Raised concentrations of lipid peroxidation products (LPO) in pregnant women with impaired glucose tolerance

Krzysztof C. Lewandowski¹,², Nemanja Stojanovic³, Martin Press⁴, Susan Tuck⁴, Andrzej Lewiński¹,², Małgorzata Karbownik-Lewińska²,⁵

¹ Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, Lodz, Poland
² Department of Endocrinology and Metabolic Diseases, Polish Mother’s Memorial Hospital – Research Institute, Lodz, Poland
³ Department of Diabetes and Endocrinology, Royal Free Hospital, Hampstead, London, UK
⁴ Department of Obstetrics and Gynaecology, Royal Free Hospital, Hampstead, London, UK
⁵ Department of Oncological Endocrinology, Medical University of Lodz, Lodz, Poland

Address for correspondence: Andrzej Lewiński, Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, Lodz, Poland
E-mail: alewin@ckk.umed.lodz.pl

www.aem.pl

INTRODUCTION

Under physiological conditions, biological cells produce low or moderate amounts of reactive oxygen species (ROS) that are required for life processes. In basal conditions, ROS are continuously detoxified by antioxidant systems and, therefore, they are not toxic [1]. Overproduction of ROS, free radicals included, results in enhanced oxidative stress and may lead to several diseases [2]. Alternatively, pathological processes in organs or tissues may, in turn, lead to an increased formation of ROS and, in consequence, to increased damage to macromolecules, such as membrane lipids [2, 3]. Oxidative damage to membrane lipid products is known as lipid peroxidation (LPO). The placenta is a major source of oxidative stress during pregnancy. As placenta is rich in polyunsaturated fatty acids which are highly susceptible to attack by ROS, then increased LPO is expected during pregnancy. This process is known as shedding. The concentration of these soluble TNFα receptors (sTNFα-Rs) is proportional to previous TNFα action. In fact, sTNFα-Rs remain elevated in plasma for longer periods of time after the administration of TNFα and are thought to be a surrogate of previous TNFα effects [15, 16]. This is because the two soluble receptor forms – sTNF-R1 and sTNF-R2 – have longer half-lives


Abstract

Introduction. Lipid peroxidation (LPO) results from oxidative damage to membrane lipids. Whereas LPO rises in normal pregnancy, the effect of gestational diabetes mellitus (GDM) on this process has not been clearly defined.

Materials and Method. Fasting blood concentrations of malondialdehyde+4-hydroxyalkenals (MDA+4-HDA), as LPO index, TNFα soluble receptors (sTNF–R1 and sTNF–R2), and soluble adhesion molecules (sICAM-1, sVCAM-1), were measured in 51 women at 28 weeks of gestation. The women were divided according to the results of 50.0 g glucose challenge test (GCT) and 75.0 g oral glucose tolerance test (OGTT): Controls (n=20), normal responses to both GCT and OGTT; Intermediate Group (IG) (n=15), abnormal GCT but normal OGTT; GDM group (n=16), abnormal both GCT and OGTT.

Results. Glucose concentrations in women diagnosed with GDM were within the range of impaired glucose tolerance. There were no significant differences in concentrations of either TNFα soluble receptors R1 and R2, or sICAM-1 or sVCAM-1. LPO concentrations [MDA+4-HDA (nmol/mg protein)] were significantly higher in women with GDM than in the other two groups [64.1 ± 24.3 (mean ± SD), 39.3 ± 23.1, 47.0 ± 18.1, for GDM, IG and Controls, respectively; p<0.05]. In multivariate analysis, the only significant independent correlation was between LPO level and glucose at 120 minutes of OGTT (rs=0.42; p=0.009).

Conclusions. Oxidative damage to membrane lipids is increased in GDM and might result directly from hyperglycaemia. Physiological significance of this phenomenon remains to be elucidated.

Key words

gestational diabetes mellitus, lipid peroxidation, glucose tolerance, insulin resistance, TNF alpha soluble receptors

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than TNFα, and their concentration may reflect TNFα activity [17]. Furthermore, recent evidence suggests that the TNFα system and serum sTNF-R1 and sTNF-R2 might be associated with the rate of glucose and lipid oxidation during hyperinsulinaemia in an opposite manner to adiponectin [18]. While serum TNFα concentrations were reported to be raised in GDM, in most [19, 20, 21, 22], though not in all studies [23], there are few data on concentrations of TNFα soluble receptors in women with GDM. Binding of monocyteic cells to vascular endothelium, one of the earliest detectable events in atherosclerotic lesion development and in inflammatory processes, arises under the influence of adhesion molecules, such as soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1) [24]. It has been shown that diabetic patients have an increase of soluble adhesion molecules (sICAM-1, sVCAM-1, sE-selectin, sL-selectin, sP-selectin) considered an integral part of the inflammatory state [25]. Cellular forms of adhesion molecules mediate specific steps of leukocyte-endothelial cell interaction and have been implicated in the pathophysiology of pre-clampsia, endothelial dysfunction [26], as well as IR [25, 27].

**Objective.** The presented study aimed to test the hypothesis that concentrations of LPO products might be altered in GDM. A further aim was to assess any potential relationships between LPO products and IR indices and the components of TNFα system (i.e. TNFα soluble receptors R1 and R2), as well as soluble forms of adhesion molecules (sICAM-1 and sVCAM-1).

**MATERIALS AND METHOD**

This was a cross-sectional study performed at 28 weeks of gestation. The study group comprised 51 women who attended either Obstetric Clinics at the Royal Free Hospital in London, UK, or The Department of Endocrinology and Metabolic Diseases at The Polish Mother’s Memorial Hospital Research Institute in Łódź, Poland, and were screened for GDM and evaluated with 50.0 g glucose challenge test (GCT) and 75.0 g oral glucose tolerance test (OGTT). Women with plasma glucose <7.8 mmol/l one hour after the GCT were regarded normal and subjected to routine antenatal care. GDM was diagnosed according to the WHO criteria [28]. The women were divided into three groups according to the results of GCT and OGTT: Controls (n=20) had normal glucose concentrations at 120 minutes of OGTT (Fig. 1), while fasting glucose levels (albeit still higher than in controls, p≤ 0.01) were still within the reference range for all but one woman with GDM. In women with GDM, glucose levels at 120 minutes of OGTT were in the range between 8.0 mmol/l – 11.6 mmol/l, with only one subject exceeding 11.1 mmol/l (i.e. the cut-off point between impaired glucose tolerance and diabetes mellitus in non-pregnant subjects). Worsening of glucose tolerance was characterised by an increase in fasting glucose in the GDM group, and fasting insulin and HOMA index in both

**RESULTS**

There were no significant differences between the subgroups, regarding age and BMI, both before and during pregnancy (Tab. 1). According to the results of the OGTT, the principal differences between women with GDM and controls pertained to glucose levels after 120 minutes of OGTT (Fig. 1), while fasting glucose levels (albeit still higher than in controls, p≤ 0.01) were still within the reference range for all but one woman with GDM. In women with GDM, glucose levels at 120 minutes of OGTT were in the range between 8.0 mmol/l – 11.6 mmol/l, with only one subject exceeding glucose concentration of 11.1 mmol/l (i.e. the cut-off point between impaired glucose tolerance and diabetes mellitus in non-pregnant subjects). Worsening of glucose tolerance was characterised by an increase in fasting glucose in the GDM group, and fasting insulin and HOMA index in both

**Table 1. Demographic characteristics (mean ± SD) of subjects participating in the study**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GDM (n=16)</th>
<th>IG (n=15)</th>
<th>CTRs (n=20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.6 ± 4.2</td>
<td>33.0 ± 4.3</td>
<td>31.9 ± 3.7</td>
<td>0.65</td>
</tr>
<tr>
<td>BMI before pregnancy [kg/m²]</td>
<td>23.7 ± 3.2</td>
<td>23.5 ± 4.8</td>
<td>23.2 ± 4.2</td>
<td>0.54</td>
</tr>
<tr>
<td>BMI current [kg/m²]</td>
<td>27.7 ± 3.6</td>
<td>26.8 ± 4.2</td>
<td>26.4 ± 4.2</td>
<td>0.34</td>
</tr>
</tbody>
</table>

P – value of the Kruskal-Wallis’ test for comparison of distributions between three groups
Intermediate (i.e., in patients with FPGCT) and in the GDM groups in comparison to Controls (p<0.01). However, there were no differences in fasting insulin, fasting glucose and HOMA index between the Intermediate and GDM groups (p>0.10 (Tab. 2). In contrast, there was no difference in the estimates of insulin resistance assessed by IRI between the Controls and Intermediate groups (0.68±0.25 vs. 0.93±0.29, p=0.23). There was, however, a marked difference in the value of IRI between the GDM and Intermediate groups (1.67±0.39 vs. 0.93±0.29, p=0.015), and between the GDM group and Controls (p<0.001).

Descriptive statistics for LPO concentrations [MDA+4-HDA (nmol/mg protein)] are presented in Table 2 and Figure 2. LPO concentrations were significantly higher in women with GDM than in the other two groups (p<0.05), but there were no differences in LPO concentrations between Controls vs. Intermediates. Concentrations of other measured parameters are also presented in Table 2. There were no
The main finding of the presented study was to demonstrate increased concentrations of LPO products in women with GDM in comparison to healthy pregnant controls, as well as with pregnant women with less pronounced abnormalities of glucose tolerance (i.e., false positive glucose challenge test, but normal results of 75.0 g OGTT). The main difference between women with GDM and those from the Control and Intermediate groups pertained predominantly to glucose levels at 120 minutes of OGTT (Fig. 1), while those from the Intermediate group were more insulin-resistant than controls (HOMA: 2.05±1.36 vs. 1.15±0.82, p<0.01).

As concentrations of LPO products did not correlate with the indices of insulin resistance (HOMA, IRI), but correlated independently with glucose concentration at 120 minutes of OGTT, then it is likely that glycaemia, rather than insulinaemia, might be the driving force behind raised concentrations of LPO products in women with GDM.

As mentioned above, in normal pregnancy LPO rises until the middle of the second trimester and generally return to non-pregnant levels in early postpartum [4, 5]; however, there are very scanty data for LPO levels in GDM. Dey et al. [31] reported a significant increase in the erythrocytic glutathione, serum total glutathione and protein thiols in GDM maternal blood when compared to controls, whereas erythrocytic superoxide dismutase exhibited a marked decrease in GDM. The authors, however, did not attempt to correlate LPO concentrations with glucose or insulin resistance indices, although they postulated that elevated glucose levels might induce oxidative stress in GDM mothers. Also, Karacay et al. [32] reported raised myeloperoxidase (MPO) in 27 women with GDM in comparison to 27 women with pre-clampsia and 29 controls, suggestive of raised LPO in GDM. These patients were tested for GDM at a later stage of pregnancy (up to 36 weeks). Again, however, there were no data on correlation with glucose, insulin, insulin resistance indices, as well as other parameters. Indeed, to the best of our knowledge, this is the first study, where concentrations of LPO products were tested for direct relationship with glucose and insulin resistance indices in pregnant women across the whole range of abnormalities of glucose intolerance (including those with false positive 50.0 g Glucose Challenge Test, i.e., our Intermediate group), as well as concentrations of soluble forms of adhesion molecules (sICAM-1 and sVCAM-1) and soluble TNFα receptors R1 and R2.

There are experimental data suggesting that glucose induces lipid peroxidation and inactivation of membrane-associated ion-transport enzymes in human erythrocytes in vivo and in vitro [33], although at concentrations higher than those observed in patients with GDM in the presented study. These data were later confirmed by other authors; for instance, Ahmed et al. [34] suggested that elevated levels of glucose induced oxidative stress that is ultimately reflected by the increased malondialdehyde (MDA) levels in erythrocyte membranes of diabetic patients. The authors also suggested that elevated concentrations of antioxidant enzymes may be considered as markers for vascular injury in patients with type 2 diabetes. Hyperglycaemia also induced an increase in antioxidant enzymes and a relationship seems to exist between diabetic complications and elevated levels of these enzymes.

There is also some evidence that oxidative stress may be involved in the progression and/or pathogenesis of GDM, as the release of 8-isoprostane (a marker of oxidative stress) was greater from placentas, subcutaneous adipose tissue, and skeletal muscles of women with GDM, in contrast to healthy pregnant controls [35]. Interestingly, elevated activity of butyrylcholinesterase, i.e. an enzyme involved in the reduction of oxidative stress, was also reported to be elevated in serum and placenta in gestational diabetes and in pregnant women with type 2 diabetes on insulin vs. women with diet-controlled GDM [36].
In contrast, data on the relationship between LPO and insulin resistance indices are less clear. For example, Facchin et al. [37] demonstrated a positive correlation between plasma lipid hydroperoxide concentrations and insulin resistance measured by the steady-state plasma insulin (SSPI) and glucose (SSPG) concentrations in response to a 180-min constant infusion of octreotide (r=0.42, p=0.01), but there was no correlation between HOMA and malondialdehyde (MDA) concentrations in women with impaired glucose tolerance [38], i.e., within the range of glucose concentrations similar to those observed in the presented study. It should also be noted that although LPO concentrations were reported to be raised in women with pre-clampsia [39], none of the women in the current study had any clinical features of pre-eclampsia at the time of testing, while pre-eclampsia subsequently developed in only one subject with GDM; therefore, the development of subsequent pre-eclampsia was unlikely to explain the elevated LPO concentrations in women with GDM.

Interestingly, the presented study failed to demonstrate differences in serum concentrations of TNFα soluble receptors in women with GDM vs. controls. This is in keeping with the results of Kinalski et al. [19, 40], but in contrast to the results of Winkler et al. [41] who reported higher serum concentrations of TNFα soluble receptors R1 and R2 in women with GDM. It must be appreciated, however, that regulation of the TNFα system in pregnancy is very complex, and that serum concentrations of TNFα soluble receptors reflect the contribution of various organs (including placenta) into the systemic circulation. Namely, components of the TNFα system may be released from placenta, adipose tissue, neutrophils and other sources [42]. There are data that in response to oxidative stress, GDM placenta releases less TNFα, in turn though, the GDM placenta is characterised by increased antioxidant gene expression, yet it appears to be less responsive to exogenous oxidative stress than tissues obtained from normal pregnant women [42]. On the other hand, high glucose concentrations induce TNFα release from the placenta and adipose tissue in women with GDM [43]. In such circumstances, the net release of TNFα and its soluble receptors from placenta may, at least in theory, depend on the balance between the severity of an exogenous oxidative stress and the degree of hyperglycaemia.

In summary, the presented study has demonstrated increased concentrations of LPO products in women with GDM, despite a relatively mild degree of glucose intolerance (if not pregnant, all but one subjects would be classified as impaired glucose tolerance only). Furthermore, the independent positive correlation with glucose levels at 120 minutes of OGTT suggests that the increased oxidative damage to lipids might result directly from hyperglycaemia. This hypothesis, however, still remains to be clinically and experimentally proven. The fact that the study failed to observe the expected differences in concentrations of other parameters (TNFα soluble receptors R1 and R2, sICAM-1, sVCAM-1) implies that an increase in lipid peroxidation might be a relatively early phenomenon in women with GDM, potentially antedating the change of concentrations of certain adipokines or inflammatory markers.

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