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Proposed Relationships between the Degree of Insulin Resistance, Serum Chromium Level/BMI and Renal Function during Pregnancy and the Pathogenesis of Gestational Diabetes Mellitus

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Abstract

Aims: A retrospective, observational, case control, pilot study investigating the relationship between insulin resistance (IR), renal function and maternal serum chromium (Cr) level in pregnancy.

Methods: 115 pregnant women in their first 12 weeks to 40 weeks were initially recruited for the study, involving an oral glucose tolerance test (oGTT) from the Day Assessment unit at Derriford Hospital using the 75 g standard glucose load recommended by WHO. 101 patients were at least 28 weeks gestation, 10 were diagnosed with gestational diabetes mellitus (GDM), 8 of these were included in the study. 34 of these patients at over 28 weeks gestation were further tested for urine and serum Cr and creatinine (GDM, n=8; non-GDM, n=26) and insulin (n=70), IR was calculated. The diagnosed GDM group (n=8) was compared to the normoglycaemic group (n=26) and control group of non-pregnant, normoglycaemic controls (n=8). No statistical significance observed between the groups with respect to age or BMI.

Results: Correlation was found between IR and serum Cr/BMI in the whole patient group (p=0.009; R=0.435, n=34) and within the non-GDM patients (R=0.416; p=0.01, n=26 two tailed) but not within the GDM patients (n=8) alone.

There was significant correlation in the GDM group only for increased urine creatinine associated with urine Cr levels (p=0.049, R=0.709, n=8) at the 0.05 two-tailed level.

Conclusion: Increased IR during pregnancy was related to an increase in serum Cr /BMI levels in non-GDM pregnant women. Deficiency of Cr status may be a pathogenic factor in the development of GDM. We propose a mechanism.

Keywords: Insulin resistance; Chromium; Gestational diabetes; Birth weight; Renal function; Creatinine

Abbreviations: IR: Insulin Resistance; Cr: Chromium; GDM: Gestational Diabetes Mellitus; GTT: Glucose Tolerance Test; GTF: Glucose Tolerance Factor

Introduction

Over the last 14-16 years, the incidence of gestational diabetes mellitus (GDM) has paralleled the epidemic of obesity by doubling in its occurrence. It has increased the risk for glucose intolerance and obesity in the offspring and the development of type 2 diabetes (T2DM) and macrosomia in the mother [1].

During a normal pregnancy, the foetus must receive a sustained supply of glucose and for this eventuality to occur, certain physiological, metabolic, biochemical, haematological and immunological changes must take place [2]. In order to maintain maternal levels of glucose between meals there must be a decrease in glucose tolerance work and in the peripheral utilisation of glucose by maternal tissues. The supply of glucose to the foetus is vital and a balance must be established between this and that of sustaining maternal normoglyceamia. Sometimes this balance is not achieved or counterbalanced by increased insulin secretion [3]. The principal energy substrate for the placenta and the fetus is glucose and is essential for normal fetal metabolism and growth. A complex set of mechanisms regulates the supply of this substrate to the tissues, maintaining a relatively constant metabolism [4].

Insulin resistance (IR) is one of those mechanisms of adaption in the mother. The decreased action of insulin on body tissue at normal concentrations of insulin in the plasma is described as IR. A number of factors are associated with this, such as, defective molecular structure of insulin, defective receptor functioning or a defective signal transduction pathway. If the condition is acquired then it is related to a change in receptor affinity or signaling induction pathway. The β-cell in the islets of Langerhans increases the production of insulin to compensate for insulin resistance resulting in hyperinsulinemia. β-cells cannot increase insulin production indefinitely and as insulin production declines, the condition progresses to glucose intolerance and subsequently, to diabetes mellitus [5]. Maternal IR leads to an increased use of fats rather than carbohydrates for energy by the mother and spares carbohydrates for fetus. Maternal glucose concentration is increased by increasing rates of maternal glucose production as well as the development of relative maternal glucose intolerance and IR. The transfer of maternal glucose to the fetus by the placenta is buffered by placental glucose utilization and finally, the production of insulin by the developing fetal pancreas enhances glucose utilization among the insulin-sensitive tissues (skeletal muscle, liver, heart, adipose tissue). Fetal mass and thus glucose need increases during late gestation [4].

In a normal pregnancy there is accretion of adipose tissue followed by increased IR. Insulin sensitivity is known to fall by 45-75% by the 28th week of pregnancy when compared to the first 13 weeks and with the 13th to the 27th week of gestation and this has been reported to increase gradually through these first and second trimesters, suggesting that there is progressive rise in insulin secretion as the pregnancy advances [6]. Normally in pregnancy, there is an approximate 50% decrease in insulin-mediated glucose disposal and a 200% to 250% increase in insulin secretion to maintain euglycemia in the mother [7].

Eventually, in some cases, beta-cell function in the production of insulin is unable to increase sufficiently to maintain normal glucose tolerance, although extremely high levels of insulin can still fail to maintain normoglycaemia during pregnancy. A number of factors are attributed to this, such as, increase in the levels of estrogen, progesterone and human placental lactogen (hPL) among other factors [8,9].

As the pregnancy advances, progesterone increases in concentration and is associated with increased inhibition of insulin-induced GLUT4 translocation and glucose uptake. There are also high estrogen concentrations in pregnancy and 17β-estradiol diminishes insulin sensitivity at high concentrations [10].

There is a distinct difference between the IR seen in pregnancy and that associated with T2DM in that the former is of short duration. The development of impaired glucose tolerance and T2DM that succeeds increased IR, however, occurs over many years and may be decades. Overall, women who develop GDM are a more IR group than women who remain glucose tolerant [11].

A coexisting insulin secretary problem may also be important in the development of GDM. Interestingly, male foetuses induced lower mean adjusted β-cell function in the mother and are associated with poorer β-cell function, higher postprandial glycaemia, and an increased risk of GDM. Thus, fetal sex potentially may influence maternal glucose metabolism in pregnancy [12].

The resultant hyperglycaemia from increased IR can be diverted to the foetus via the placenta as an augmented nutritional supply resulting in increased foetal growth or macrosomia. The frequency of macrosomia has been found to increase in previous studies but was not found to be statistically significant [13].

In considering the role of chromium (Cr) in insulin sensitivity during pregnancy, it is noted that the biochemistry of Cr has proved to be an enigma for the last fifty years since its discovery [14,15]. The establishment of a link between the nutrient Cr and the symptoms at a molecular level has remained controversial [16]. It's essential role in human nutrition was suggested when it was found that Cr supplementation reversed glucose intolerance in hospitalized patients receiving long-term total parenteral nutrition [17,18].

There are two biomolecules that to are known to bind Cr: transferrin and low-molecular weight Cr-binding substance (LMWCr) [19]. The latter has been proposed to be biologically active and has the ability to potentiate the activity of insulin-activated insulin receptor *in vitro*. Cr increases tissue sensitivity and potentiates *in vivo* action of insulin by increasing insulin receptor expression of cell surface membranes and/or by increasing insulin receptor affinity for its ligand [20,21]. The binding of insulin to the cell surface may be increased in glucose intolerant individuals by administration of Cr. This has been seen on human erythrocytes obtained from individuals who are glucose intolerant nutritionally supplemented with Cr, where insulin binding was elevated, indicating that Cr increases insulin binding to the cell surface. There is also an increase in insulin receptor number [20]. The skeletal muscle of obese KK/HlJ diabetic mice had enhanced insulin signalling after the administration of Cr [21]. Once insulin binds to the α-subunit of the insulin receptor, there is a specific phosphorylation of the β-subunit through a cascade of intermolecular phosphorylation reactions. Tyrosine kinase is the enzyme responsible for the phosphorylation that leads to an increase in insulin sensitivity and Glucose Tolerance Factor (GTF) binding activates this [22-24].

GTF is synthesized *in vivo* from absorbed dietary Cr, binding to insulin and acting as a physiological enhancer of insulin activity, potentiating its action by about three-fold [22]. The role of GTF, in insulin potentiation appears to occur after the hormone binds to its receptor. Cr potentiates insulin at, or before, the transport of carbohydrate into the cell. In rats, insulin-mimetic and insulin-potentiating effects have been induced by GTF, both *in vivo* and *in vitro* [23-26].

The insulin-like effect acts downstream of the insulin receptor on cellular signals. GTF induced a dose-dependent increase in deoxy-glucose transport into myoblasts and fat cells similar to insulin. The cellular effect of GTF on the insulin-signaling pathway was investigated *in vitro* in rats. GTF produces an insulin-like effect by acting on cellular signals downstream of the insulin receptor [23-25].

Historically, it has been difficult to identify the Cr status of an individual, as little is known about how the metal is stored in tissue and serum levels are extremely variable between individuals. Attempts have been made to address this by analysing hair samples for Cr and differences have been identified between diabetic and non-diabetic groups [27,28]. Individuals with diabetes appear to have lower Cr levels in hair than those that are asymptomatic. There appears to be an age-related decline in Cr levels in hair samples. Levels of Cr in the hair of pregnant women have been found to be lower in those with GDM than normal pregnancies. Hair Cr analysis is thought to give an indication of Cr status [28].

Factors affecting tissue levels of Cr, such as, diet and losses due to kidney function may be factors to consider. Urine creatinine can be an indication of the glomerular filtration rate, the leakage of protein through the kidney and thus an indication of renal function [29-31]. Thus creatinine in the urine can thus be associated with a renal lesion and its increase could be a possible cause of excessive Cr loss in patients with diabetes. Plasma creatinine and urea concentrations are known to decrease during pregnancy due to a haemodilution effect caused by increases in blood volume in the mother. As maternal haemodynamics change due to pregnancy, urine levels increase with the increase in glomerular filtration rate and raised blood pressure possibly contributing to increased Cr losses to the mother [30].

This case control pilot study preluded a longitudinal prospective study of mothers and babies where patients were recruited in the summer of 2000 and examines the data retrospectively for protective factors against GDM. The pilot study was set up to test a protocol for the larger study of mothers and babies, where both groups were to be followed up over a ten year period and onwards, observing the effects of hyperglycaemia on the participants. Glucose tolerance, insulin resistance and the onset of diabetes, in both mothers and offspring, were of particular interest. Identifying epigenetic events that may occur in those affected was also part of the study and will be reported on at a later date.

HbA1c, fasting and postprandial blood sugar, blood lipids, creatinine (urine and serum), diet analysis, gestational age at birth and birth weight were all collated for this study. Only serum and urine Cr, glycaemic status, insulin resistance and creatinine are considered and compared in this paper. Although lipids and diet were analysed in the original pilot study observations, these variables were not tested in the subsequent prospective, longitudinal study in relation to glycaemia and insulin resistance.

Patients

The case control study recruited patients who were pregnant with a high random glucose test during routine tests at several GP practices in

the Plymouth, Southwest area. The patients were referred because they presented with glycosuria, had previous glycosuria, random blood test with raised glucose levels, a history of hyperglycaemia, macrosomia or a medical history of GDM. All attendants to the clinic for this procedure were interviewed and logged onto the database as potential participants for the study. Patients in their 28th to 40th week gestation were identified for further testing but patients with blood borne diseases were excluded from the study. Patients with conditions that affect the test results as outliers, such as, polycystic ovary syndrome (insulin) were also excluded. One postnatal patient attended the clinic for oGTT but was not included for further analysis.

115 patients were scheduled to be given an oral Glucose Tolerance Test (oGTT) at the Day Assessment Ward in the maternity department at Derriford Hospital, Plymouth using the 75 g standard oral glucose load recommended by WHO for GDM diagnosis and were recruited for the case control study. Patients referred to the unit had fasted since midnight (except for water). All patients were assessed for fasting blood glucose (FBS) and postprandial glucose (BS2). Those patients with postprandial glucose of >7.8 were diagnosed as GDM patients. Some patients in their third trimester, were further tested for insulin and Cr although one patient was excluded because she was HIV positive. They were all informed both orally and by a written document of the purposes of the study and gave signed consent to participate. The South West Local Research and Development Ethics Committee approved the study.

96 patients were in their 28th week of gestation and over, 11 were between 13 to 27th week of pregnancy and 3 in their first 13 weeks of gestation. Out of the 96 patients, 10 patients at 28 weeks and over gestation were diagnosed as having GDM. Although 70 patients (both GDM and non-GDM) were further tested for insulin (not standard practice with oGTT patients), 8 patients with GDM and 26 of the normoglycaemic patients were included and tested for Cr and creatinine levels in blood and urine, resulting in a matched group of patients. One of the patients with GDM had polycystic ovary syndrome and was excluded from the final analysis, as their insulin levels (161 μIU/ml) were several times higher than any of the other patients, thus was considered to be an outlier in the data. Another of the 10 patients with GDM was HIV positive and was also excluded for further testing (heroine user) and was excluded from the study resulting in 8 patients with GDM being included. The included patients had their insulin resistance calculated and Cr levels standardised with the patient BMI. 34 of the patients who were 27-40 weeks gestation were successfully analysed for Cr levels in serum and urine in the spectroscopy laboratory in Plymouth University (26 non-GDM; 8 GDM).

The baby's gender, gestational age at birth and birth-weight were also recorded after the mother's confinement. The birth weights were corrected according to the gestational age at birth using a Cole correction.

Control Group

We also had a control group of eight volunteers who agreed to undertake an oGTT of normal, healthy, non-pregnant controls, recruited from the members of staff from the PCMD research department, John Bull building, Derriford, Plymouth; insulin was analysed for 8 of these controls and 7 of them had Cr levels determined (one sample was lost during testing). Urine Cr and creatinine were also compared. These control individuals signed a written consent form and were fully informed of the procedures involved and the reasons for the study, as required by ethics approval.

Methods

Glucose measurements were determined by enzymic reference method with hexokinase and insulin by an immunometric assay using a chemiluminescent substrate at Derriford Hospital Combined Laboratory. Creatinine was determined spectraphotometrically.

IR was determined using the HOMA calculation in 60 patients of the 3rd T patients and 8 non-pregnant controls.

A graphite furnace atomic absorption method was employed to measure Cr levels in blood and urine in the analytical laboratory of the Department of Environmental Sciences Department of Plymouth University.

The maternal pre-pregnancy weight was recorded and BMI was calculated from the patients' height and pre-pregnancy weight details measured at the beginning of the pregnancy by the midwife. This record of weight was considered to be a more stable indication of a patient's metabolic status and individual build, whereas the pregnant weight was subject to many variables, such as, differences in blood volume, water retention, amount of amniotic fluid, foetal weight and the exact day of gestation.

Birth weight was corrected as an estimate of the expected weight at term by using average weight gain per day and gestational age at birth. All corrected weights approximate the expected weight at 40 weeks gestation.

The results were analysed statistically with ANOVA and a bivariate Pearson correlation or linear regression within 95 and 99% confidence limits using packages on an SPSS program. Statistical significance was defined as when the p value=0.05 or less at a 95% confidence limit. The p value was corrected for the number of groups in comparison to ensure a reliable indication of significance.

The university medical statistician (Dr Steve Shaw) performed a power calculation to determine the optimum sample sizes that were required to find significant statistical difference in this cohort of patients. We recruited 115 patients in order to achieve the number of patients with GDM past their 27th week gestation required for statistical significance. The statistician predetermined the optimum number of participants needed for this study of patients (n≈100) and controls (n≈8).

Relationships were explored within the data and also by further dividing the 3rd T group into three groups according to their insulin values and made some comparisons. (Group 1=0-<10 mmol/l; Group 2=10-<20 mmol/l; Group 3= >20 mmol/l). Similarly, postprandial glucose groups were subdivisions of the 3rdT group used to compare the data (Group 1= >3-5; Group 2= >5-<7; Group 3= >7) (Figure 1).

Results

The average age of the non-GDM group was 27.85 ± SEM 0.667; the GDM group was 30.70 ± SEM 1.453 (Table 1). The mean BMI of the GDM group was 23.70 ± 1.45; that of the non-GDM group was 24.89 ± SEM 0.772 and the control group was mean 32.63 ± SEM 4.01 (Table 2). There was no statistically significant difference between these three groups, which may reduce confounding factors resulting from gender or age differences. We did not account for socioeconomic or environmental differences but the diet analysis contribute to addressing this confounding effect. The effects of diet are not reported in this paper.

The BS2 was significantly higher in the GDM group than the normoglycaemic group (GDM mean 8.69 ± 0.49, non GDM mean 5.68 ± 0.11, p=0) and there was also significant difference for this between the pregnant and non-pregnant groups (pregnant mean 6.00 ± 0.15, non pregnant mean 4.72 ± 0.18, p=0.024).

The GDM group was statistically higher for insulin than the other normoglycaemic groups (mean 30.47 ± 16.43, p=0.023). Related to this, IR was higher in the GDM group than the normoglycaemic groups (mean 11.53 ± 5.92, p=0.003). As anticipated, the GDM group had a higher HbA1c than the normoglycaemic group (GDM, mean 5.43 ± 0.10; 3rd T non-GDM, mean 5.05 ± 0.04, p=0.002) (Tables 3 and 4).

Study No.	Sex	BW/ gms	Cole Corr. BW/gms	GA at birth/ days	BMI/ Kg/ m²	FBS/ mmol/L	BS2/ mmol/L	HbA1c/ %	HbA1c/ mmol/mol	Insulin/ µIU/ml	IR	CrS/BMI
7	F	3620	3646	278	23.00	4.50	7.2	4.9	30.1	11.5	3.68	0.0113
10	F	3120	2982	293	46.00	4.10	6.5	5.2	33.3	12.9	3.73	0.0096
15	M	3850	3642	296	26.00	3.80	5.8	4.8	29.0	9.6	2.47	0.0204
23	M	3740	3922	267	25.00	5.50	7.3	5.8	39.9	14.4	4.67	0.0116
26	F	3205	3094	290	23.00	3.90	4.9	4.7	27.9	9.0	1.96	0.0000
41	F	3780	3649	290	28.00	4.30	5.6	5.0	31.1	12.8	3.19	0.0000
56	M	4060	4118	276	22.00	4.00	4.2	5.1	32.2	9.4	1.75	0.0000
57	M	3750	3596	292	24.00	4.40	6.6	3.9	19.1	8.5	2.49	0.0000
59	F	3080	3147	274	24.00	4.40	6.2	4.7	27.9	10.6	2.92	0.0000
61	M	4100	4028	285	26.00	3.70	7.4	4.9	30.1	9.6	3.16	0.0100
62	F	3315	3168	293	22.00	4.20	5.1	5.4	35.5	2.6	0.59	0.0000
71	M	2280	2418	264	19.00	4.30	5.1	4.9	30.1	5.6	1.27	0.0000
73	F	3400	3364	283	20.00	3.20	4	4.8	29.0	5.3	0.94	0.0085
80	M	2800	2947	266	31.00	3.90	7.7	4.9	30.1	13.0	4.45	0.000
85	F	3150	3219	274	21.00	4.70	6.4	-	-	12.7	3.61	0.000
86	F	3630	3709	274	20.00	3.90	4.2	-	-	10.7	2.00	0.000
92	M	4460	4306	290	23.00	4.20	4.4	4.9	30.1	21.1	4.13	0.0126
93	F	3955	4012	276	23.00	4.40	5.4	4.8	29.1	25.4	6.10	0.0130
96	F	3680	3747	275	20.00	4.70	4.6	5.2	33.3	21.8	4.46	0.0160
98	F	4210	4195	281	19.00	4.20	4.3	4.4	24.6	8.6	1.64	0.0000
100	F	4080	4008	285	22.00	4.90	5	5.3	34.4	11.8	2.62	0.000
102	M	3820	3861	277	29.00	4.80	5.3	5.3	34.4	19.1	4.50	0.0138
106	M	3060	2914	294	21.00	3.50	6.9	5.0	31.1	12.6	3.86	0.0119
108	F	3770	3717	284	30.00	4.40	5.5	5.3	34.4	10.7	2.62	0.0133
111	M	4000	4103	273	28.00	4.60	5.1	5.0	31.1	10.2	2.31	0.0054
114	M	4570	4323	296	25.00	4.00	7	4.6	26.8	15.2	4.73	0.0088
n=26												

Table 1: Normoglycaemic patient data. It shows the laboratory test results and measurements taken for normoglycaemic pregnant patients in their third trimester, presenting at Derriford Hospital Maternity Unit for oGTT. They were referred to the hospital due to a raised random blood sugar test in a routine clinic visit but after the oGTT were found to be were normoglycaemic. 86 tested for FBS, 85 for BS2 and 60 of these patients were tested for insulin levels and 26 of these were randomly selected to be further tested for Cr and analysed by SPSS. These 26 were included for comparison in the study.
GA is gestational age at birth; BW is birth weight (Cole BW has been corrected for gestational age); the sex of the baby is also recorded (F-female, M-male). IR is insulin resistance; FBS is the fasting blood sugar; BS2 in the postprandial serum glucose; CrS/BMI is the serum chromium corrected for BMI. The average age of the non-GDM group was 27.85 ± SEM 0.667. The mean BMI of the non-GDM group was 24.89 ± SEM 0.772

Patient Study No	Sex	BTHWT/ gms	Cole BW/ gms	Gest at birth (days)	BMI	FBS/ mmol/L	BS2 mmol/L	HbA1c/ %	HbA1c/ mmol/mol	IR	Insulin/ µIU/ ml	CrS/ BMI
2	F	3475	3320.82	293	36.00	5	7.9	5.9	41.0	6.57	18.70	0.0067
9*	F	4260	4229.78	282	23.00	4.4*	8.1*	5.2	33.3	57.96*	161.00*	0.0209*
11	M	3960	4129.79	269	22.00	4.2	8.1	5.4	35.5	3.24	9.00	0.0155
40	F	3270	4380.86	209	20.00	6.1	12.9	5.8	39.9	14.56	25.40	0.0150
46	M	3580	3492.68	287	25.00	4.3	8.8	5.5	36.6	-	-**	-**
77	M	3420	3432.26	279	20.00	3.6	7.8	-	-	3.99	11.50	0.0080
91	F	3515	3785.38	260	23.00	4.4	8.2	5.5	36.6	3.83	10.50	0.0078
110	F	3780	3687.81	287	22.00	3.7	8.1	5.0	31.1	2.74	7.60	0.0000
113	M	2710	2769.34	274	24.00	4.3	9.2	5.5	36.6	7.77	19.00	0.0000
115	****	****	****	280	32.00	4.9	7.8	5.1	-	4.7	11.5	0.0088
6****	***	***	***	284	31.00	6.8	13.1	6.0	-	-	15.5	***
n=	9	9	9	11	11	11	11	10	8	9	10	9
Included in study	8	8	-	-	8	8	8	8	-	8	8	8

Table 2: Laboratory test results for glucose, insulin and chromium of patients diagnosed with GDM 3rdT
*Patient with polycystic ovary syndrome and outlying insulin level (patient number 9) excluded from further analysis.
**Patient HIV +ve
***1st Trimester diagnosed with GDM (11 weeks gestation)
****Variable not reported

Diagnosis	Serum Cr/BMI × 10 mg/L ± SEM	Cr n=	Insulin/mmol/L ± SEM	Insulin n=	IR ± SEM	Av BMI Kg/m² ± SEM
Non-GDM	6.41 ± 1.24	26	12.10 ± 0.75	26**	3.13 ± 0.22	24.9 ± 0.8
GDM	8.20 ± 2054	8	30.47 ± 16.43	8	11.63 ± 5.91	23.7 ± 1.5
All patients	6.59 ± 1.11	34	14.81 ± 2.53	34	4.41 ± 0.95	24.7 ± 0.7
Controls	7.41 ± 2.78	7*	8.62 ± 1.53	8	1.76 ± 0.28	22.6 ± 0.9

Table 3: Mean values table for IR with serum Cr and BMI respectively.
*One control sample unsuccessfully tested for serum Cr.
**26 patients were randomly selected for serum Cr out of the total non-GDM cohort and compared for insulin when included in the statistical analysis

Serum chromium is not necessarily the most effective way of assessing the chromium status of patients. BMI is an indicator of the tissue quantity (weight) over the skeletal frame (height), including adipose tissue and muscle. BMI is known to increase blood volume and to reduce the concentration of serum metal ions, such as, iron and zinc. We postulated that serum Cr and Cr status correlate in a linear manner, influenced, in part, by BMI. In order to make the serum chromium levels more relative to chromium status and comparable between individuals taking into account the effects of BMI and to standardise serum Cr levels between individual values, Cr values were divided by the patient BMI, as each patient has their own set of variables involving height, weight, muscle/adipose mass, and Cr status at tissue level.

Diagnosis	Insulin, R=	P=	IR, R=	p=	n=
Non-GDM	00.516	<0.01	0.503	<0.01	8
GDM	0.253	0.545	0.374		26
All patients	0.440	<0.01	0.435	<0.01	34

Table 4: Correlation of insulin and IR with serum CR/BMI. Regression analysis of insulin values and IR in GDM patient group and all patients together. Significant correlation of serum Cr/BMI with both insulin and IR in the non-GDM group and all patients. No correlation in the GDM group.

A relationship was found between the concentration of serum Cr/BMI and the degree of IR observed in the patient. This relationship was present within the whole group of patients (R=0.435; p=0.009; n=34 two tailed Pearson correlation at the 0.01 level) and within the patients that were not diagnosed with GDM (R=0.416; p=0.01, n=26 two tailed). The GDM group showed no significant relationship between IR and serum Cr/BMI (Tables 3 and 4).

A statistically significant difference was observed in the comparison of serum creatinine between pregnant (mean 57.39 ± SEM 0.59) and the non-pregnant control group (mean 67.43 ± SEM 2.65, p=0.000). There was significant correlation only in the GDM group tested separately for correlation, showing urine creatinine to be associated with urine Cr levels (p=0.027, R=810) and a significant difference between Cr and urine creatinine means between the GDM and non-GDM groups (p=0.049, R=0.709 at a two-tailed level) (Table 5).

Much further analysis is required to compare dietary data and other variables measured.

Discussion and Conclusion

In a previous study, involving healthy non-pregnant volunteers during an oral glucose challenge, an inverse relationship was observed between

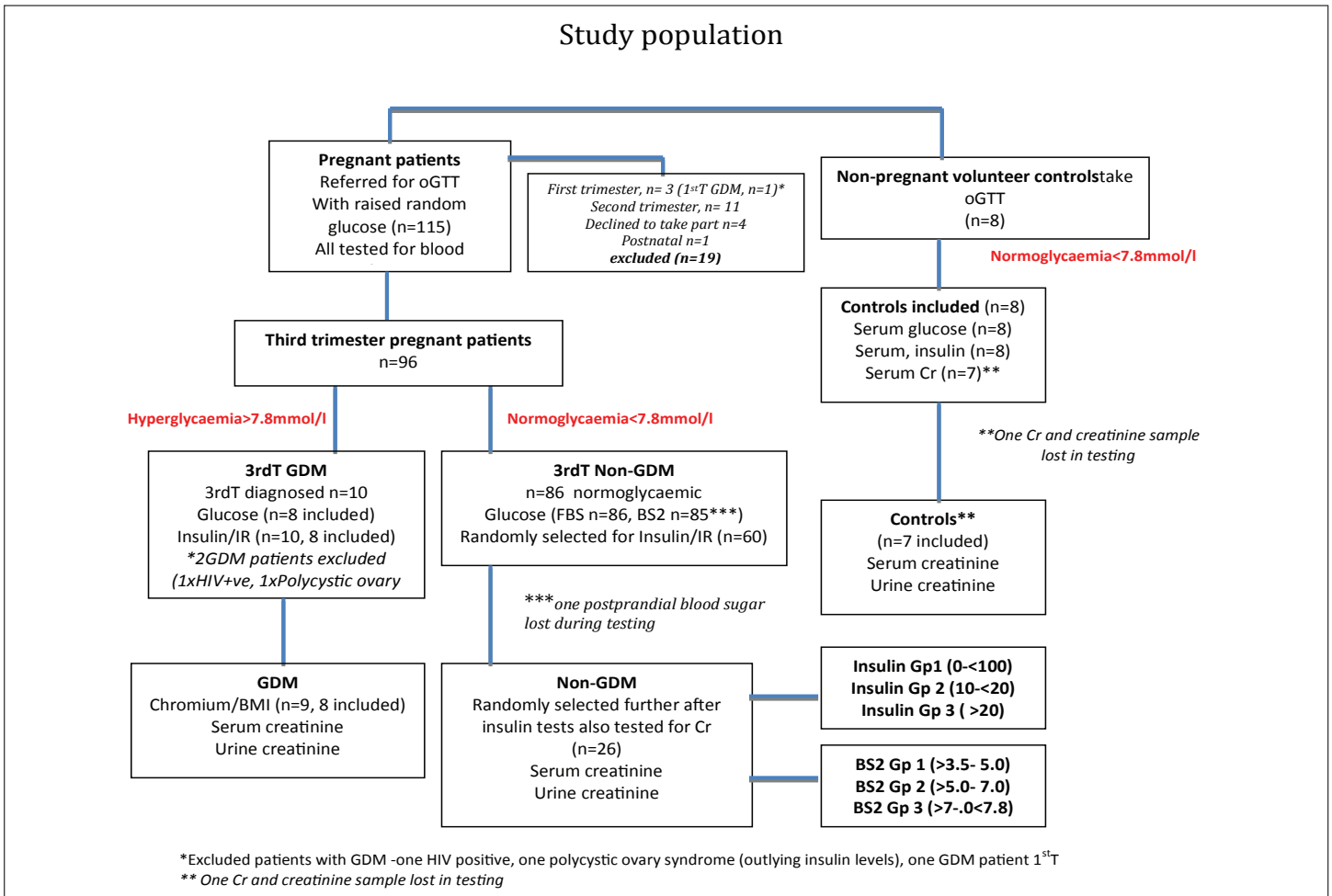

Figure 1: Study population. 115 patients were recruited for the study, involving an oral glucose tolerance test (oGTT) from the Day Assessment unit at Derriford Hospital using the 75 g standard glucose load recommended by WHO. 86 3rd T patients were included and 10 3rd T patients were diagnosed with gestational diabetes mellitus (GDM), 8 of these were included in the study for statistical analysis and comparison. 69 3rd T patients were successfully tested for insulin (GDM, n=9; non-GDM, n=60) and IR calculated, those tested also for Cr were included. 26 eligible patients were randomly selected and tested for Cr. Fasting blood sugar (FBS), n=96 and 2 hour blood glucose (BS2) successfully tested in 95 patients, 85 were found to be normoglycaemic (one BS2 lost), serum creatinine and urine creatinine in tested in non-GDM.

Diagnosis	n=	Mean urine Cr/mg/l ± SEM	Mean urine creatinine/mg/d ± SEM	R=	p=
Controls	7	1.27 ± 0.18	5.76 ± 2.51	-	-
All patients	34	-	-	-0.110	0.535
Non-GDM	26	1.43 ± 0.24	9.72 ± 2.33	-0.175	0.384
GDM	8	1.14 ± 0.75 (p=0.049)	4.36 ± 4.22 (p=0.049)	0.810	0.027

Table 5: Correlation of urine Cr with urine creatinine. Statistically significant correlation of urine Cr with urine creatinine where GDM patients' urine Cr is significantly correlated with urine creatinine (GDM Cr mean1.14 ± 0.75, creatinine mean 4.36 ± 4.22, R=0.810, p=0.027) 0.05 Two tailed level significant difference for mean values within the GDM group only between urine creatinine and urine Cr p=0.049, R=7.09.

plasma Cr levels and insulin levels while patients with diabetes maintained low plasma Cr levels; several other papers describe findings of serum Cr and glucose tolerance [32-42].

Our findings showed that fasting Cr/BMI serum levels increased with increased insulin levels in pregnant women, unlike most studies, which focus on absolute serum Cr levels. This is the first study of its type to consider serum Cr, BMI, IR and renal function (creatinine) together in patients with GDM, building a wider picture of events.

In terms of external validity, the patients and controls recruited were from a wide socioeconomic sample of the population in the Southwest of England and were randomly selected with different dietary choices and living conditions. Differences due to ethnicity, social status, income or environment have not been considered in this study. There may, however, be some geographical differences or genetic differences that could affect the findings of this kind of study that we have not accounted for and a UK wide investigation of this kind may better establish this. A larger GDM group may further corroborate our findings. Ethnicity may have been an influential factor in the results and has not been accounted for in this analysis, requiring further investigation in future studies.

There is a migration of Cr from blood to insulin sensitive tissue compartments. Redistribution studies have revealed differences in Cr levels in these different compartments and, although it is not fully understood how Cr is stored in the body, serum levels of Cr may be augmented but still causing a depletion of Cr stores.

We propose a model where, in the pregnant woman, serum Cr/BMI increases with insulin in an approximately linear fashion until tissue stores are depleted. At this point the serum Cr/BMI does not rise in a linear fashion and potentiation of insulin is further impaired, resulting in further IR and hyperglycaemia leading to the diagnostic criteria associated with GDM. If nutritional intake of Cr is low, the stores are not replenished. Eventually, after much depletion of stored tissue Cr, the serum Cr levels cannot continue to increase with the rise in insulin even during a glucose load, as was previously observed in non-pregnant patients with diabetes mentioned earlier (Figures 2 and 3).

The mechanism for the decrease in insulin sensitivity in pregnancy is not fully understood and is part of the natural process during pregnancy, although the insulin signalling pathway can be interrupted by several factors, such as increased levels of serum cortisol, Tumor Necrosis Factor α and some interleukin cytokines, leading to IR, during normal pregnancy [43]. It may be that the phenomena of increased Cr with insulin can only be seen in normal pregnancies to maintain glucose tolerance and may not be observed in non-pregnant individuals. This should be further investigated.

The lack of association between Cr/BMI and IR may indicate that the patients with GDM were unable to increase serum Cr levels adequately from depleted tissue stores, to correspond with insulin requirements in contrast to the normoglycaemic group [39].

A simple graphical representation of our interpretation of these findings is demonstrated in figure 3. This does not take into account insulin deficiency that may be the result of β-cell failure or destruction in the pancreas due to excessive insulin production or any other diabetic complications nor does it account for any conditions that can affect insulin production or insulin sensitivity. In figure 3, we try to present the factors in graphical form that could initiate and increase IR and how this may decrease Cr status and lead to further loss of insulin potentiation possibly causing the hyperglycaemia seen in GDM.

In the GDM group, only, was an increase of urine Cr with urine creatinine found to be mildly associated but with very few GDM urine creatinine results to compare, nevertheless suggesting that renal function could affect Cr status. This relationship did not occur in the normoglycaemic group and might be a further indication that Cr loss through a renal lesion could contribute towards GDM. Also there was a strong correlation between serum creatinine and serum Cr/BMI, which is a confusing result and much more investigations into renal function, Cr, IR and pregnancy are needed with larger GDM cohorts to make sense of this.

There is little doubt that the Cr status of an individual is important in maintaining normoglycaemia. The action of glucose tolerance factor along with evidence based on parenteral nutrition [17,38] and other experiments involving Cr supplementation show improvements in the IR and glycaemic status of insulin resistant or diabetic patients [26-31].

In order to maintain normoglycaemia in pregnancy, it may be important that Cr serum levels increase with increasing insulin levels. If Cr status is compromised and levels are not able to increase correspondingly with the insulin then hyperglycaemia and GDM may be the result.

Renal function may be an additional factor in Cr status and Cr losses in pregnancy leading to GDM due to increased glomerular filtration rate and increases in blood pressure. Cr status may be further compromised if a renal lesion is present in the mother.

From this data, Cr deficiency may not be associated with the initial IR seen during pregnancy but may be pathogenic in the further development of hyperglycaemia in the development of GDM in maternity patients (Figure 2). Thus further clarification of the mechanisms of Cr action and determining if the relationships observed in pregnancy can be extrapolated into T2DM are required.

This phenomenon of Cr levels and serum insulin correlation must be further investigated in order to have a better understanding of the pathogenesis of GDM and to find out if it has any relevance in insulin action for Type 2/1 diabetes (Figure 3).

A clearer understanding of the role of Cr deficiency as an accelerating factor towards hyperglycaemia and GDM could prevent some of the infant mortality due to the toxicity of hyperglycaemia. Much controversy has developed around this subject largely hinged on the establishment of norms of Cr status in an individual and toxicity factors around prescribing Cr supplementation [30,31]. Serum levels in one study reported that Cr levels could not predict glucose tolerance in pregnancy [38]. In a Cochrane review of CrPicolinate as a treatment for obesity, searching The Cochrane Library, MEDLINE, EMBASE, ISI Web of Knowledge, the Chinese Biomedical Literature Database, the China Journal Full text Database and

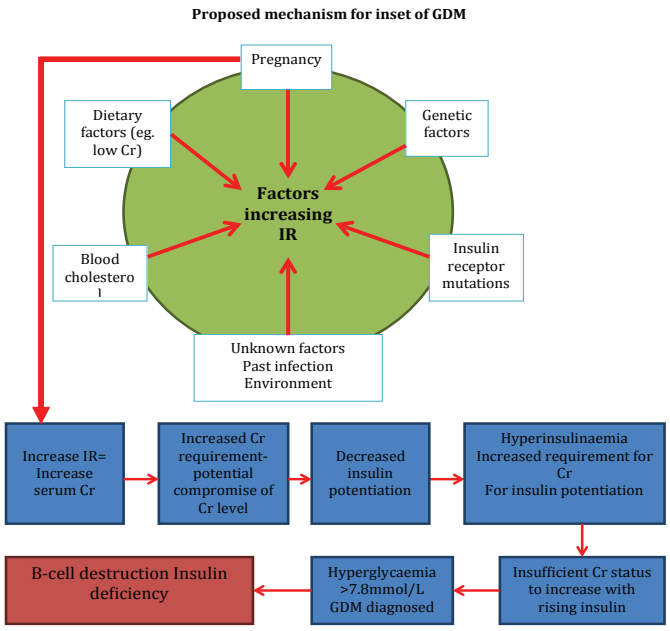

Figure 2: Multiple factors are thought to be responsible for increased insulin resistance including pregnancy. The results of this study suggest a series of events concerning Cr levels and renal function that may contribute to the development of GDM.

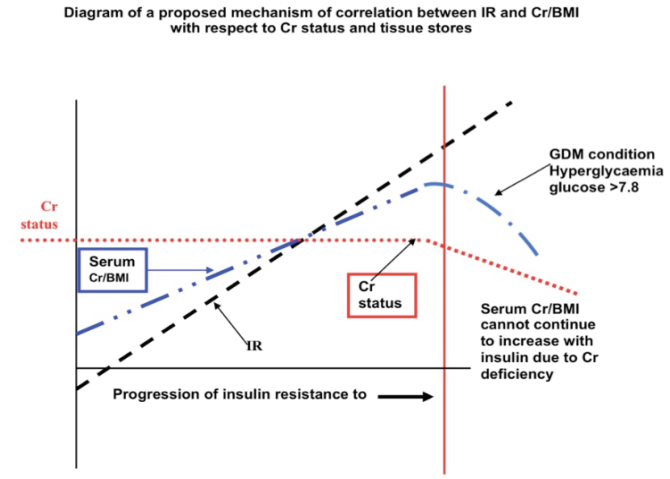

Figure 3: Diagram of a proposed mechanism of correlation between IR and Cr/BMI with respect to Cr status and tissue stores

the Chinese Scientific Journals Full text Database, no significant adverse events were attributed to Crpicolinate and biotin supplementation.

It has been suggested that investigating the nutrigenomics may enlighten us as to how Cr supplementation may affect on Cr-gene interactions and genes regulated by Cr, possibly providing strategies that may prevent insulin resistance-related disorders [32]. It has also been suggested recently that levels of Cr deficiency are relatively common in patients with pre-diabetes, and it is necessary to screen patients with diabetes and pre-diabetes according to the American Diabetes Association guidelines, with regard to the Cr level and action should be taken to eliminate the Cr deficiency in these patients [24,40-42].

More research in this field could contribute towards healthier pregnancies and reduce costs due to the treatment of GDM.

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