

Modulation of Adipokines and Cytokines in Gestational Diabetes and Macrosomia

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Context/Objective: Not much is known about the implication of adipokines and different cytokines in gestational diabetes mellitus (GDM) and macrosomia. The purpose of this study was to assess the profile of these hormones and cytokines in macrosomic babies, born to gestational diabetic women.

Design/Subjects: A total of 59 women (age, 19–42 yr) suffering from GDM with their macrosomic babies (4.35 ± 0.06 kg) and 60 healthy age-matched pregnant women and their newborns (3.22 ± 0.08 kg) were selected.

Methods: Serum adipokines (adiponectin and leptin) were quantified using an obesity-related multiple ELISA microarray kit. The concentrations of serum cytokines were determined by ELISA.

Results: Serum adiponectin levels were decreased, whereas the concentrations of leptin, inflammatory cytokines, such as IL-6 and TNF- α , were significantly increased in gestational diabetic mothers

compared with control women. The levels of these adipocytokines were diminished in macrosomic babies in comparison with their age-matched control newborns. Serum concentrations of T helper type 1 (Th1) cytokines (IL-2 and interferon- γ) were decreased, whereas IL-10 levels were significantly enhanced in gestational diabetic mothers compared with control women. Macrosomic children exhibited high levels of Th1 cytokines and low levels of IL-10 compared with control infants. Serum IL-4 levels were not altered between gestational diabetic mothers and control mothers or the macrosomic babies and newborn control babies.

Conclusions: GDM is linked to the down-regulation of adiponectin along with Th1 cytokines and up-regulation of leptin and inflammatory cytokines. Macrosomia was associated with the up-regulation of Th1 cytokines and the down-regulation of the obesity-related agents (IL-6 and TNF- α , leptin, and adiponectin). (*J Clin Endocrinol Metab* 91: 4137–4143, 2006)

GESTATIONAL DIABETES mellitus (GDM), defined as a carbohydrate intolerance of varying severity, is the most frequent metabolic disorder of pregnancy, affecting 1–10% of all pregnancies (1). Although most of the women with GDM return to normal glucose tolerance after delivery, they have an increased risk of developing diabetes, mainly type 2 diabetes mellitus (DM), later on, with an incidence ranging from 6–62%, depending on the population examined and the length of the follow-up considered (2). The offspring of women with GDM are prone to adverse side effects such as macrosomia, which is strongly associated with fetal death, prematurity, birth trauma, and respiratory distress syndrome; and equally important, these offspring have a high risk of developing obesity, impaired glucose tolerance, and type 2 diabetes in adulthood (3).

Cytokines, through their ability to interfere with insulin signaling, have been implicated in insulin resistance in type 2 DM (4). Adiponectin, a physiologically active polypeptide hormone derived from adipose tissue, exhibits insulin-sen-

sitizing, antiatherogenic, and antiinflammatory properties (5). Although the effect of adiponectin in insulin sensitivity has been studied, limited data are available on the association between adiponectin and pregnancy-induced insulin resistance (6). Moreover, hypoadiponectinemia is associated with the pathogenesis of GDM and macrosomia (6).

Because adipocytokines may play an important role in the early defects of type 2 diabetes (4), women with GDM represent an ideal population model to study these interrelationships. A recent study of 15 subjects has suggested a role for TNF- α (7) in this pregnancy-induced insulin resistance. Furthermore, another study has found an association between TNF- α and fasting C-peptide levels (8). Dandona *et al.* (9) have proposed that TNF- α may provide a mechanism for mediating insulin resistance. To date, few studies have reported that TNF- α might be elevated in GDM (8, 10). Leptin, another adipocytokine, is also produced by the placenta and involved in weight regulation and lipid metabolism. Contradictory results have been reported on its secretion in GDM. Indeed, Kautzky-Willer *et al.* (11) have observed elevated leptin levels in gestational diabetic women, whereas Simmons and Breier (12) did not find any change. However, Festa *et al.* (13) have found that leptin level was reduced in GDM.

IL-6 can also be involved in the pathogenesis of insulin resistance, type 2 DM, abnormal adiposity, or lipid disorders

First Published Online July 18, 2006

Abbreviations: BMI, Body mass index; DM, diabetes mellitus; GDM, gestational DM; HbA1c, glycosylated hemoglobin; IFN, interferon; Th, T helper.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

(14). The observation that 10–35% of the body's basal circulating IL-6 is derived from adipose tissue has stimulated interest in this cytokine as a possible mediator of metabolic processes. Furthermore, the correlation between circulating IL-6 and adiposity has been shown (14). Moreover, positive correlation has been found between insulin resistance and circulating IL-6 levels (15), which were elevated in the plasma of patients with type 2 DM (16, 17).

Through different experimental models of diabetes, it has been well established that the secretion of cytokines plays an important role in the regulation of tolerance of islet antigens (18). The production of these cytokines during the islet inflammatory response may, in part, explain the ability of CD4⁺ Th (T helper) cells alone to cause β -cell destruction (18). On the basis of production of cytokines, Th cells can be classified into two principal populations, Th1 and Th2. Th1 cells support cell-mediated immunity and as a consequence promote inflammation, cytotoxicity, and delayed-type hypersensitivity; whereas Th2 cells support humoral immunity and down-regulate the inflammatory actions of Th1 cells (19). Th1 cells secrete IL-2, IFN- γ , and TNF- β ; whereas Th2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13. Th1 cytokines, mainly IFN- γ , play a pathogenic role; whereas Th2 cytokines, mainly IL-4 and IL-10, assure regulatory function and thus mediate protection during diabetes (20, 17). Th2 cytokines, especially IL-4, are also involved in allergic responses (19).

Thus, the current study was undertaken to investigate the implication of adipocytokines (adiponectin and leptin) and proinflammatory mediators (TNF- α and IL-6) in gestational diabetic women and their macrosomic newborns. Because T cells play an important role in the onset of gestational diabetes, we quantified the concentrations of principal T cell cytokines in both mothers with GDM and macrosomic infants.

Subjects and Methods

Subjects

A total of 59 gestational diabetic mothers with their macrosomic babies were recruited in the Gynecology Department, Hôpital Universitaire Farhat Hached, Sousse, Tunisia. In GDM, the pathology appeared in the second or third trimester of pregnancy. These women were be-

tween 19 and 42 yr old, and 75% of the diabetic mothers had an episiotomy during delivery (Table 1). They were hyperglycemic and hyperinsulinemic. As control subjects, 60 healthy age-matched pregnant women and their newborn babies were selected.

Newborn babies were immediately weighed after delivery. Babies from diabetic mothers whose birth weight was 2 SD greater than the mean birth weight of the control infants were considered as macrosomic infants and included in the study. The mean birth weight of macrosomic babies in this study was 4.35 ± 0.06 kg, whereas that of control infants was 3.22 ± 0.08 kg with a respective body mass index (BMI) of 33.84 ± 0.65 and 13.38 ± 0.22 kg/m² (Table 1).

Selected control women had no significant history of illness, no pregnancy-related complications, and no risk factor for gestational diabetes. They had normal glucose tolerance tests during the first and third trimesters of pregnancy. An attempt was made to match these women to diabetic subjects, at least regarding maternal age, BMI as determined by the weight and height of patients, parity, gestational age, and mode of delivery. Both diabetic and control mothers were offered regular examinations of their offspring. The characteristics of mothers and newborns are shown in Table 1.

The protocol was approved by the Sousse Farhat Hached Hospital Committee for Research on Human Subjects (Tunisia). Informed written consent was obtained from all of the subjects.

Blood samples

From each patient or control subject, fasting venous blood samples were collected at delivery in tubes containing or not EDTA to obtain plasma and serum, respectively. The cord blood samples of the babies were collected at delivery. Serum or plasma was obtained by centrifugation ($1000 \times g$ for 20 min). Plasma was immediately used for glucose and glycosylated hemoglobin (HbA1c) determinations. Serum was aliquoted and frozen at -80 C for additional determinations of insulin, lipids and adipocytokines, and T cell cytokine concentrations.

Determination of plasma glucose and HbA1c and serum insulin and lipid concentrations

Serum triglycerides, total cholesterol, and free cholesterol concentrations were determined by using enzymatic methods, according to the instructions furnished with the kit (Boehringer, Mannheim, Germany). Plasma fasting glucose was determined by the glucose oxidase method using a glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma HbA1c levels were determined by isolab column chromatography (21). Serum concentrations of insulin were determined by using the Insulin IRMA kit (Ref. IM3210; Immunotech, Beckman Coulter Inc., Fullerton, CA) with a detection limit of $0.5 \mu\text{IU/ml}$. The interassay coefficients of variability were 3.3 and 4%, respectively, for the concentrations 13 and 54 IU/ml.

TABLE 1. Characteristics of mothers and their offspring

	Mothers		Newborns	
	Control	Diabetic	Control	Macrosomic
N	60	59	60	59
Female/male ratio	60/0	59/0	31/29	36/33
Age	19–42 yr	22–42 yr	<1 month	<1 month
Body weight (kg)			3.22 ± 0.08	4.35 ± 0.06^a
BMI (kg/m ²)			13.38 ± 0.22	33.84 ± 0.65^a
HbA1c (%)	4.3 ± 0.20	6.6 ± 0.30^a		
Cranial perimeter (cm)			34.17 ± 0.21	35.87 ± 0.29
Macrosomia history (%)	0	43		
Episiotomy (%)	35	75		
Fasting glucose (mmol/liter)	4.86 ± 0.71	6.87 ± 0.63^a	5.51 ± 0.38	4.99 ± 0.38
Insulinemia ($\mu\text{IU/ml}$)	5.98 ± 1.13	11.41 ± 4.71^a	5.77 ± 0.88	7.78 ± 3.15^a
Triglycerides (mM)	3.07 ± 0.22	3.07 ± 0.25	1.02 ± 0.04	1.30 ± 0.04^a
Total cholesterol (mM)	6.33 ± 0.32	5.37 ± 0.65	0.83 ± 0.21	1.34 ± 0.17^a
Free cholesterol (mM)	12.11 ± 0.34	9.30 ± 0.93^a	6.66 ± 0.30	7.55 ± 0.15^a

Values are means \pm SD. n = 60 control mothers/babies; n = 59 gestational diabetic mothers/macrosomic babies.

^a Significant difference between diabetic mothers or macrosomic newborns and their corresponding controls: $P < 0.01$.

Determination of serum adipocytokines, IL-6, and TNF- α levels

The levels of serum adipocytokines, IL-6, and TNF- α were measured by using an obesity-related multiple ELISA array (Phoenix Pharmaceuticals, Inc., Belmont, CA), according to the manufacturer's instructions. The enzyme-substrate reaction was imaged by a CCD-based microarray scanner capable of quantitatively measuring chemiluminescence. The quantified intensity of the spots was directly proportional to the amount of human adipokines in the standard solution or samples.

Determination of serum cytokine levels

The determination of cytokine concentrations was performed on serum samples that were stored at -80°C . The repeated freeze-thaw cycles were avoided. The cytokines were quantified by ELISA, using eBio-science human Th1/Th2 ELISA Ready-Set-Go kit, according to the manufacturer's instructions.

Statistical analysis

Values are means \pm SD. Statistical analysis of data was carried out using STATISTICA (version 4.1; Stat-Soft, Paris, France). Data were evaluated by ANOVA. Duncan's multiple-range test was employed for the comparison between gestational diabetic patients or macrosomic newborns and their corresponding control subjects. Differences were considered significant when $P < 0.05$.

Results

Blood HbA1c, insulin, and glucose levels

Plasma HbA1c levels were higher in women with GDM than the nondiabetic mothers (Table 1). Gestational diabetic women exhibited higher fasting glycemia and insulinemia compared with healthy pregnant mothers. The macrosomic babies, as well as their age-matched controls, were normoglycemic, but the former were hyperinsulinemic (Table 1).

Serum lipid levels

Triglyceride and total cholesterol did not differ between gestational diabetic and control mothers. Free cholesterol was lower in diabetic women than control mothers. Triglyceride, total cholesterol, and free cholesterol were significantly higher in macrosomic babies compared with control offspring (Table 1).

Serum adipocytokine levels

Serum adiponectin concentration was decreased, whereas leptin, IL-6, and TNF- α levels were significantly increased in gestational diabetic mothers compared with pregnant control women (Figs. 1 and 2). All of these adipocytokine levels were diminished in macrosomic babies in comparison with their age-matched control newborns. TNF- α , leptin, and IL-6 levels in GDM were positively correlated with insulin, fasting glucose concentrations, and lipid parameters. Adiponectin levels in GDM maternal circulation were negatively correlated with insulin and fasting glucose. TNF- α , leptin, adiponectin, and IL-6 levels in macrosomic infants were inversely correlated with insulin and BMI.

Serum T cell cytokine levels

Serum IL-2 and IFN- γ concentrations were diminished in women with GDM compared with control mothers (Fig. 3), whereas these cytokines were increased in their macrosomic

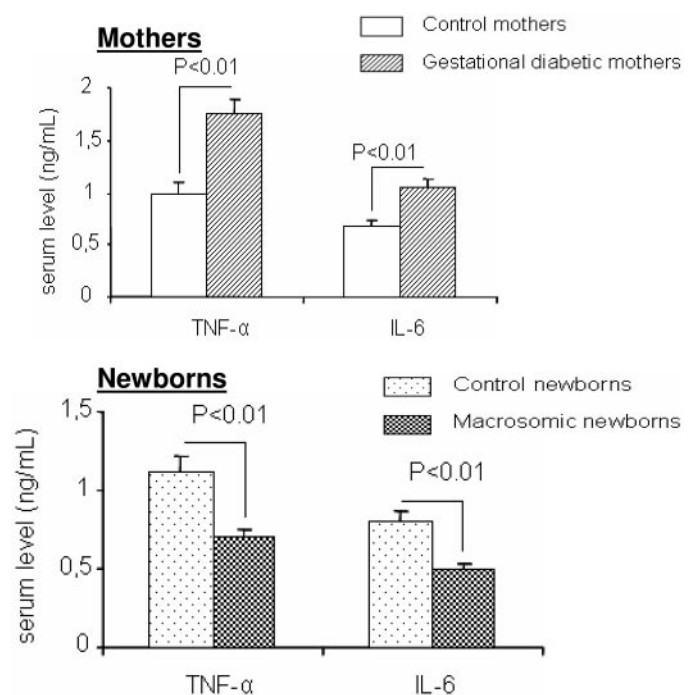


FIG. 1. Serum TNF- α and IL-6 concentrations in gestational diabetic and control women and their newborns. Serum TNF- α and IL-6 concentrations were determined as described in *Subjects and Methods*. Values are means \pm SD; $n = 60$ control mothers/babies; $n = 59$ gestational diabetic mothers/macrosomic babies.

babies (Fig. 4). No difference was observed in serum IL-4 concentrations between control and gestational diabetic mothers and between control and macrosomic newborns. IL-10 concentrations were up-regulated in gestational diabetic mothers (Fig. 3) but down-regulated in macrosomic offspring (Fig. 4). The Th1/Th2 ratio (as measured by IL-2/IL-4, IL-2/IL-10, IFN- γ /IL-4, and IFN- γ /IL-10) demonstrate down-regulation and up-regulation of the Th1 profile, respectively, in gestational diabetic mothers and macrosomic newborns (Table 2).

Discussion

In the present study, the diabetic pregnant women were hyperinsulinemic and hyperglycemic, reflecting a decrease in insulin sensitivity in these individuals, in accordance with several reports (22). Although these subjects with GDM were also normolipidemic, they exhibited high HbA1c levels, indicating a poorly controlled diabetic condition (23). However, their macrosomic infants were only hyperinsulinemic. Indeed, it has been shown that during GDM, the mother's glucose, after its passage via the fetoplacental barrier, induces the release of insulin from fetal pancreas and, thereby, produces fetal hyperinsulinemia (22).

Recent data have shown that the plasma concentration of inflammatory mediators, such as TNF- α and IL-6, is increased in the insulin-resistant states of obesity and type 2 diabetes (24). The observation that 10–35% of the body's basal circulating IL-6 is derived from adipose tissue has stimulated interest in this cytokine as a possible mediator of metabolic processes (14, 25). In the present study, we have

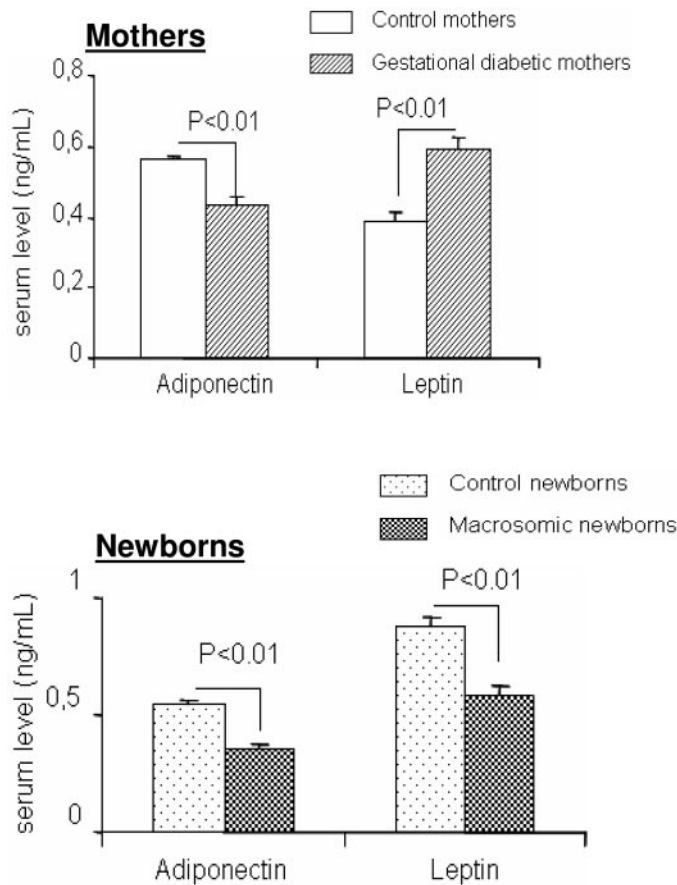


FIG. 2. Serum adiponectin and leptin concentrations in gestational diabetic and control women and their newborns. Serum adiponectin and leptin concentrations were determined as described in *Subjects and Methods*. Values are means \pm SD; n = 60 control mothers/babies; n = 59 gestational diabetic mothers/macrosomic babies.

noticed that the concentrations of TNF- α and IL-6 are increased in women with GDM. It has been suggested that the increases in TNF- α and IL-6 in diabetic conditions might be a result of oxidative stress and inflammatory changes caused by hyperglycemia (26). In fact, Mohanty *et al.* (27) have shown that the ingestion of glucose in normal subjects induces a fall in α -tocopherol concentrations and an increase in p47^{phox} expression in peripheral mononuclear cells and a peak of reactive oxygen species generation of more than 200% of the basal levels. Hence, increased concentrations of TNF- α and IL-6 might not only diminish insulin sensitivity by suppressing insulin signal transduction but also interfere with the antiinflammatory effect of insulin in these subjects (24). These inflammatory mediators may also interfere with adipokines (see below).

Adipokines, secreted by adipose tissue, are required for a number of physiological and metabolic processes (28). Despite the potential importance of these agents as putative mediators of metabolic disorders, little is known about their implications in GDM and macrosomia. In the present study, we have observed that the levels of adiponectin, an antiinflammatory agent (29), are decreased in women with GDM. Our results are in accordance with those obtained by Meller *et al.* (30), who have reported that adiponectin concentrations

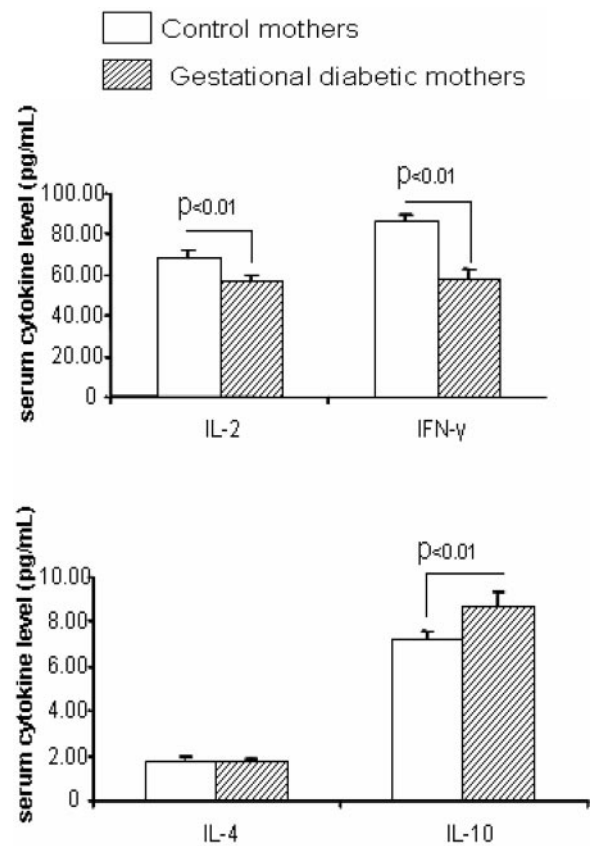


FIG. 3. Serum IL-2, IFN- γ , IL-4, and IL-10 concentrations in gestational diabetic and control women. Serum Th1 and Th2 cytokine concentrations were determined as described in *Subjects and Methods*. Values are means \pm SD; n = 60 control mothers; n = 59 gestational diabetic mothers.

are decreased in human pregnancies complicated with DM compared with nondiabetic pregnancies. Is there any physiological importance of concomitant high TNF- α levels and low adiponectin concentrations in women with GDM? Hence, we can state that these two agents might counteract with their mechanisms of actions. Indeed, it has been shown that adiponectin and TNF- α produce opposite effects on insulin signaling, with TNF- α inhibiting (31) and adiponectin increasing (32) tyrosine phosphorylation of the insulin receptor. Besides, it is also possible that TNF- α may be responsible for the lowered synthesis of adiponectin in GDM subjects because Ruan and Lodish (33) have suggested that the former inhibits the synthesis of the latter. According to Lihn *et al.* (34), both the mediators, TNF- α and IL-6, downregulate adiponectin expression. Adiponectin has been shown to enhance insulin sensitivity, although its mechanism of action remains unclear (29). The low concentrations of adiponectin may also be responsible for the lack of diminution of hyperglycemia in GDM women. We would like to mention the study of Tsai *et al.* (35), who have demonstrated that decreased maternal adiponectin concentration and insulin sensitivity may increase risk of fetal overgrowth in women suffering from GDM. Finally, we can state that TNF- α and IL-6 may also be involved in the pathogenesis of insulin resistance, type 2 diabetes, abnormal adiposity, or lipid disorders.

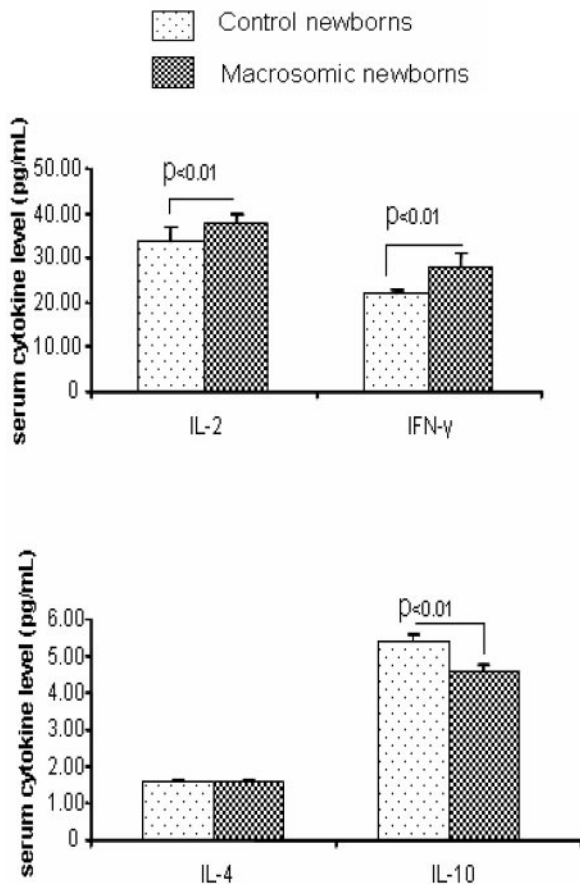


FIG. 4. Serum IL-2, IFN- γ , IL-4, and IL-10 cytokine concentrations in macrosomic and control newborns. Serum Th1 and Th2 cytokine concentrations were determined as described in *Subjects and Methods*. Values are means \pm SD; n = 60 control babies; n = 59 macrosomic babies.

Leptin is principally produced by adipocytes and secreted into the bloodstream (36). It is an appetite suppressant agent, and it exerts its effects by interacting with neuropeptide Y, MSH, and the melanocortin-4 receptor in the hypothalamus (37). In the present study, we have observed that the concentrations of leptin were higher in women with GDM than the control pregnant mothers. There is a controversy as far as the levels of leptin in GDM are concerned. Leptin levels have been reported either elevated (11) or unaltered (12) or reduced (13) in GDM pregnancy, albeit a recent study (38) has shown that maternal leptin concentrations are high in women with GDM. This discrepancy could be a result of the

TABLE 2. The ratios of serum Th1 and Th2 cytokine concentrations in GDM mothers and their newborns

	IL-2/IL-4	IL-2/IL-10	IFN- γ /IL-4	IFN- γ /IL-10
Control mothers	39.05	9.41	49.87	12.02
GDM mothers	31.61 ^a	6.54 ^a	31.76 ^a	6.57 ^a
Control newborns	20.93	6.22	13.67	4.06
Macrosomic newborns	23.56 ^a	8.18 ^a	17.34 ^a	6.02 ^a

Values are the ratios of serum Th1 and Th2 cytokine concentrations; n = 60 control mothers/babies; n = 59 GDM mothers/macrosomic babies.

^a Significant difference between diabetic mothers or macrosomic newborns and their corresponding controls: $P < 0.01$.

differences in the time of the maternal blood collection (*i.e.* gestational age). However, elevated leptin concentrations during diabetic pregnancy may be a result of its secretion by the adipocytes in the presence of elevated estrogen (39) and placenta (40). In fact, leptin, acting as a signal for sufficient energy supply, is persistently increased in women with GDM after delivery and associated with hyperglycemia and insulin resistance (11). It is also possible that hyperleptinemia in GDM might have favored the development of macrosomia in the fetuses. Indeed, Yura *et al.* (41) have reported that mice born to dams that had abnormal placental leptin levels developed accelerated weight gain and adiposity. Previously, we have mentioned that hyperglycemia-induced oxidative stress may be responsible for the production of TNF- α and IL-6. Again, increased leptin levels observed in women with GDM might induce oxidative stress that may be subsequently involved in the release of inflammatory mediators (42).

It is important to notice that the concentrations of TNF- α , IL-6, adiponectin, and leptin are decreased in macrosomic infants compared with control babies. A plausible explanation for these changes is not available. However, we can cite the study of Weiss *et al.* (43), who have shown that circulating adiponectin levels are significantly lower in obese children than the nonobese infants. Low leptin levels in these macrosomic babies may contribute to weight gain because it has been shown that leptin-deficient rodents (37) and humans (44) develop marked obesity.

It has been well propounded that during normal pregnancy, Th1 cytokines are down-regulated, whereas cytokines belonging to Th2 cells are up-regulated (45). Besides, a shift of Th1 phenotype to Th2 during pregnancy has been shown to encourage vigorous production of antibodies that not only combat infections during pregnancy but also offer passive immunity to the fetus (46). In the present study, we have observed that serum IL-2 and IFN- γ concentrations are down-regulated, whereas IL-4 concentrations are not altered in gestational diabetic mothers. Interestingly, the levels of IL-10, a Th2 cytokine, are increased in these diabetic mothers. Our observations suggest that diminished concentrations of Th1 cytokines and increased IL-10 levels may be implicated in maintaining the pregnancy in gestational diabetic women. However, the lack of changes in circulating IL-4 levels may be responsible for the induction of diabetes mellitus. Our idea can be supported with the observations of Muller *et al.* (17) who have shown that diabetes susceptibility was more associated with reduction of IL-4 than with induction of IFN- γ in islets of BALB/c male mice rendered diabetic. Similarly, Wood *et al.* (20) have reported diminished expression of IL-4 in thymocytes of diabetic mice. We have recently shown that a decrease in IL-4, but not in IL-10, favors the onset of diabetic pregnancy in rats (47) and mice (48). The high secretion of IL-10 may be because of an elevated concentration of cortisol during pregnancy (49). Indeed, it has been shown that cortisol, concentrations of which are increased in pregnant women (49), induces an increase in IL-10 secretion (50). Moreover, in the present study, the ratio of cytokines demonstrates that in gestational diabetic women, the Th1 phenotype is down-regulated. In this context, we have recently demonstrated that Th1 phenotype is down-

regulated during diabetic pregnancy in rats (47) and mice (48).

In macrosomic rats (47), we have previously demonstrated an increase in the concentrations of Th1 cytokines (IL-2 and IFN- γ) and a decrease in IL-10 levels without any modifications in IL-4 concentrations. However, comparing the ratios of Th1/Th2 cytokines, we noticed an increase in Th1 phenotype in these macrosomic babies. The physiological importance of Th1 phenotype in macrosomia is not well understood. Ours is the first study to show a Th1 phenotype of T cells in human macrosomia, although fully activated T cells are detected in the cord blood of infants and mothers with type I diabetes but not in infants from normal mothers (51). Moreover, from birth up to 15 yr of age, the percentage of total T cells was higher in children of type 1 diabetic mothers than in those of healthy mothers (52). We have shown that T cells of macrosomic pups also present a defect in intracellular calcium signaling (53). Because macrosomic infants in the present study exhibited up-regulation of Th1 cytokines, it is possible that these activated T cells may contribute, in part, to the development of diabetes and obesity in a later stage of life in these infants (54, 55).

To sum up, we can state that GDM is associated with hyperinsulinemia, hyperglycemia, high concentrations of leptin and inflammatory mediators such as TNF- α and IL-6, and low adiponectin levels. These GDM subjects are associated with a down-regulated Th1 phenotype of T cells. The macrosomic offspring of women with GDM exhibit hyperinsulinemia and low levels of leptin, adiponectin, TNF- α , and IL-6, along with an up-regulated Th1 phenotype of T cells. Additional studies are required to explore the implication of T cell subtypes in the onset of inflammation-related diabetes/obesity and their role in the modifications of adipokines in GDM and macrosomia.

Acknowledgments

We thank the Ministry of Higher Education, Republic of Benin, which granted a scholarship to J.-M.A. We express our sincere thanks to the French Embassy at Cotonou, Benin, and the Islamic Development Bank for the sanction of a scholarship to one of the authors (A.Y.).

Received May 8, 2006. Accepted July 12, 2006.

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