mikuni T, Taniguchi H: Protective effect of butylated hydroxytoluene against induction of gastric cancer by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in Wistar rats. *Int J Cancer* 32:253–254, 1983
16. Williams GM, Tanaka T, Maeura Y: Dose-related inhibition of aflatoxin B1 induced hepatocarcinogenesis by the phenolic antioxidants, butylated hydroxyanisole and butylated hydroxytoluene. *Carcinogenesis* 7:1043–1050, 1986

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17. McCormick DL, May CM, Thomas CF, Detrisac CJ: Anti carcinogenic and hepatotoxic interactions between retinyl acetate and butylated hydroxytoluene in rats. *Cancer Res* 46:5264–5269, 1986
18. Eriksson UJ: Importance of genetic predisposition and maternal environment for the occurrence of congenital malformations in offspring of diabetic rats. *Teratology* 37:365–374, 1988
19. Eriksson UJ, Dahlström E, Larsson KS, Hellerström C: Increased incidence of congenital malformations in the offspring of diabetic rats and their prevention by maternal insulin therapy. *Diabetes* 31:1–6, 1982
20. Terao J, Magarian RA, Brueggemann G, King MM: Methods of extraction and high-performance liquid chromatographic analysis of butylated hydroxytoluene from the tissues and serum of rats. *Anal Biochem* 151:445–454, 1985
21. Ostle B: *Statistics in Research.* 2nd ed. Ames, IA, Iowa State University Press, 1963
22. Winer BJ: *Statistical Principles in Experimental Design.* New York, McGraw-Hill, 1971
23. Horii K, Watanabe G, Ingalls TH: Experimental diabetes in pregnant mice: prevention of congenital malformations in offspring by insulin. *Diabetes* 15:194–204, 1966
24. Baker L, Egler JM, Klein SM, Goldman AS: Meticulous control of diabetes during organogenesis prevents congenital lumbosacral defects in rats. *Diabetes* 30:955–959, 1981
25. Goldman AS, Baker L, Piddington R, Marx B, Herold R, Egler J: Hyperglycemia-induced teratogenesis is mediated by a functional deficiency of arachidonic acid. *Proc Natl Acad Sci USA* 82:8227–8231, 1985
26. Viana M, Herrera E, Bonet B: Teratogenic effects of diabetes mellitus in the rat. Prevention with vitamin E. *Diabetologia* 39:1047–1054, 1996
27. Simán CM, Borg LAH, Eriksson UJ: Vitamin E treatment blocks dysmorphogenesis in embryos of diabetic rats. *Diabetologia* 38 (Suppl. 1):A22, 1995
28. Suzuki N, Svensson K, Eriksson UJ: Effects of increased glucose concentration and antioxidants on rat cranial neural crest cell development in vitro. *Diabetologia* 39:401–411, 1996
29. Eriksson UJ, Borg LAH, Hagay Z, Groner Y: Increased superoxide dismutase (SOD) activity in embryos of transgenic mice protects from the teratogenic effects of a diabetic environment. *Diabetes* 42 (Suppl. 1):85A, 1993
30. Hagay ZJ, Weiss Y, Zusman I, Peled-Kamar M, Eriksson UJ, Groner Y: Prevention of hyperglycemia-associated embryopathy by embryonic overexpression of the free radical scavenger copper zinc superoxide dismutase gene. *Am J Obstet Gynecol* 173:1036–1041, 1995
31. Mills J, Baker L, Goldman AS: Malformations in infants of diabetic mothers occur before the seventh gestational week. *Diabetes* 28:292–293, 1979
32. Eriksson UJ, Bone AJ, Turnbull DM, Baird JD: Timed interruption of insulin therapy in diabetic BB/E rat pregnancy: effects on maternal metabolism and fetal outcome. *Acta Endocrinol* (Copenhagen) 120:800–810, 1989
33. Eriksson RSM, Thunberg L, Eriksson UJ: Effects of interrupted insulin treatment on fetal outcome of pregnant diabetic rats. *Diabetes* 38:764–772, 1989
34. Thorogood P, Ferretti P: Heads and tales: recent advances in craniofacial development. *Br Dent J* 173:301–306, 1992
35. Davis WL, Crawford LA, Cooper OJ, Farmer GR, Thomas DL, Freeman BL: Ethanol induces the generation of free radicals by neural crest cells in vitro. *J Craniofacial Genet Dev Biol* 10:277–293, 1990
36. Cagliero E, Forsberg H, Sala R, Lorenzi M, Eriksson UJ: Maternal diabetes induces increased expression of extracellular matrix components in rat embryos. *Diabetes* 42:975–980, 1993
37. Frawley JP, Kohn FE, Kay JH, Calandra JC: Progress report on multigeneration reproduction studies in rats fed butylated hydroxytoluene (BHT). *Food Cosmet Toxicol* 3:377–386, 1965
38. Stokes JD, Scudder CJ: The effect of butylated hydroxyanisole and butylated hydroxytoluene on behavioral development of mice. *Dev Psychobiol* 7:343–350, 1974
39. Vorhees CV, Butcher RE, Brunner RL, Sobotka TJ: Developmental neurobehavioural toxicity of butylated hydroxytoluene in rats. *Food Cosmet Toxicol* 19:153–162, 1981
40. Tanaka T, Oishi S, Takahashi O: Three generation toxicity study of butylated hydroxytoluene administered to mice. *Toxicol Lett* 66:295–304, 1993
41. Witschi H, Williamson D, Lock S: Enhancement of urethane tumorigenesis in mouse lung by butylated hydroxytoluene. *J Natl Cancer Inst* 58:301–305, 1977
42. Malkinson AM, Beer DS: Pharmacological and genetic studies on the modulatory effects of butylated hydroxytoluene on mouse lung adenoma formation. *J Natl Cancer Inst* 73:925–933, 1984
43. Bolton JL, Valerio LG Jr, Thompson JA: The enzymatic formation and chemical reactivity of quinone methides correlate with alkylphenol-induced toxicity in rat hepatocytes. *Chem Res Toxicol* 5:816–822, 1992
44. Brown WD, Johnson AR, O'Halloran MW: The effect of the level of dietary fat on the toxicity of phenolic antioxidants. *Aust J Exp Biol* 37:533–548, 1959
45. Simán CM, Eriksson UJ: Effect of butylated hydroxytoluene on α-tocopherol in liver and fat tissue of rats. *Toxicol Lett* 87:103–108, 1996
46. Bolton JL, Thompson JA: Oxidation of butylated hydroxytoluene to toxic metabolites: factors influencing hydroxylation and quinone methide formation by hepatic and pulmonary microsomes. *Drug Metab Disposition* 19:467–472, 1991
47. Powell CJ, Connolly AK: The site specificity and sensitivity of the rat liver to butylated hydroxytoluene-induced damage. *Toxicol Appl Pharmacol* 108:67–77, 1991
48. Flyvholm MA, Menné T: Sensitizing risk of butylated hydroxytoluene based on exposure and effect data. *Contact Dermatitis* 23:341–345, 1990
49. Styrud J, Thunberg L, Nybacka O, Eriksson UJ: Correlations between maternal metabolism and deranged development in the offspring of normal and diabetic rats. *Pediatr Res* 37:343–353, 1995

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