Global DNA methylation profiling in South African women with gestational diabetes mellitus

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Abstract

Background/Objective: Recently, several studies have reported that placental global DNA methylation is associated with gestational diabetes mellitus (GDM), sparking interest in the potential use of global DNA methylation as a biomarker for this disorder. This study investigated whether global DNA methylation is associated with GDM in South African women.

Methods: Global DNA methylation was quantified in the peripheral blood of women with (n=63) or without (n=138) GDM using the MDQ1 Imprint® DNA Quantification Kit.

Results: Global DNA methylation levels were not different between women with or without GDM and were not associated with fasting glucose nor insulin concentrations. However, levels were 18% (p=0.012) higher in obese compared to non-obese pregnant women, and inversely correlated with serum adiponectin concentrations (p=0.005).

Discussion: Contrary to our hypothesis, global DNA methylation was not associated with GDM in our population. Our findings suggest that despite being a robust marker of overall genomic methylation that offers opportunities as a biomarker, global DNA methylation profiling may not offer the resolution required to detect methylation differences in the peripheral blood of women with GDM. Further studies are required to explore the candidacy of a more targeted approach using gene-specific methylation as a biomarker for GDM in our population.

Words: 200

Key words: Gestational diabetes mellitus; global DNA methylation; biomarker; peripheral blood; South Africa, obesity

Clinical Significance

- The prevalence of gestational diabetes mellitus (GDM) is rapidly increasing globally
- The 75 g 2 hr oral glucose tolerance test remains the gold standard for GDM diagnosis, however, several challenges hamper its use
- Risk factor-based screening has poor sensitivity for detecting GDM
- Thus, a more robust, non-invasive, simple and cost effective screening tool is needed
- Altered DNA methylation patterns in peripheral blood holds potential as a biomarker for GDM screening

Introduction

Gestational diabetes mellitus (GDM), defined as glucose intolerance that is first diagnosed during pregnancy, is a significant source of morbidity and mortality. In South Africa, a middle-income country, the prevalence of GDM has rapidly increased over the last few years (Mamabolo *et al.* 2007, Adam and Rheeder 2017, Macaulay *et al.* 2018), paralleling the rising obesity epidemic. Recently, it was reported that 68% of South African women over the age of 15 years were either overweight or obese (Statistics South Africa 2017), a serious cause for concern since obesity is a major risk factor for the development of GDM. GDM is associated with adverse perinatal outcomes (Jensen *et al.* 2000) and increases susceptibility to future metabolic disorders in both mothers and their offspring (Damm 2009), thus posing a significant burden to the already struggling and over-burdened South African health system.

The oral glucose tolerance test (OGTT), conducted between 24-28 weeks of gestation is the gold standard for the diagnosis of GDM (WHO 1999). However, the test is cumbersome to conduct and has several challenges including high costs, requirement for fasting, multiple blood draws, and is associated with nausea and vomiting. Currently, universal screening for GDM is recommended for all pregnant women (Hod *et al.* 2015). However, due to limited

resources, selective screening based on traditional GDM risk factors such as obesity (body mass index (BMI) ≥ 30 kg/m^2), advanced maternal age (> 35 years), family history of diabetes, history of GDM, previous macrosomic (baby weighing ≥ 4000 g) pregnancy, glycosuria, or previous adverse pregnancy outcomes (congenital abnormalities, unexplained still birth or recurrent pregnancy loss) is often performed in low and middle income countries. Unfortunately, these risk factors have poor sensitivity for detecting GDM in our population (Adam and Rheeder 2017), resulting in a large number of GDM cases being missed. The identification of simple and cost effective biomarkers to detect women with GDM could offer an alternative to the OGTT. Although a number of circulating biomarkers such as adiponectin, sex hormone globulin, C-reactive protein (CRP) and glycosylated fibronectin have been explored as biomarkers for GDM, none have yet achieved clinical applicability (Smirnakis *et al.* 2007, Nanda *et al.* 2011, Rasanen *et al.* 2013, Adam *et al.* 2018).

Epigenetics reflect gene-environment interactions and is increasingly being implicated in the pathophysiology of metabolic diseases (Gu *et al.* 2013, Martín-Núñez *et al.* 2014). DNA methylation, the most widely studied and best characterized epigenetic mechanism, refers to the addition of a methyl group to the fifth carbon position of a cytosine residue within CpG dinucleotides, often leading to transcriptional repression (Lim and Maher 2010). The process is reversible thus offering opportunities for risk stratification and intervention, and has accordingly received considerable interest as biomarkers of disease. Although both gene-specific and global DNA methylation profiling have been explored, global DNA methylation, which gives an estimate of overall genomic methylation can be quantified using non-invasive, inexpensive and simple methods, thus making it an attractive target for biomarker discovery. Several studies have reported that global DNA methylation is altered during hyperglycemia (Matsha *et al.* 2016, Pinzón-Cortés *et al.* 2017) and in placental tissue of women with GDM (Nomura *et al.* 2014, Reichetzeder *et al.* 2016). We hypothesized that global DNA methylation

in the peripheral blood of black South African women is altered during GDM, and accordingly has potential as a biomarker for GDM in our population.

Materials and methods

Participants

The study was approved by the Health Sciences Ethics Committee of the University of Pretoria (180/2012). Pregnant women were recruited at a primary care clinic in Johannesburg, South Africa (Adam and Rheeder 2017). Written informed consent was obtained from all participants. Women of black ethnicity, who were less than 26 weeks pregnant and who had a singleton pregnancy were included in this study. Women with pre-existing diabetes, random glucose level >11.1 mmol/L, measured with a glucometer (Roche Diagnostics, Mannheim, Germany) or glycated hemoglobin (HbA1c) level > 6.5%, measured with the point-of-care Afinion system (Alere Technologies, Oslo, Norway) were excluded. Of the 1000 women who were recruited, the 75 g 2 hr OGTT was conducted on 554 women (Figure 1). Human Immunodeficiency Virus (HIV) positive women and those with an unknown HIV status were excluded from the current study. For this case control study, 63 women with GDM and 138 women without GDM were matched individually according to age, BMI and gestational age as far as possible, and were selected.

Clinical and biochemical characteristics

Demographic information was obtained from a standardized questionnaire and anthropometric measurements were assessed according to standard procedures (Adam *et al.* 2018). The OGTT was conducted according to the International Association of Diabetes in Pregnancy Study Group (IADPSG) criteria (Metzger *et al.* 2010). Briefly, women were given a 75 g glucose drink to ingest, and blood was collected for glucose measurements at 0 hr, 1 hr and 2 hr. At the time of OGTT, HbA1c concentrations were measured again by an accredited laboratory (Vermaak and Partners, Pretoria, South Africa). For comparative analysis, GDM was classified using the National Institute for Health and Care Excellence (NICE) and the World Health

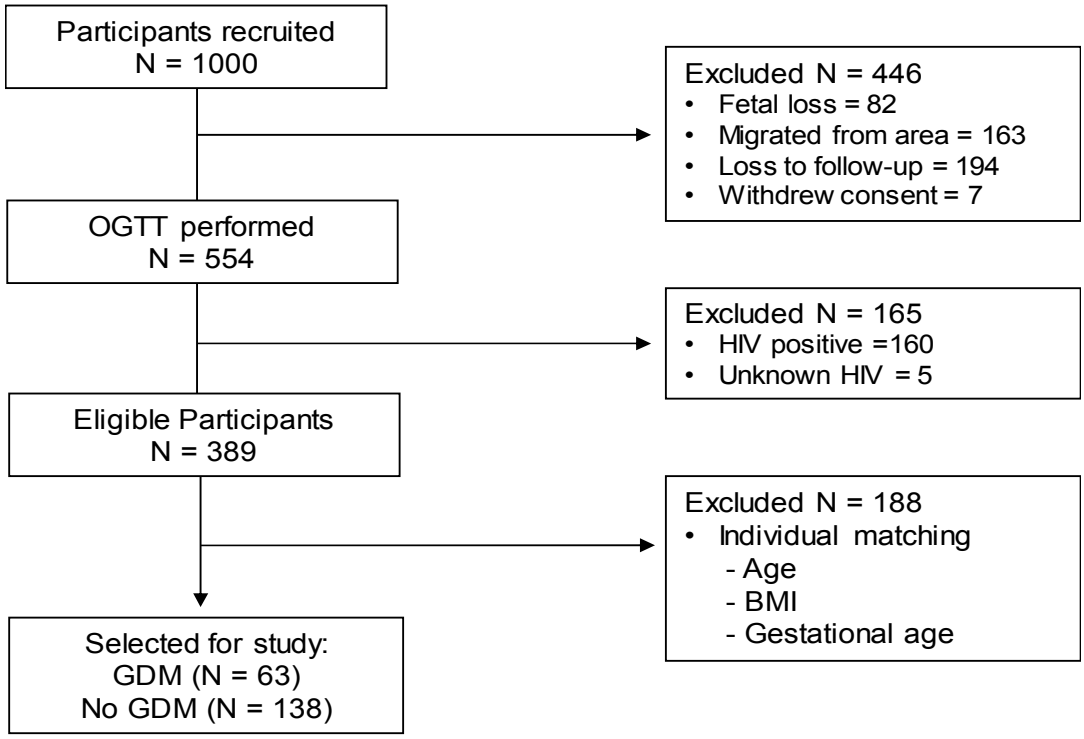

Figure 1. Flow diagram for study participants. For the current study, women with (n=63) or without (n=138) GDM were selected from a prospective cohort study in which 1000 pregnant women were recruited.

Table 1: GDM diagnostic criteria commonly used in South Africa

	Glucose concentration (mmol/L)		
Time*	IADPSG	NICE	WHO 1999
0 hr OGTT	5.1	5.6	7.0
1 hr OGTT	10	-	-
2 hr OGTT	8.5	7.8	7.8

*Time after ingesting 75 g glucose drink; OGTT: oral glucose tolerance test; hr: hour; GDM: gestational diabetes mellitus; IADPSG: International Association of Diabetes in Pregnancy Study Group; NICE: National Institute for Health and Care Excellence; WHO: World Health organization (WHO 1999, Metzger *et al.* 2010, NICE guidelines 2015)

Organization (WHO) 1999 criteria (Table 1) (WHO 1999, NICE guidelines 2015). Fasting insulin and CRP concentrations were measured in stored serum samples (Pathcare laboratories, Cape Town, South Africa). Serum adiponectin concentrations were quantified using the human adiponectin enzyme-linked immunosorbent assay (ELISA) (Merck, Darmstadt, Germany). The homeostatic model assessment (HOMA), a measure of insulin resistance was calculated using the equation: (glucose x insulin)/22.5, using fasting plasma glucose and fasting serum insulin concentrations. Whole Blood for DNA methylation analysis was stored at -80^{o} C.

Global DNA methylation

DNA was extracted from 2 ml of stored whole blood in Ethylenediaminetetraacetic acid (EDTA) tubes using the QIAmp DNA Blood Midi Kit according to the manufacturer's instructions (Qiagen, Hilden, Germany), and concentrations were measured using the Qubit Flourometer dsDNA Broad Range Assay Kit (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. Global DNA methylation was quantified using the MDQ1 Imprint® Methylated DNA Quantification Kit according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, USA) as previously described (Pheiffer *et al.* 2014). Briefly, 100 ng of DNA was allowed to bind to the ELISA plate, where after the methylated fraction of DNA was detected using a 5-methylcytosine monoclonal antibody, and the absorbance was measured at 450 nm on a BioTek® ELX 800 plate reader (BioTek Instruments Inc., Winooski, USA). Global DNA methylation levels were calculated relative to the methylated positive control which was included in the kit. All samples were analysed in duplicate.

Statistical analysis

Statistical analysis was conducted using STATA 14 (StataCorp, Texas, USA). Data were expressed as the mean ± standard error of the mean (SEM), or as the median and interquartile range (25th and 75th percentiles) for data that were not normally distributed. Categorical data

were expressed as count (n) and percentage (%). The Shapiro-Wilk test was used to test for normality. The unpaired student t test or the Mann-Whitney test was used to compare variables across GDM groups, and the Chi-square test was used to analyze categorical variables. Spearman's rank correlation (r_s) was used to evaluate the relationship between global DNA methylation and serum adiponectin concentrations. A $p \leq 0.05$ was considered statistically significant.

Results

The clinical characteristics of participants are presented in Table 2. As expected, fasting blood glucose ($p<0.001$), 1 hr OGTT ($p<0.001$), 2 hr OGTT ($p<0.001$) and HbA1c ($p=0.008$) concentrations were significantly higher in women with GDM compared to women without GDM. Similarly, fasting insulin ($p=0.067$) and HOMA ($p<0.001$) levels were increased in women with GDM. In contrast, women with GDM had lower concentrations of serum adiponectin than women without GDM ($p=0.018$).

Since the extent of hyperglycemia may influence the association between global DNA methylation and GDM, GDM was classified using IADPSG, WHO and NICE criteria. Glucose concentrations differed significantly between women with GDM compared to women without GDM, using all three diagnostic criteria (Table 3). Fasting plasma glucose values were significantly lower in women with GDM using the WHO criteria compared to the IADPSG ($p=0.014$) and NICE ($p=0.005$) criteria, while the 2 hr OGTT values were significantly higher in women with GDM using the WHO criteria compared to the IADPSG criteria and NICE criteria ($p<0.001$) (Table 3). However, no difference in global DNA methylation levels between women with or without GDM were observed when the different diagnostic criteria were used ($p>0.05$) (Figure 2A-C). Global DNA methylation levels were 18% ($p=0.012$) higher in obese compared to non-obese pregnant women (Figure 3) and were inversely correlated with serum adiponectin concentrations ($r_s=-0.243$, $p=0.005$) (Figure 4).

Table 2: Clinical characteristics of the study population stratified according to GDM using the IADPSG criteria

Variable	GDM (n=63)	No GDM (n=138)	*p*-value
Age (years)[a]	28.0 (24.0-32.0)	28.0 (24.0-32.0)	0.810
BMI (kg/m^2)[a]	27.4 (23.4-31.2)	25.8 (23.5-29.8)	0.180
Fasting glucose (mmol/L)[a]	5.5 (5.2-5.9)	4.3 (4.0-4.6)	< 0.001
OGTT 1 hr (mmol/L)[a]	6.5 (5.5-8.3)	5.5 (4.7-6.5)	< 0.001
OGTT 2 hr (mmol/L)[a]	6.1 (5.2-7.2)	5.2 (4.6-5.9)	< 0.001
HbA1c (%)[b]	5.2 (0.4)	5.1 (0.3)	0.008
Fasting insulin (mIU/L)[a]	6.5 (4.8-9.4)	5.7 (3.8-8.0)	0.067
HOMA[a]	1.6 (1.2-2.4)	1.1 (0.8-1.7)	< 0.001
C-reactive protein (mg/L)[a]	6.9 (3.7-9.9)	5.4 (3.1-8.5)	0.209
Adiponectin (µg/ml)[a]	7.6 (4.9-11.8)	9.8 (6.6-14.7)	0.018
Education: n (%)[c] <grade 12 ≥grade 12	 29.0 (46.7) 33.0 (53.3)	 66.0 (49.6) 67.0 (50.4)	0.769
Risk factors: n (%)[c] None ≥1 Risk factors n (%)	 27.0 (42.8) 36.0 (57.2)	 79.0 (57.3) 59.0 (42.7)	0.143

BMI: body mass index; OGTT: oral glucose tolerance test; HbA1c: glycated hemoglobin; HOMA: homeostatic model assessment.

Data are expressed as the [a]median (25th–75th percentiles); [b]mean ± standard error of the mean or as [c]count (percentage).

P-values for continuous data were calculated using the Mann-Whitney or the unpaired student *t* test.

P-values for categorical data were calculated using the Chi-square test.

Table 3: GDM defined using the IADPSG, NICE and WHO 1999 diagnostic criteria

Glucose concentration (mmol/L)									
	IADPSG			NICE			WHO 1999		
Time*	GDM	No GDM	*p*-value‡	GDM	No GDM	*p*-value‡	GDM	No GDM	*p*-value‡
0 hr OGTT	5.5 (5.2-5.9)[a]	4.3 (4.0-4.6)	< 0.001	5.8 (5.5-6.0)[b]	4.4 (4.0-4.8)	< 0.001	5.2 (4.7-5.4)[a,b]	4.5 (4.1-5.1)	<0.05
1 hr OGTT	6.5 (5.5-8.3)	5.5 (4.7-6.5)	< 0.001	-	-		-	-	
2 hr OGTT	6.1 (5.2-7.2)[c]	5.2 (4.6-5.9)	< 0.001	6.6 (5.6-8.0)[d]	5.3 (4.7-5.9)	< 0.001	8.6 (8.0-9.7)[c,d]	5.3 (4.7-6.0)	< 0.001

*Time after ingesting 75 g glucose drink; ‡significant difference between women with or without GDM in each diagnostic criteria; similar superscripts indicate significant difference between groups; OGTT: oral glucose tolerance test; hr: hour; GDM: gestational diabetes mellitus; IADPSG: International Association of Diabetes in Pregnancy Study Group; NICE: National Institute for Health and Care Excellence; WHO: World Health Organization (WHO 1999, Metzger *et al.* 2010, NICE guidelines 2015).

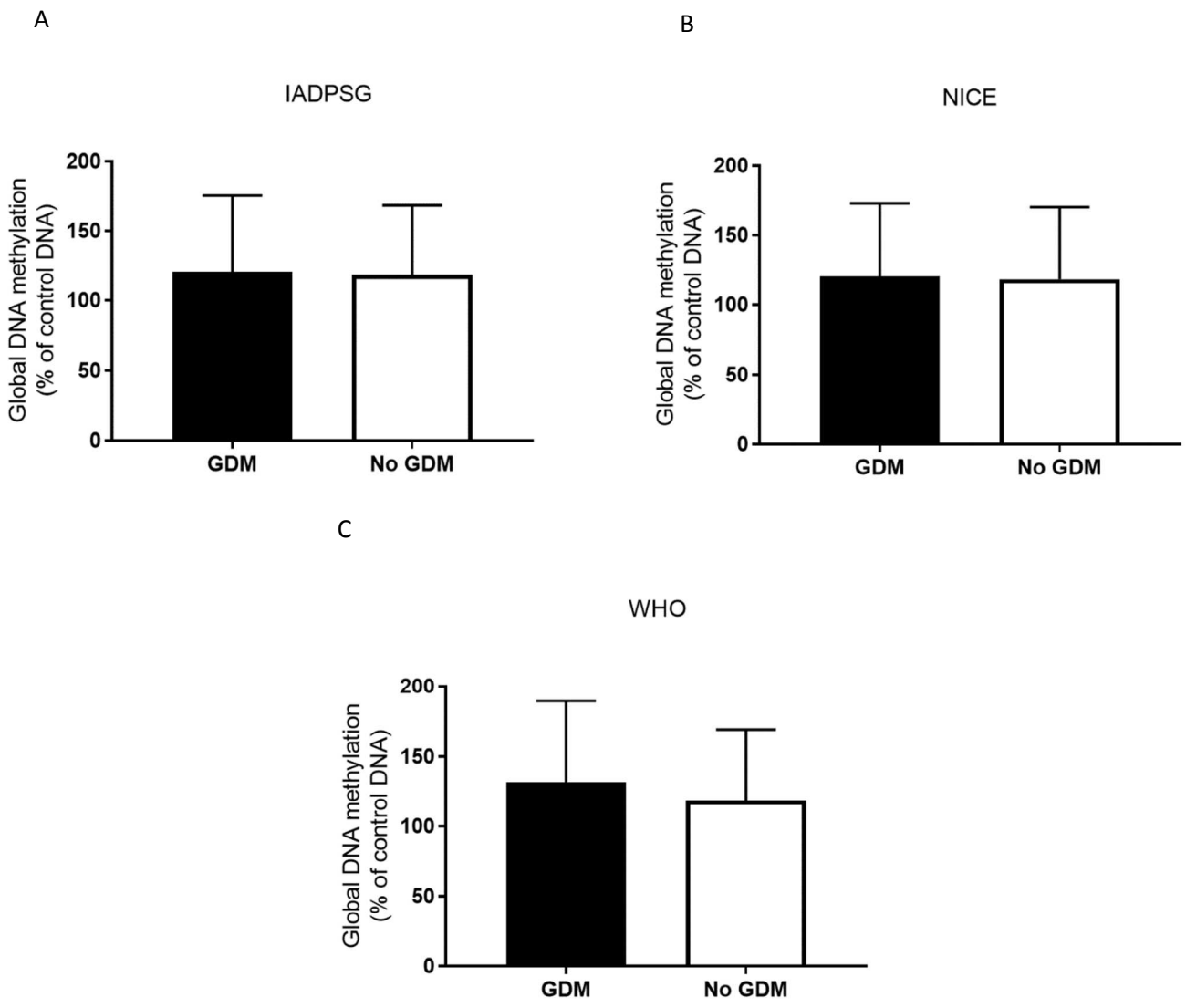

Figure 2. Global DNA methylation levels according to GDM status. Global DNA methylation was measured in the peripheral blood of women with or without GDM according to the IADPSG (A), the NICE (B) and the WHO (C) criteria, using the MDQ1 Imprint® Methylated DNA Quantification Kit. Global DNA methylation levels were calculated relative to the methylated positive control which was included in the kit. Data are represented as the mean ± standard error of mean (SEM).

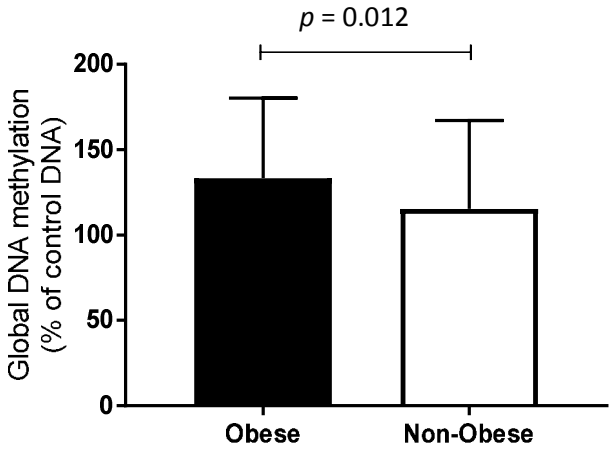

Figure 3. Global DNA methylation levels varies according to obesity status. Global DNA methylation was measured in the peripheral blood of obese (n=51) and non-obese (n=138) pregnant women using the MDQ1 Imprint® Methylated DNA Quantification Kit. Global DNA methylation levels were calculated relative to the methylated positive control which was included in the kit. Data are represented as the mean ± standard error of mean (SEM).

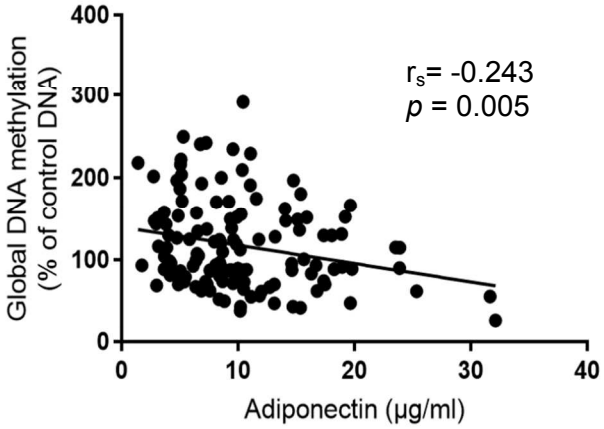

Figure 4. Global DNA methylation is inversely correlated with serum adiponectin concentrations. Each data point represents an individual (n=139) and indicates global DNA methylation levels relative to a methylated positive control and adiponectin concentration.

Discussion

This study investigated whether global DNA methylation profiling has potential as a screening tool for GDM in black South African women. Contrary to our hypothesis, global DNA methylation levels in the peripheral blood of black South African women were not associated with GDM. The IADPSG criteria is stringent, and the failure to observe differences in methylation between women with or without GDM may be due to small glucose concentration differences between groups. However, no difference in methylation was observed when GDM was classified according to NICE and WHO criteria, where glucose concentration differences between women with or without GDM were more pronounced. Previous studies have reported that global DNA methylation is associated with GDM (Nomura *et al.* 2014, Reichetzeder *et al.* 2016), however, these were conducted on placental tissue and used different methods to quantify global DNA methylation, possibly accounting for the discrepancies observed. Biological source affects global DNA methylation (Reinius *et al.* 2012), thus our failure to observe an association between GDM and global DNA methylation could be due to the use of peripheral blood rather than placenta. Furthermore, using liquid chromatography–mass spectrometry Reichetzeder *et al.* demonstrated that placental DNA methylation was increased during GDM (Reichetzeder *et al.* 2016), while using a luminometric methylation assay Nomura *et al.* reported that placental DNA methylation is decreased during GDM (Nomura *et al.* 2014), illustrating that method of quantification influences results. The ELISA, as used in this study, offers several advantages over other methods of quantifying global DNA methylation. It is cost-effective and does not require specialized equipment and expertise, making it more amenable for screening in low-and middle income countries (Kurdyukov and Bullock 2016). Several studies have reported that the ELISA is able to detect aberrant global DNA methylation patterns during disease

(Nakano *et al.* 2012, Keller *et al.* 2014, Kagohara *et al.* 2015, Ramos *et al.* 2016), and in response to environmental factors (Guerrero-Preston *et al.* 2010, Tellez-Plaza *et al.* 2014, Ivorra *et al.* 2015, Sánchez *et al.* 2015).

Global DNA methylation levels were higher in obese compared to non-obese pregnant women. It has been widely reported that global DNA methylation is associated with obesity (Cash *et al.* 2011, Jintaridth *et al.* 2013, Piyathilake *et al.* 2013, Na *et al.* 2014), however studies in pregnant women are limited (Herbstman *et al.* 2013, Nomura *et al.* 2014). Consistent with our results, Nomura *et al.* reported that global DNA methylation was higher in obese compared to non-obese pregnant women (Nomura *et al.* 2014). However, in contrast to our findings, Herbstman *et al.* reported that global DNA methylation is decreased during pre-pregnancy obesity, while Michels *et al.* failed to see an association between global DNA methylation and obesity (Michels *et al.* 2011, Herbstman *et al.* 2013). These findings confirm the variability in assessing global DNA methylation levels according to biological source and methods of quantification.

Intriguingly, global DNA methylation was inversely correlated with serum adiponectin concentrations. Adiponectin is an adipokine with insulin-sensitizing properties, which is dysregulated during obesity and metabolic disease (Cao 2014). Similar to our findings, several studies have reported that adiponectin concentrations are decreased during GDM (Lacroix *et al.* 2013, Pala *et al.* 2015, Adam *et al.* 2018). Recently, it was reported that altered methylation at the adiponectin gene *(ADIPOQ*) locus is inversely correlated with circulating adiponectin concentrations during pregnancy (Bouchard *et al.* 2012), and is associated with decreased *ADIPOQ* gene expression levels in adult offspring of women with GDM (Houshmand-Oeregaard *et al.* 2017). To further explore the significance of the association between global DNA

methylation and adiponectin, pyrosequencing of *ADIPOQ* is currently being conducted in our laboratory.

Although quantification of global DNA methylation is a robust method to assess overall genomic DNA methylation, and has potential as a biomarker to facilitate risk stratification and intervention (Ramos *et al.* 2016), it may not offer the resolution required to detect subtle methylation differences in women with or without GDM. Another limitation of the study is the use of peripheral blood, which consists of a mixture of different cell types such as erythrocytes, lymphocytes and platelets which may confound methylation analysis (Reinius *et al.* 2012). Future studies should consider purification of blood cell populations to separate specific cell types (Reinius *et al.* 2012). It has been widely reported that DNA methylation is affected by environmental factors such as diet, smoking, alcohol consumption and physical activity (Joubert *et al.* 2012, Lim and Song 2012, Ling and Rönn 2014, Pauwels *et al.* 2017, Miyake *et al.* 2018). Thus, the lack to account for these environmental factors, pose a significant limitation to our study. However, women were recruited from the same community with similar life experiences, suggesting that they were likely to have similar environmental exposures.

Conclusion

Contrary to our hypothesis, global DNA methylation was not associated with GDM in our population. Our findings suggest that despite being a robust marker of overall genomic methylation that offers opportunities as a biomarker, global DNA methylation profiling may not offer the resolution required to detect subtle methylation differences in the peripheral blood of women with GDM. Further studies are required to explore the candidacy of a more targeted approach using gene-specific methylation as a biomarker for GDM in our population. To our knowledge, this is the first study to investigate the association between global DNA methylation and GDM in South Africa.

Disclosure of statement

No potential conflict of interest was reported by the authors.

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References

Adam, S., Pheiffer, C., Dias, S., and Rheeder, P., 2018. Association between gestational diabetes and biomarkers: a role in diagnosis. *Biomarkers: Biochemical Indicators of Exposure, Response, and Susceptibility to Chemicals*, 23 (4), 386–391.

Adam, S. and Rheeder, P., 2017. Screening for gestational diabetes mellitus in a South African population: Prevalence, comparison of diagnostic criteria and the role of risk factors. *South African Medical Journal*, 107 (6), 523–27.

Bouchard, L., Hivert, M.-F., Guay, S.-P., St-Pierre, J., Perron, P., and Brisson, D., 2012. Placental adiponectin gene DNA methylation levels are associated with mothers' blood glucose concentration. *Diabetes*, 61 (5), 1272–1280.

Cao, H., 2014. Adipocytokines in Obesity and Metabolic Disease. *The Journal of endocrinology*, 220 (2), T47–T59.

Cash, H.L., McGarvey, S.T., Houseman, E.A., Marsit, C.J., Hawley, N.L., Lambert-Messerlian, G.M., Viali, S., Tuitele, J., and Kelsey, K.T., 2011. Cardiovascular disease risk factors and DNA methylation at the LINE-1 repeat region in peripheral blood from Samoan Islanders. *Epigenetics*, 6 (10), 1257–1264.

Damm, P., 2009. Future risk of diabetes in mother and child after gestational diabetes mellitus. *International Journal of Gynecology & Obstetrics*, 104 (suppl. 1), S25–S26.

Gu, T., Gu, H.F., Hilding, A., Sjöholm, L.K., Ostenson, C.-G., Ekström, T.J., and Brismar, K., 2013. Increased DNA methylation levels of the insulin-like growth factor binding protein 1 gene are associated with type 2 diabetes in Swedish men. *Clinical Epigenetics*, 5 (1), 21.

Guerrero-Preston, R., Goldman, L.R., Brebi-Mieville, P., Ili-Gangas, C., LeBron, C., Witter, F.R., Apelberg, B.J., Hernández-Roystacher, M., Jaffe, A., and Halden, R.U., 2010. Global DNA hypomethylation is associated with in utero exposure to cotinine and perfluorinated alkyl compounds. *Epigenetics*, 5 (6), 539–546.

Herbstman, J.B., Wang, S., Perera, F.P., Lederman, S.A., Vishnevetsky, J., Rundle, A.G., Hoepner, L.A., Qu, L., and Tang, D., 2013. Predictors and Consequences of Global DNA Methylation in Cord Blood and at Three Years. *PLOS ONE*, 8 (9), e72824.

Hod, M., Kapur, A., Sacks, D.A., Hadar, E., Agarwal, M., Di Renzo, G.C., Cabero Roura, L., McIntyre, H.D., Morris, J.L., and Divakar, H., 2015. The International

Federation of Gynecology and Obstetrics (FIGO) Initiative on gestational diabetes mellitus: A pragmatic guide for diagnosis, management, and care. *International Journal of Gynaecology and Obstetrics*, 131 Suppl 3, S173-211.

Houshmand-Oeregaard, A., Hansen, N.S., Hjort, L., Kelstrup, L., Broholm, C., Mathiesen, E.R., Clausen, T.D., Damm, P., and Vaag, A., 2017. Differential adipokine DNA methylation and gene expression in subcutaneous adipose tissue from adult offspring of women with diabetes in pregnancy. *Clinical Epigenetics*, 9 (13), 37.

Ivorra, C., Fraga, M.F., Bayón, G.F., Fernández, A.F., Garcia-Vicent, C., Chaves, F.J., Redon, J., and Lurbe, E., 2015. DNA methylation patterns in newborns exposed to tobacco in utero. *Journal of Translational Medicine*, 13, 25.

Jensen, D.M., Sørensen, B., Feilberg-Jørgensen, N., Westergaard, J.G., and Beck-Nielsen, H., 2000. Maternal and perinatal outcomes in 143 Danish women with gestational diabetes mellitus and 143 controls with a similar risk profile. *Diabetic Medicine*, 17 (4), 281–286.

Jintaridth, P., Tungtrongchitr, R., Preutthipan, S., and Mutirangura, A., 2013. Hypomethylation of Alu Elements in Post-Menopausal Women with Osteoporosis. *PLoS ONE*, 8 (8), e70386.

Joubert, B.R., Håberg, S.E., Nilsen, R.M., Wang, X., Vollset, S.E., Murphy, S.K., Huang, Z., Hoyo, C., Midttun, Ø., Cupul-Uicab, L.A., Ueland, P.M., Wu, M.C., Nystad, W., Bell, D.A., Peddada, S.D., and London, S.J., 2012. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environmental Health Perspectives*, 120 (10), 1425–1431.

Kagohara, L.T., Schussel, J.L., Subbannayya, T., Sahasrabuddhe, N., Lebron, C., Brait, M., Maldonado, L., Valle, B.L., Pirini, F., and Jahuira, M., 2015. Global and gene-specific DNA methylation pattern discriminates cholecystitis from gallbladder cancer patients in Chile. *Future Oncology*, 11 (2), 233–249.

Keller, M., Kralisch, S., Rohde, K., Schleinitz, D., Dietrich, A., Schön, M.R., Gärtner, D., Lohmann, T., Dreßler, M., Tönjes, A., Stumvoll, M., Kovacs, P., Fasshauer, M., Blüher, M., and Böttcher, Y., 2014. Global DNA methylation levels in human adipose tissue are related to fat distribution and glucose homeostasis. *Diabetologia*, 57 (11), 2374–2383.

Kurdyukov, S. and Bullock, M., 2016. DNA Methylation Analysis: Choosing the Right Method. *Biology*, 5 (1).

Lacroix, M., Battista, M.-C., Doyon, M., Ménard, J., Ardilouze, J.-L., Perron, P., and Hivert, M.-F., 2013. Lower Adiponectin Levels at First Trimester of Pregnancy Are Associated With Increased Insulin Resistance and Higher Risk of Developing Gestational Diabetes Mellitus. *Diabetes Care*, 36 (6), 1577–1583.

Lim, D.H.K. and Maher, E.R., 2010. DNA methylation: a form of epigenetic control of gene expression. *The Obstetrician & Gynaecologist*, 12 (1), 37–42.

Lim, U. and Song, M.-A., 2012. Dietary and lifestyle factors of DNA methylation. *Methods in Molecular Biology*, 863, 359–376.

Ling, C. and Rönn, T., 2014. Epigenetic adaptation to regular exercise in humans. *Drug Discovery Today*, 19 (7), 1015–1018.

Macaulay, S., Ngobeni, M., Dunger, D.B., and Norris, S.A., 2018. The prevalence of gestational diabetes mellitus amongst black South African women is a public health concern. *Diabetes Research and Clinical Practice*, 139, 278–287.

Mamabolo, R.L., Alberts, M., Levitt, N.S., Waal, H.A.D. de, and Steyn, N.P., 2007. Prevalence of gestational diabetes mellitus and the effect of weight on measures of insulin secretion and insulin resistance in third-trimester pregnant rural women residing in the Central Region of Limpopo Province, South Africa. *Diabetic Medicine*, 24 (3), 233–239.

Martín-Núñez, G.M., Rubio-Martín, E., Cabrera-Mulero, R., Rojo-Martínez, G., Olveira, G., Valdés, S., Soriguer, F., Castaño, L., and Morcillo, S., 2014. Type 2 diabetes mellitus in relation to global LINE-1 DNA methylation in peripheral blood: a cohort study. *Epigenetics*, 9 (10), 1322–1328.

Matsha, T.E., Pheiffer, C., Mutize, T., Erasmus, R.T., and Kengne, A.P., 2016. Glucose Tolerance, MTHFR C677T and NOS3 G894T Polymorphisms, and Global DNA Methylation in Mixed Ancestry African Individuals [online]. *Journal of Diabetes Research*. Available from: https://www.hindawi.com/journals/jdr/2016/8738072/ [Accessed 12 Jan 2018].

Metzger Boyed E, Persson B, IADPSG panel, and Gabbe SG, 2010. International Association of Diabetes and Pregnancy Study Groups Recommendations on the Diagnosis and Classification of Hyperglycemia in Pregnancy. *Diabetes Care*, 33 (3), 676.

Michels, K.B., Harris, H.R., and Barault, L., 2011. Birthweight, Maternal Weight Trajectories and Global DNA Methylation of LINE-1 Repetitive Elements. *PLOS ONE*, 6 (9), e25254.

Miyake, K., Kawaguchi, A., Miura, R., Kobayashi, S., Tran, N.Q.V., Kobayashi, S., Miyashita, C., Araki, A., Kubota, T., Yamagata, Z., and Kishi, R., 2018. Association between DNA methylation in cord blood and maternal smoking: The Hokkaido Study on Environment and Children's Health. *Scientific Reports*, 8 (1), 5654.

Na, Y.K., Hong, H.S., Lee, D.H., Lee, W.K., and Kim, D.S., 2014. Effect of Body Mass Index on Global DNA Methylation in Healthy Korean Women. *Molecules and Cells*, 37 (6), 467–472.

Nakano, K., Boyle, D.L., and Firestein, G.S., 2012. Regulation of DNA Methylation in Rheumatoid Arthritis Synoviocytes. *The Journal of Immunology*, 1202572.

Nanda, S., Savvidou, M., Syngelaki, A., Akolekar, R., and Nicolaides, K.H., 2011. Prediction of gestational diabetes mellitus by maternal factors and biomarkers at 11 to 13 weeks. *Prenatal diagnosis*, 31 (2), 135–141.

NICE guidelines, 2015. Diabetes in pregnancy: management from preconception to the postnatal period. [online]. Available from: https://www.nice.org.uk/guidance/ng3 [Accessed 15 Jun 2018].

Nomura, Y., Lambertini, L., Rialdi, A., Lee, M., Mystal, E.Y., Grabie, M., Manaster, I., Huynh, N., Finik, J., Davey, M., Davey, K., Ly, J., Stone, J., Loudon, H., Eglinton, G., Hurd, Y., Newcorn, J.H., and Chen, J., 2014. Global Methylation in the Placenta and Umbilical Cord Blood From Pregnancies With Maternal Gestational Diabetes, Preeclampsia, and Obesity. *Reproductive Sciences*, 21 (1), 131–137.

Pala, H.G., Ozalp, Y., Yener, A.S., Gerceklioglu, G., Uysal, S., and Onvural, A., 2015. Adiponectin levels in gestational diabetes mellitus and in pregnant women without glucose intolerance. *Advances in Clinical and Experimental Medicine*, 24 (1), 85–92.

Pauwels, S., Ghosh, M., Duca, R.C., Bekaert, B., Freson, K., Huybrechts, I., Langie, S.A.S., Koppen, G., Devlieger, R., and Godderis, L., 2017. Dietary and supplemental maternal methyl-group donor intake and cord blood DNA methylation. *Epigenetics*, 12 (1), 1–10.

Pheiffer, C., Dias, S., Muller, C., and Louw, J., 2014. Decreased global DNA methylation in the white blood cells of high fat diet fed vervet monkeys (Chlorocebus aethiops). *Journal of Physiology and Biochemistry*, 70 (3), 725–733.

Pinzón-Cortés, J.A., Perna-Chaux, A., Rojas-Villamizar, N.S., Díaz-Basabe, A., Polanía-Villanueva, D.C., Jácome, M.F., Mendivil, C.O., Groot, H., and López-Segura, V., 2017. Effect of diabetes status and hyperglycemia on global DNA methylation and hydroxymethylation. *Endocrine Connections*, 6 (8), 708–725.

Piyathilake, C.J., Badiga, S., Alvarez, R.D., Partridge, E.E., and Johanning, G.L., 2013. A Lower Degree of PBMC L1 Methylation Is Associated with Excess Body Weight and Higher HOMA-IR in the Presence of Lower Concentrations of Plasma Folate. *PLOS ONE*, 8 (1), e54544.

Ramos, R.B., Fabris, V., Lecke, S.B., Maturana, M.A., and Spritzer, P.M., 2016. Association between global leukocyte DNA methylation and cardiovascular risk in postmenopausal women. *BMC Medical Genetics*, 17, 71.

Rasanen, J.P., Snyder, C.K., Rao, P.V., Mihalache, R., Heinonen, S., Gravett, M.G., Roberts Jr, C.T., and Nagalla, S.R., 2013. Glycosylated fibronectin as a first-trimester biomarker for prediction of gestational diabetes. *Obstetrics & Gynecology*, 122 (3), 586–594.

Reichetzeder, C., Dwi Putra, S.E., Pfab, T., Slowinski, T., Neuber, C., Kleuser, B., and Hocher, B., 2016. Increased global placental DNA methylation levels are associated with gestational diabetes. *Clinical Epigenetics*, 82 (8).

Reinius, L.E., Acevedo, N., Joerink, M., Pershagen, G., Dahlén, S.-E., Greco, D., Söderhäll, C., Scheynius, A., and Kere, J., 2012. Differential DNA Methylation in Purified Human Blood Cells: Implications for Cell Lineage and Studies on Disease Susceptibility. *PLOS ONE*, 7 (7), e41361.

Sánchez, I., Reynoso-Camacho, R., and Salgado, L.M., 2015. The diet-induced metabolic syndrome is accompanied by whole-genome epigenetic changes. *Genes & Nutrition*, 10 (4), 21.

Smirnakis, K.V., Plati, A., Wolf, M., Thadhani, R., and Ecker, J.L., 2007. Predicting gestational diabetes: choosing the optimal early serum marker. *American journal of obstetrics and gynecology*, 196 (4), 410–e1.

Statistics South Africa, 2017. *South Africa demographic and health survey, 2016: key indicators report*.

Tellez-Plaza, M., Tang, W., Shang, Y., Umans, J.G., Francesconi, K.A., Goessler, W., Ledesma, M., Leon, M., Laclaustra, M., Pollak, J., Guallar, E., Cole, S.A., Fallin, M.D., and Navas-Acien, A., 2014. Association of Global DNA Methylation and Global DNA Hydroxymethylation with Metals and Other Exposures in Human Blood DNA Samples. Environmental Health Perspectives, 122 (9), 946–954.

WHO, 1999. Definition, diagnosis and classification of diabetes mellitus and its complications. Geneva: World Health Organization; 1999. [online]. Available from: http://apps.who.int/iris/handle/10665/66040 [Accessed 12 Jan 2018].