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Features of endothelial dysfunction in umbilical cord vessels of women with gestational diabetes

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Abstract *Background and aims:* Gestational diabetes (GDM) is associated with increased oxidative stress and overexpression of inflammatory cytokines, both of which might lead to endothelial dysfunction and vascular disease. As such, GDM could be viewed as a sort of 'short lived' metabolic syndrome. As umbilical cord vessels represent a suitable model for the study of vascular alterations brought about by GDM, the aim of the present work was to characterize the phenotype of human umbilical vein endothelial cells (HUVECs) chronically exposed to hyperglycaemia and to a pro-inflammatory environment during pregnancy so as to identify molecular modifications of cellular homoeostasis eventually impacting on endothelial dysfunction.
Methods and result: Tissue specimens and HUVECs were obtained from umbilical cords of GDM and control women. As compared to controls, GD-HUVEC exhibited enhanced monocyte adhesion and vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) expression and exposure on plasma membrane after tumour necrosis factor-alpha (TNF-α) stimulation (Western blot, flow cytometer). As compared to control cells, GD-HUVEC in basal conditions exhibited enhanced monocyte adhesion, nitric oxide synthase (NOS) expression and activity (eNOS Real-Time polymerase chain reaction, Western Blot for eNOS total protein and monomers/dimers ratio, conversion of [3H]-L-arginine in [3H]-L-citrulline), increased O_2^- generation together with increased NT levels (immunofluorescence) and reduced NO bioavailability (guanosine 3′,5′-monophosphate (cGMP) production, EIA). Furthermore, immunohistochemistry revealed increased eNOS and NT immunoreactivity in GD umbilical cords.
Conclusion: Endothelial cells exposed in vivo even transiently to hyperglycaemia, oxidative stress and inflammation exhibit durable pro-atherogenic modifications.

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Introduction

Women with history of gestational diabetes (GDM) exhibit impaired endothelial function and an elevated risk of cardiovascular (CV) and/or metabolic diseases [1]. A subclinical inflammatory state associated with GDM might represent the link between GDM, future type 2 diabetes (T2DM) and metabolic syndrome and it might be involved in the increased CV risk [2]. Recent studies have also suggested that the development of adult CV disease might be correlated with maternal metabolic dysfunctions causing modifications in the intrauterine environment. Although the pathogenetic mechanisms of this so-called 'foetal programming' remain to be fully elucidated, an insult occurring during a crucial developmental period can elicit lasting structural or functional effects leading to adult morbidities [3]. GDM does induce chronic maternal stress and inflammation. Indeed, in pregnancy complicated by GDM and/or obesity, plasma concentration of circulating inflammatory molecules (such as C reactive protein (PCR), tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)) is increased, while plasma levels of anti-inflammatory molecules such as IL-10 and adiponectin are reduced [4]. GDM is thus characterized by an imbalance between pro- and anti-inflammatory molecules. Furthermore, GDM is associated with oxidative stress and overexpression of inflammatory mediators, both of which might lead to vascular disease [5]. As such, GDM could be viewed as a sort of short-lived metabolic syndrome.

As far as endothelial dysfunction is considered, one of its main facets is the alteration of mechanisms involved locally in the control of endothelial NO synthesis and release and thus in the control of vascular tone [6]. As in metabolic syndrome and in T2DM, also in GDM altered glucose homoeostasis might impact on endothelial NO release and availability by inducing a host of pathophysiological changes such as reactive oxygen species (ROS) intracellular accumulation, modifications in endothelial nitric oxide synthase (eNOS) expression and activation, cell apoptosis, inflammation, etc., all contributing to accelerated endothelial damage and dysfunction [7].

A few studies have indeed showed alterations in NO synthesis and release in endothelial cells exposed in vitro to high glucose concentration [8] or in endothelial cells explanted from umbilical cords obtained from GDM mothers [9]. However, a comprehensive investigation of potential atherogenic changes in vascular tissues exposed to the metabolic milieu of GDM has not been so far carried out.

Umbilical cord vessels should represent a suitable model for such an investigation, that is, the study of vascular alterations related to the metabolic abnormalities and the inflammatory stress brought about by GDM. Therefore, the aim of the present work was to characterize the phenotype of human umbilical vein endothelial cells (HUVECs) chronically exposed to hyperglycaemia and to a pro-inflammatory environment during pregnancy so as to identify molecular modifications of cellular homoeostasis eventually impacting on NO synthesis and bioavailability and potentially leading to vascular insult and atherosclerosis.

Methods

All methods are described briefly, for details see supplementary material.

Umbilical cords harvesting and donor characteristics

Experimental procedures involving human tissue and cell samples were reviewed and approved by the Pescara Town Hospital and G. d'Annunzio University, Chieti (Italy) Ethics Committees (EC), in accordance with Declaration of Helsinki. All pregnant women signed a written informed consent, approved by the ECs with the experimental protocol.

Donor parameters are described in Table 1: normotensive GD and C women, matched for age and body mass index (BMI), underwent a 100 g 3 h oral glucose tolerance test (OGTT) during 24–28th gw according to guidelines. Moreover, each woman performed a 7-point/day blood glucose self-monitoring on 3 different days during the week at 34–36th gw to compare fasting and post-prandial (either 1 or 2 h after meal) capillary glucose levels.

Table 1 Clinical characteristics of Control (C) and Gestational Diabetic (GD) women.

Characteristic	C women	GD women
Age (years)	34.6 ± 4.93 (29–42)	35.4 ± 4.48 (29–43)
Height (m)	1.69 ± 0.05 (1.62–1.78)	1.60 ± 0.07 (1.47–1.70)
Pre-gestational Weight (kg)	63.05 ± 14.47 (44–93)	66.75 ± 29.28 (46–134)
OGTT values (mmol/L)		
Basal glycaemia	4.22 ± 0.46 (3.7–4.9)	*5.20 ± 1.26 (3.88–7.38)
1 h glycaemia	7.10 ± 1.49 (5.1–9.1)	†11.30 ± 1.59 (9.3–13.8)
2 h glycaemia	5.59 ± 1.16 (4.3–7.8)	‡9.80 ± 1.51 (8.5–13.5)
3 h glycaemia	5.33 ± 0.53 (4.4–6.1)	*7.18 ± 2.41 (3.8–11.9)
OGTT gestational week	28.1 ± 2.85 (25–34)	28.6 ± 6.08 (12–32)
Self-monitoring values (mmol/L)		
Fasting glycaemia	4.13 ± 0.36 (3.6–4.4)	§5.25 ± 0.38 (4.9–5.72)
1 h postprandial glycaemia	5.92 ± 0.74 (5.2–6.6)	‖7.22 ± 5.53 (6.7–7.2)
2 h postprandial glycaemia	5.40 ± 0.12 (5.2–5.5)	‖6.42 ± 0.55 (6–7.1)

Data are expressed as mean ± S.D. and range; group differences of continuous variables were compared using ANOVA: *$p < 0.05$; †$p < 0.00002$; ‡$p < 0.000005$; §$p < 0.006$; ‖$p < 0.03$.