Elevated Acetoacetate and Monocyte Chemotactic Protein-1 Levels in Cord Blood of Infants of Diabetic Mothers

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Key Words
Infant of diabetic mother · Oxidative stress · Monocyte chemotactic protein-1 · Acetoacetate · Hyperketonemia

Abstract
Background: Infants of diabetic mothers (IDMs) are at increased risk for metabolic complications. Type 1 and some type 2 diabetic patients have elevated levels of the ketone bodies acetoacetate (AA) and β-hydroxybutyrate (BHB). Objective: The aim of this study was to examine how hyperketonemia in diabetic mothers affects markers of inflammation and oxidative stress in their offspring. Methods: Blood was obtained from 23 diabetic mothers and 13 healthy mothers and their infants’ umbilical cords at delivery. Interleukin-8, monocyte chemotactic protein-1 (MCP-1) and protein carbonyl (protein oxidation) levels were determined by ELISA. U937 human monocyte cell culture was used to examine the effect of AA and BHB on secretion of MCP-1. Results: There was a significant increase in the levels of AA in cord blood of IDMs compared with cord blood of infants of healthy mothers. A significant increase in the levels of protein oxidation (p < 0.05) and MCP-1 levels (p < 0.05) was observed in the cord blood of IDMs. The level of MCP-1 correlated significantly (r = 0.51, p = 0.01) with the concentration of AA in the IDMs. In further experiments with cultured monocytes treated with exogenous AA (0–4 mM), a significant increase in MCP-1 secretion was observed in AA- but not BHB-treated monocytes. Conclusion: Blood levels of AA and MCP-1 are elevated in IDMs, which may contribute to the development of the metabolic complications seen in IDMs.

Introduction
Maternal hyperglycemia leads to elevated blood glucose levels in the fetus and a metabolically abnormal fetal milieu. This may result in birth defects, spontaneous abortions, macrosomia, asphyxia, respiratory distress syndrome and other metabolic complications [1–3]. Infants of diabetic mothers (IDMs) may be at an increased risk for developing diabetes and/or obesity later in life [1]. Controlling hyperglycemia during pregnancy reduces these complications in their offspring. In addition to hyperglycemia, hyperketonemia also occurs in type 1 and some type 2 diabetic patients [4]. Hyperketonemia can cause increased circulating levels of proinflammatory cytokines [5–7]. Vascular inflammation plays an important role in the development of many complications associat-
ed with diabetes mellitus during pregnancy and in the newborn period [8, 9]. Even though ketonemia is associated with markers of inflammation in adult diabetic patients [5–7], the effect of ketonemia on the inflammatory markers in IDMs is unknown. Dahlgren et al. [10] reported that exposure of animals to cytokines prenatally in utero can induce gender-specific programming of neuroendocrine regulation with subsequent sequelae in adult life. In another study, ketonemia during pregnancy was associated with a lower intelligence quotient in children [11]. Ketone bodies are increased in maternal and fetal plasma of streptozotocin-treated diabetic pregnant ewes [12]. However, no study has examined if the cord blood of IDMs has elevated ketone levels. The objective of this study was to examine whether ketone levels are elevated in IDMs and the possible effects of hyperketonemia on proinflammatory cytokines and oxidative stress markers. This study measured ketone blood levels [acetoacetate (AA), β-hydroxybutyrate (BHB)], the levels of inflammatory markers [interleukin (IL)-8, monocyte chemotactic protein-1 (MCP-1)] and protein oxidation in diabetic mothers and healthy mothers (controls) as well as in the blood from umbilical cords of their respective infants at the time of delivery.

Methods

Subjects

The study was approved by the Louisiana State Health Sciences Center-Shreveport Institutional Review Board. Recruitment began in November 2008 and concluded in April 2010. Written informed consent was obtained from all study subjects. Subjects were divided into 2 groups, namely pregnant women with diabetes mellitus (study group, n = 23) and healthy non-diabetic pregnant women (control group, n = 13). The study group consisted of pregnant women with type 1 diabetes (n = 12), type 2 diabetes (n = 3) and gestational diabetes (n = 8). All the study group patients showed ketonemia. Data forms were completed for all patients. They included information on the following: age, type of diabetes, complications during any previous pregnancy, medications, substance abuse, chorioamnionitis, premature rupture of membranes, meconium, mode of delivery, gestational age, infants’ Apgar score at 5 min and infants’ complications at birth. Immediately prior to delivery, 10 ml of venous blood was drawn from all mothers, and the umbilical vein of the placenta after delivery. The sample was divided into three aliquots: 2 ml was used for the glycated hemoglobin (HbA1c) assay, 2 ml was used for the fructosamine measurements in the mother’s blood, and the remaining 6-ml aliquot was centrifuged (2,500 rpm for 20 min), and the plasma was recovered and frozen at −80°C. Immediately after delivery of the placenta, 6 ml of cord blood was drawn from the umbilical vein and centrifuged, and plasma was frozen at −80°C.

Results

Table 1 gives the maternal age and fructosamine and HbA1c levels, as well as the birth weight, gestational age and length of hospital stay of the newborns. Maternal age in the two groups was similar. Although IDMs were larger than normal infants, the difference in their birth weights was not statistically significant. However, IDMs...
had slightly shorter gestational ages compared to those of the normal infants. The HbA1c and fructosamine levels of diabetic mothers were higher compared with those of healthy mothers (p < 0.01). Apgar scores were similar in the two groups, and none of the infants had meconium-stained amniotic fluid. None of the mothers in the diabetes or control (healthy) groups had chorioamnionitis or a history of substance abuse. Sepsis was neither suspected nor diagnosed in the newborn infants. Amongst the infants born to diabetic mothers, 6 had hypoglycemia which resolved within a few hours, 5 had macrosomia and 3 had congenital malformations (1 cleft lip and palate, 1 hypospadias, 1 ear anomaly).

AA and BHB levels in IDMs and infants of healthy mothers are shown in Table 2. There was a significant increase in plasma AA levels in IDMs compared with those of infants born to healthy mothers. Total ketone levels were obtained by adding the concentrations of AA and BHB. There was a significant correlation in levels of total ketones (r = 0.50, p = 0.016) and BHB (0.49, p = 0.018) but not AA (0.26, p = 0.22) between the mothers and their respective infants’ cord blood in the diabetic group. There was no relationship to any ketone levels between mothers and cord blood in the healthy group. Table 2 also gives MCP-1 and protein oxidation levels in IDMs and infants of healthy mothers. There was a significant increase in protein oxidation and MCP-1 levels in IDMs compared with infants born to healthy mothers (p < 0.05). The level of MCP-1 correlated significantly with that of total ketone bodies (r = 0.41, p = 0.05) and AA (r = 0.51, p = 0.01). When data from cord blood of all diabetic patients was separated into type 1 diabetes versus gestational and type 2 diabetes groups, there was an increase in MCP-1 levels in cord blood of infants of type 1 diabetic mothers (713 ± 211 pg/ml) versus cord blood of infants of normal mothers (269 ± 32 pg/ml), but it was not significant (p = 0.097). The MCP-1 levels in cord blood of infants of mothers with gestational diabetes (493 ± 162 pg/ml) was similar to those in infants of healthy mothers. No relationship with gestational age was seen with MCP-1 (r = 0.15, p = 0.50) in cord blood samples. Similarly, there was no relationship between blood MCP-1 levels and birth weight in this study (r = 0.14, p = 0.51). Thus, differences in gestational age or birth weight are unlikely to have any effect on changes in MCP-1. There was no relationship between MCP-1 levels and maternal fructosamine or HbA1c levels at delivery. The increase in plasma levels of IL-8 in IDMs compared with infants of healthy mothers was not statistically significant (table 2).

Figure 1 illustrates that treatment of monocytes with AA resulted in a concentration-dependent increase in secretion of MCP-1, suggesting that AA can stimulate

### Table 1.

<table>
<thead>
<tr>
<th></th>
<th>IDM (n = 23)</th>
<th>Infants of healthy mothers (n = 13)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother’s age, years</td>
<td>26.8 ± 1.3</td>
<td>26.5 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Mother’s HbA1c, %</td>
<td>7.0 ± 0.3</td>
<td>5.6 ± 0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Mother’s fructosamine, mM</td>
<td>198 ± 8</td>
<td>166 ± 3</td>
<td>0.01</td>
</tr>
<tr>
<td>Newborn birth weight, g</td>
<td>3,737 ± 118</td>
<td>3,221 ± 81</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>37.6 ± 0.2</td>
<td>39.3 ± 0.3</td>
<td>0.05</td>
</tr>
<tr>
<td>Newborn length of stay, days</td>
<td>3.9 ± 0.5</td>
<td>2.2 ± 0.1</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data are given as means ± SEM. NS = Not significant.

### Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Diabetic mothers (n = 23)</th>
<th>Healthy mothers (n = 13)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>IL-8, pg/ml</td>
<td>7.95 ± 2.6</td>
<td>9.65 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>MCP-1, pg/ml</td>
<td>277 ± 51</td>
<td>328 ± 63</td>
<td>NS</td>
</tr>
<tr>
<td>AA, mM</td>
<td>0.38 ± 0.12</td>
<td>0.33 ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td>BHB, mM</td>
<td>2.4 ± 0.47</td>
<td>1.38 ± 0.41</td>
<td>NS</td>
</tr>
<tr>
<td>Total ketones, mM</td>
<td>2.8 ± 0.5</td>
<td>1.71 ± 0.52</td>
<td>NS</td>
</tr>
<tr>
<td>Protein carbonyl, μg/ml</td>
<td>0.38 ± 0.07</td>
<td>0.31 ± 0.14</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are given as means ± SEM. NS = Not significant.
MCP-1 secretion. In contrast, BHB treatment (1–5 mM) did not show any effect on MCP-1 secretion (data not given here). Neither AA nor BHB treatment had an effect on cell viability (data not shown here). There was no change in pH of the culture medium after addition of AA. Air plus CO₂ was used during cell culture incubation studies. The present study revealed AA levels in the range of 0.1–0.93 mM in cord blood of IDMs and up to 1.96 mM in the mother’s blood. However, studies in the literature have reported more than 4 mM AA levels in the blood of diabetic patients [13]. Thus, an AA concentration of 1 mM is quite close to the physiological range. Our previous study showed an increase in MCP-1 after AA treatment of monocyte-rich human peripheral blood mononuclear cells isolated from human blood without activation with LPS [7]. Stimulation of monocytes with LPS has been used by many investigators for cell culture studies.

Discussion

Approximately 100,000 IDMs are born in the USA annually, some of whom may be at increased risk for metabolic and other complications [1]. The biochemical mechanisms leading to these complications are not completely understood. Recent studies suggest that hyperketonemia, oxidative stress and vascular inflammation may each play a role in the development of fetal complications during diabetic pregnancies. Ketone bodies can cross the placenta, and their levels have been found to be elevated in fetuses of diabetic mothers in pregnant ewes [12]. At birth, an increase in inflammatory markers such as C-reactive protein and intercellular adhesion molecule-1 has been reported in the offspring of type 1 diabetic mothers [14, 15]. However, the effect of hyperketonemia on markers of vascular inflammation and oxidative stress in IDMs has not been investigated. For the first time, this study demonstrates increased levels of MCP-1 and protein oxidation in the cord blood of IDMs (p < 0.05) but not in the cord blood of infants born to normal mothers. The level of MCP-1 correlated significantly (p = 0.01) with the concentration of AA in the cord blood of IDMs. A significant correlation of MCP-1 with AA levels in the blood and increased secretion of MCP-1 in AA-treated monocytes suggests that hyperketonemia may contribute to elevated MCP-1 levels in IDMs.

MCP-1 is a chemokine secreted by different cell types [16]. It attracts monocytes into an area of inflammation and then activates them. Several studies have examined its role during normal and abnormal pregnancies and during the neonatal period [17, 18]. Denison et al. [18] found that MCP-1 is important in the development of the placenta and thus maintaining a normal pregnancy. Briana et al. [19] estimated maternal and cord blood MCP-1 levels and reported significantly lower levels in growth-retarded infants compared to healthy controls. MCP-1 levels are increased in women with type 1 and 2 diabetes [20, 21]. Two separate studies from Poland reported that women with gestational diabetes showed increased levels of the chemokine MCP-1, possibly leading to adverse pregnancy outcomes [22, 23]. The present study did not find a significant increase in the levels of IL-8 in the cord blood of IDMs or the cord blood of infants born to normal mothers.

This study observed elevated levels of protein carbonyl, a marker of oxidative stress. The study by Kinalski et al. [24] in diabetic mothers suggests that their fetuses experience increased oxidative stress. The results show that AA, but not BHB, increased MCP-1 secretion in U937 monocytes exposed to high glucose. The reasons that one ketone body as opposed to the other affects inflammation differently remain unclear. We speculate that effects caused by AA may be due to the generation of oxygen radicals either directly or indirectly. It has been shown that AA but not BHB can generate oxygen radicals in a cell-free system [25–27], which may ultimately lead to an oxidative environment. It is also possible that AA is indirectly producing reactive oxygen species by...
being fed into the electron transport chain, creating an overload of electrons similar to that seen with hyperglycemia [25]. Since studies have also shown a decrease in the mitochondrial membrane potential in monocytes treated with AA, it seems likely that the mitochondria would be the second source of reactive oxygen species in monocytes treated with AA. Another possibility is that AA is converted to BHB, which in turn can alter the redox state of the cell by affecting both nicotinamide adenine dinucleotide and glutathione levels. Structurally, the two compounds are very similar, but there is no ketone functional group present in BHB. AA contains two keto groups whereas BHB does not, suggesting that the difference in chemical structure of the two ketone bodies may play a role in mediating the effects caused by AA and not BHB. AA is much less stable than BHB. Previous studies have also reported that the ketone body AA can increase oxygen radical formation and oxidative stress [25–27]. However, we found no correlation between levels of AA and protein oxidation levels in IDMs. The long-term outcomes of IDMs exposed to oxidative stress are unknown.

In conclusion, this study demonstrates that increases in circulating MCP-1 and protein oxidation levels in the cord blood of IDMs compared to normal infants suggest increased inflammation and oxidative stress. The hyperglycemic environment reflected by higher HbA1c and fructosamine levels in diabetic mothers and the presence of the AA ketone may be a factor in the increased MCP-1 levels in the cord blood of IDMs. The increase in oxidative stress may also result from elevated AA levels and/or glycosylation of antioxidative defense enzymes due to hyperglycemia. The role of elevated MCP-1 levels in the metabolic complications common to diabetic pregnancies needs further investigation in a larger patient population.

Acknowledgements

The authors thank Ms. Georgia Morgan for excellent editing of the manuscript. The authors are supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases, the Office of Dietary Supplements of the National Institutes of Health (ROI DK072433) and the Malcolm Feist Endowed Chair in Diabetes.

Disclosure Statement

None of the authors has any financial interest in the publication of this paper, nor have they received any money from any sources other than the National Institutes of Health or Louisiana State University Health Sciences Center.

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