Occurrence of diabetogenic changes in pregnancy among black women in an urban setting

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Abtract

Objective: To find out if pregnancy in black Zimbabwean women is a diabetogenic state using basal blood levels of cortisol, insulin, C-peptide and glucose.

Methods: 111 women (28 non-pregnant, 29 first trimester, 26 second trimester and 28 third trimester) aged between 18 and 35 years were recruited for the study. Fasting plasma cortisol, insulin, C-peptide and glucose were determined by standard methods. The glucose/insulin ratio was used as an index of insulin sensitivity and the C-peptide/glucose ratio as well as the homeostasis assessment model (HOMA) as an index of insulin resistance.

Results: The means of fasting plasma cortisol levels were significantly elevated, $p < 0.0001$ among the four groups (non-pregnant, first, second and third trimester women). Fasting plasma insulin levels peaked during the third trimester and significant differences were noted among all women, $p < 0.05$. Similar data was obtained for C-peptide levels (a better indicator of beta-cell insulin secretory activity) among the groups, $p < 0.01$. The means of fasting plasma glucose levels were significantly decreased with advancing gestation, $p < 0.0001$. Significantly lower glucose/insulin ratios, a measure of insulin sensitivity and elevated C-peptide/glucose ratios, an index of insulin resistance, were demonstrated among the women, ($p < 0.05$ and < 0.01 respectively).

Conclusion: The basal data presented in this paper clearly demonstrates that the diabetogenic effects of pregnancy are also expressed by Zimbabwean black women, especially in late gestation.

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Introduction

It is widely accepted that there is an alteration in carbohydrate metabolism during pregnancy and many studies have demonstrated an impaired glucose tolerance with advancing gestation.[1,2] This deterioration may lead to the development of gestational diabetes in three to 11% of all pregnancies, depending on the population studied and the diagnostic criteria used.[3,4] Thus the affected women are unable to counteract the insulin resistance of pregnancy. Although this diabetogenic effect of pregnancy is well documented in Caucasian pregnancy,[5-7] data from black African women is less clear and the majority of the studies report an improved glucose tolerance.[8-10] A study in the Zimbabwean population suggested normal basal insulin secretion and clearance in all trimesters of pregnancy.[10] This is despite the fact that African-American pregnant women have been found to be more insulin resistant and that gestational diabetes is more prevalent in Afro-British women than in their Caucasian counterparts.[11,12]

Glucose homeostasis is normally maintained by a balance between insulin release and action as well as the counter regulatory hormonal responses. The impaired glucose tolerance in pregnancy may be due to defective enzyme-related peripheral utilisation of glucose or to the diabetogenic effect of other hormones such as human placental lactogen, (hPL) and cortisol. Fasting levels of blood insulin in Caucasian women are significantly elevated in late pregnancy.[1,7] There is scant data on the levels of these hormones in black populations.

Carbohydrate metabolism is a complex phenomenon that embraces numerous physiological and hormonal regulatory mechanisms. Although recent data suggest tumour necrosis factor-alpha (TNF-alpha) and leptin as important predictors of insulin resistance in human pregnancy,[14-16] it is still vital to further investigate the roles

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of placental anti-insulin hormones, the pituitary hormones, glucagon and the adrenal hormone cortisol in glucose metabolism and the pregnancy-related insulin resistant state.

The main objective of this study was to examine differences in insulin/C-peptide/glucose dynamics (for insulin sensitivity and resistance), using indices derived from basal plasma levels of insulin, C-peptide, and glucose in healthy Zimbabwean black pregnant and non-pregnant women. Plasma cortisol, a counter-regulatory hormone to insulin was also measured.

Materials and Methods

The study population was made up of urban, black pregnant women and healthy non-pregnant women from high density suburbs of similar age, socio-economic class, reporting at a private antenatal clinic in Harare. Socio-economic, demographic and lifestyle data was obtained by an interview. Women who had a previous pregnancy with a perinatal loss, a previous large infant (>4 kg), an excessive maternal weight gain; were obese (>30 kg/m^2); had a family history of diabetes or other endocrine disorders; were taking any medications, and were greater than 35 years of age were excluded from participation. In addition, non-pregnant women who were, or had been on hormonal contraception in the previous six months were not recruited for the study. The design and protocol of the study was explained to the women. All consecutive women who gave their informed written consent to participate in the study, and met our inclusion criteria were recruited. Permission to carry out the study was obtained from the local Medical Research Council.

The women were instructed to have a normal carbohydrate diet (at least 250 g carbohydrate) for at least three days before fasting blood samples were withdrawn from them. After an overnight fast of at least 12 hours, the women reported at the antenatal clinic the following morning (between 7.30 and 8.30), and they were allowed to rest for at least 30 minutes. Venous blood samples were obtained from all the participants and the blood was aliquoted into an EDTA vial for cortisol, insulin and C-peptide assays and a potassium oxalate/sodium fluoride vial for glucose analysis and immediately sent to the laboratory for separation of plasma.

Plasma glucose analysis was carried out within two hours of blood collection on a Beckman ASTRA 8 analyzer by the glucose oxidase method and the EDTA plasma was stored at -80° C. Cortisol, insulin and C-peptide analysis was performed within two months of sample collection. Cortisol measurement was performed using an AMERLEX competitive radioimmunoassay kit supplied by Ortho-Clinical Diagnostics (UK). The intra and inter assay coefficients of variation for the cortisol method were 4.3 and 7.7% respectively. A double antibody competitive radioimmunoassay kit from EURO/DPC LTD (UK) was used to determine C-peptide levels. Intra and inter assay coefficients of variation for the C-peptide method were 5.6 and 8.2% respectively. The Abbott IMX insulin assay, a microparticle enzyme immunoassay which shows no cross-reactivity with proinsulin was utilized to carry out plasma insulin measurement on an Abbott IMX analyzer. Intra and inter assay coefficients of variation for the insulin method were 3.1 and 3.8% respectively. The fasting plasma glucose/insulin ratio was used as an index of glucose sensitivity, whilst the fasting plasma C-peptide/glucose ratio and the homeostasis model assessment (HOMA-IR)[17] were used as indices of insulin resistance.

Assuming the prevalence of glucose intolerance to be 7.5% in non pregnant women and glucose intolerance of 50% in pregnant women with an a of 0.05 and a power of 95%, at least 26 subjects were needed in each test group. The reason for using 50% for the pregnant women is because there was no estimate for glucose intolerance from previous studies.

All continuous data are expressed as mean and standard deviation (SD). Differences between the means in the four groups of women (non-pregnant, first, second and third trimester) were tested with the t test by ANOVA for homogenous data and non-normal data by Kruskal-Wallis analysis. Statistical significance was set at a p value < 0.05. The World Health Statistical package, Epi-Info version 6 was used.

Results

One hundred and eleven pregnant and non-pregnant women, (28 non-pregnant, 29 first trimester, 26 second trimester and 28 third trimester) were recruited. The mean ages were 22.929 (±3.066), 24.897 (±2.498), 25.115(±4.236) and 24.643 (±3.674) years in non-pregnant, first trimester, second trimester and third trimester women respectively and the differences were not statistically significant, p= 0.073. The average age of the women was 24 years. All the women and their spouses had secondary education (maximum four years), as well as being in employment.

Table I shows the means and SD of fasting plasma glucose levels, which were significantly lowered with gestational age among the four groups, p < 0.0001. The inter-group comparisons were significant in all combinations except between women in the first and second trimesters. The fasting plasma cortisol levels gradually rose with advancing gestation reaching a peak in the third trimester, when they were three times higher than in non-pregnant women. The means of the cortisol levels among the four groups of women were significantly different p < 0.0001 (Table I). All possible comparisons among the groups were highly significant, except between women in the second and third trimesters.

Table I: Data of basal plasma glucose, cortisol, insulin and C-peptide levels in non-pregnant and pregnant women.

Group	No. women	Substance assayed Glucose Mean±SD	Cortisol Mean±SD	Insulin Mean±SD	C-peptide Mean±SD
Non-pregnant	28	4.1 (0.5)	10.4 (2.6)	5.5 (2.4)	1.16 (0.36)
1st trimester	29	3.8 (0.3)	15.5 (3.9)	4.7 (2.0)	0.91 (0.31)
2nd trimester	26	3.7 (0.4)	28.2 (8.6)	5.5 (2.1)	1.07 (0.30)
3rd trimester	28	3.5 (0.3)	33.5 (11.5)	6.4 (2.5)	1.19 (0.36)
p values of the comparison of means among the groups					
All groups		<0.0001	<0.0001	0.035	0.008
Group 1 vs 2		0.003	< 0.001	ns	0.007
Group 1 vs 3		0.002	<0.0001	ns	ns
Group 1 vs 4		< 0.0001	<0.0001	ns	ns
Group 2 vs 3		ns	<0.0001	ns	ns
Group 2 vs 4		0.001	<0.0001	0.005	0.003
Group 3 vs 4		0.014	0.055	ns	ns

Groups 1, 2, 3 and 4 represent the non-pregnant, first trimester, second trimester and third trimester women respectively. Units for glucose, mmol/L for cortisol, mg/100m for insulin, mU/ml and for C-peptide, ng/ml.

Table II: Derived data of insulin sensitivity and resistance in pregnant and non-pregnant women.

Group	n	Ratio of compared biochemical analytes Glucose/ insulin Mean±SD	C-peptide/ glucose Mean±SD	HOMA-IR Mean±SD
Non-pregnant	29	0.88 (0.37)	0.28 (0.09)	1.02 (0.48)
1st trimester	30	0.95 (0.41)	0.24 (0.08)	0.78 (0.35)
2nd trimester	26	0.77 (0.31)	0.29 (0.07)	0.92 (0.39)
3rd trimester	28	0.65 (0.31)	0.34 (0.10)	1.00 (0.41)
p values of the comparison of means among the groups				
All groups		0.011	0.001	0.130
Group 1 vs 2		ns	ns	0.038
Group 1 vs 3		ns	ns	ns
Group 1 vs 4		0.016	0.0201	ns
Group 2 vs 3		ns	0.032	ns
Group 2 vs 4		0.003	<0.001	0.035
Group 3 vs 4		ns	0.024	ns

Groups 1, 2, 3 and 4 represent the non-pregnant, first trimester, second trimester and third trimester women respectively.
Units for glucose, mmol/L, for insulin, mU/ml and for C-peptide, ng/ml.

Significant differences were demonstrated in the means of fasting plasma insulin among the four groups, $p < 0.05$ (Table I). The significance difference was attributed to differences during the first and third trimesters. Plasma insulin levels were at their lowest during the first trimester and peaked at the third trimester. The statistical data shows a similar pattern for fasting plasma C-peptide and insulin levels among the four groups of women. The means of the C-peptide levels among all the groups were significant, $p < 0.01$ (Table I), and this was due to differences between the non-pregnant women and those in the first trimester as well as between women in the first and third trimesters.

There were significant differences in the means of the fasting plasma glucose/ insulin ratios among all women, $p < 0.05$ (Table II). Decreased levels were evident during the second and third trimesters and were attributed to the variation in non-pregnant women and women in their last trimester as well as between women in the first trimester and the last trimester. There was a significant increase in the means of the C-peptide/glucose ratios with advancing gestation among the four groups, ($p < 0.01$). All possible comparisons among the groups were significant, except between non-pregnant women versus first and second trimester women. Although there was a gradual increase in the means of HOMA-IR levels with gestation, no significant differences were demonstrated among the four groups ($p > 0.05$), Table II. However, inter-group comparisons showed significant differences between non-pregnant and first trimester women as well as between the first and third trimester women.

Discussion

Our data demonstrates improved glucose homeostasis in the first trimester of pregnancy and an insulin resistant state in late pregnancy. Significant gestational age-related alterations in blood cortisol, insulin, C-peptide, and glucose levels were observed in the pregnant women. All the blood analytes, except glucose, exhibited a gradual elevation with advancing gestation. The gradual decline in fasting plasma glucose levels as gestation progresses could be partly due to physiological haemodilution of pregnancy. It may be useful to re-evaluate the reference ranges of fasting glucose used in the diagnosis of glucose intolerance and gestational diabetes in this population, since the current ranges are derived from the general population. The reported hormonal changes in the current study can only contribute to, or worsen the insulin resistant state associated with pregnancy. Our results clearly suggest that the diabetogenic effects of pregnancy are also present in urban, black Zimbabwean women.

Insulin plays a central role in human gestation and alterations that result in decreases in maternal insulin sensitivity as pregnancy advances have been reported.[18] C-peptide levels are a useful measure of insulin secretion and are expected to be elevated when there is pancreatic beta-cell hyperfunction, and thus can be used to evaluate glucose tolerance. In Caucasian pregnancy fasting plasma insulin is elevated in late pregnancy compared with normal pregnant women[13] and our results demonstrate a significant elevation of both basal plasma insulin (16%) and C-peptide levels (26%) with advancing gestation. As fasting plasma insulin levels can be used as a surrogate of insulin resistance, these results confirm the presence of an insulin resistant state in pregnancy.

Cortisol, an anti-insulin hormone, is known to induce a wide range of (placental) enzymes and some of these could be responsible for carbohydrate and lipid metabolism.[19] The elevated blood cortisol levels of pregnancy in this population may promote visceral fat deposition, which is known to stimulate insulin resistance, hence

hyperinsulinaemia and glucose intolerance. Some studies have suggested that the glucocorticoid, cortisol may be responsible for the development of peripheral and hepatic insulin resistance.[20, 21]

The hypercortilism of pregnancy could arise from the low levels of glucose in late pregnancy due to the persistent stimulation of the hypothalamic-pituitary-adrenal axis. It is also possible that the counter regulatory cortisol response to fasting-hypoglycaemia in pregnancy is diminished, and this may contribute to the low fasting blood glucose levels observed with advancing gestation in the study women. Caucasian pregnancy is associated with a two-fold increase in blood cortisol,[22] however, we found a three-fold elevation in black pregnancy. Differences in hypothalamic-pituitary-adrenal axis in black and white women have previously been reported,[23] but the physiological significance of these differences has not been investigated.

The indication that the above changes in hormone levels are a result of insulin resistance is confirmed by the significant decrease of the glucose/insulin ratio and the elevation of the C-peptide/glucose ratio, measures of insulin sensitivity and insulin resistance, respectively. Although HOMA-IR is a non-invasive and convenient method for measuring insulin resistance, it appears that it is not a very sensitive model to use in pregnancy. This is in agreement with recent data from studies investigating clinically useful estimates of insulin sensitivity during pregnancy.[24, 25]

The limitation to the study is that insulin production and resistance were measured indirectly, rather than using C-peptide decay kinetics and the hyperinsulinaemic clamp technique, as this was not possible under the conditions of our study. However, indices of insulin sensitivity and insulin secretion have been found to strongly correlate with estimates from hyperinsulinaemic clamp studies.[26] It was also not possible to follow the same women from the first to the third trimester for compliance and follow up reasons, since the majority of the women were reluctant to have their blood withdrawn in each trimester. As a result the women were age-matched.

Conclusion

Our data confirms reduced insulin sensitivity and elevated insulin resistance with advancing gestation, suggesting that the diabetogenic effects of pregnancy are also present in urban black Zimbabwean women. Further studies to establish the insulin resistant state during pregnancy, such as the effects of elevated hormones like placental lactogen, progesterone, oestrogen and growth hormone, as well dietary effects are essential to confirm these findings. It may be useful to re-evaluate the reference ranges of fasting blood glucose levels used in the diagnosis of glucose intolerance and gestational diabetes in this population, since the current ranges are derived from the general population.

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