

Determinants of incident hyperglycemia 6 years after delivery in young rural Indian mothers:  
the Pune Maternal Nutrition Study (PMNS)

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**Running title:**

Incident hyperglycemia in rural Indian mothers.

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**Abstract:**

**Objective:** To study determinants of incident hyperglycaemia in rural Indian mothers 6 years after delivery.

**Research Design and Methods:** The Pune Maternal Nutrition Study (PMNS) collected information in 6 villages near Pune on pre-pregnant characteristics, and nutrition, physical activity and glucose tolerance during pregnancy. An oral glucose tolerance test (OGTT) was repeated 6 years after delivery.

**Results:** Five hundred and ninety seven mothers had an OGTT at 28 weeks gestation; 3 had gestational diabetes (GDM, WHO 1999 criteria). Six years later, 42 of 509 originally normal glucose tolerant mothers were hyperglycemic (8 diabetic, 20 IGT and 14 IFG). The hyperglycemic women had shorter legs and thicker skinfolds before pregnancy ( $p < 0.01$ , both) and were less active and more hyperglycemic (2-hour plasma glucose 4.8 vs. 4.4 mmol/L,  $p < 0.001$ ) during pregnancy. They had gained more weight during follow up (6.0 vs. 2.7 kg,  $p < 0.001$ ). Multivariate analysis revealed that total leucocyte count and blood pressure during pregnancy were additional independent predictors of 2h glucose concentration at follow up.

**Conclusions:** Our results suggest that compromised linear growth, adiposity, inflammation and less physical activity predispose to hyperglycemia in young rural Indian women. International cutpoints of diabetes risk factors are largely irrelevant in these women

India has the largest number of diabetic patients in any one country (1) and has been called the 'diabetes capital' of the world. Risk factors for diabetes in Indians are mostly derived from cross-sectional surveys (2); there are only a few prospective studies of incident diabetes (3). Classic risk factors for type 2 diabetes include a family history of diabetes, higher age, greater obesity (total and central) and insulin resistance. Recent research has revealed a role for new genetic markers (4), adverse intrauterine environment and accelerated childhood growth (5), inflammation (6) and endothelial dysfunction (7). Interestingly, short height and short legs have also been associated with risk of type 2 diabetes (8).

There is growing recognition that pregnancy is a window revealing future metabolic and cardiovascular (CV) risk for the mother (9). Hormonal, metabolic and inflammatory stresses of pregnancy unmask underlying susceptibility to diabetes. The original definition of gestational diabetes mellitus (GDM) was based on the level of glycemia which predicts future risk of diabetes (10). Over 2/3rds of GDM women in our clinic were hyperglycaemic within 4 years of delivery (11), one of the highest rates anywhere. There is little community-based information in India on the predictive value of measurements during pregnancy for future risk of diabetes.

The Pune Maternal Nutrition Study (PMNS) is a community-based study of maternal nutrition, metabolism and fetal growth in 6 villages near Pune, Maharashtra, India (12). Women were enrolled before pregnancy and during pregnancy anthropometric, nutritional, and biochemical-metabolic measurements were made, including a 75g oral glucose tolerance test (OGTT). An OGTT was performed 6 years after delivery, providing

an opportunity to study risk factors in pregnancy for future hyperglycemia.

### **Research Design and Methods:**

The study design and methods of the PMNS have been described (12). We identified 2,675 married non-pregnant women for possible enrolment, 2,466 agreed. Field workers recorded menstrual dates, detailed anthropometry, and socio-economic status using a standardized questionnaire. Women missing 2 successive periods underwent an ultrasound examination; singleton pregnancies of less than 21 weeks gestation were included.

#### *Maternal measurements in pregnancy:*

At 18 $\pm$ 2 and 28 $\pm$ 2 weeks gestation we measured: anthropometry and dietary intakes using a semi-weighed 24-hour recall method and a food frequency questionnaire. A structured questionnaire was used to record typical daily routine which included farming and domestic activities. Using published data on the energy cost of various activities, a weighted total daily score was derived (13). At 28 weeks an OGTT (75g anhydrous glucose) was done, venous blood samples were obtained fasting and 2-hours post glucose.

Blood measurements included: hemoglobin, total leucocyte count (TLC) and platelet count (Beckman Coulter T540, Miami, Florida); plasma glucose, total cholesterol and triglyceride concentrations using standard kits; insulin, proinsulin and 32-33 split proinsulin using a DELPHIA assay. Insulin resistance was calculated from the homeostasis model assessment equation (HOMA-R) (14). Glucose tolerance was classified by WHO 1999 criteria (15). At delivery we made detailed measurements of baby's size. Enrolment started in June 1994 and the last delivery occurred in November 1996.

*Maternal measurements at 6-year follow-up:*

The women were invited for an OGTT 6 years after the delivery (2000-2002). They arrived the evening before, were given a standard dinner and rested overnight (only water by mouth). A 75 g OGTT was carried out next morning. Information on medical events and pregnancies since the index delivery was recorded by trained medical officer using a questionnaire. Anthropometric measurements included height and sitting height to the nearest 1 mm using a Harpenden stadiometer. Blood pressure was recorded in the supine position using an automated machine (UA 767PC, A and D Instruments Ltd, Abingdon, Oxford, UK). Socio-economic status (SES) before pregnancy was assessed using the Kuppuswamy score (16) and at 6 year follow-up using the National Family Health Survey-2 standard of living index (SLI) (17).

**Definitions and calculations:**

Impaired fasting glycemia (IFG), impaired glucose tolerance (IGT) and diabetes mellitus (DM) were diagnosed as per WHO 1999 criteria (15). Metabolic syndrome was defined using International Diabetes Federation (IDF) 2005 criteria (18). Fat mass was calculated from 4 skinfolds by Durnin's method (19) and leg length by subtracting sitting height from total height. Preterm birth was defined as a delivery before 37 weeks gestation. Babies weighing <2500g at birth were defined as low birth weight (LBW). Babies with weight above the gestation and gender-specific 90<sup>th</sup> percentile were defined as large for gestational age (LGA) and those below 10<sup>th</sup> percentile as small for gestational age (SGA). Adverse fetal outcome was defined as a history of any of the following:  $\geq 2$  spontaneous abortions, a stillbirth, neonatal death or preterm delivery.

Ethical permission for the study was granted by the KEM Hospital Ethical

Committee and by the local village leaders. The women signed an informed written consent.

**Statistical Methods:**

Data are presented as mean  $\pm$  SD. Variables with skewed distributions (subscapular and triceps skinfold thickness and maternal insulin concentrations) were log transformed to satisfy assumptions of normality. Pearson correlation coefficients were used to study associations. Multiple linear and logistic regression and ANOVA models were used to assess whether associations were independent of potential confounding factors. Analyses were carried out using STATA (version 7.0).

**Results:**

Out of 814 pregnancies enrolled in the PMNS 770 mothers delivered normal, live, single babies. Five hundred and ninety seven women had an OGTT. Three were diagnosed GDM and excluded from the analysis of incident hyperglycemia. Of 594 normal glucose tolerant mothers 4 who delivered stillbirths and 28 whose children died were not followed up. Seven mothers died, 11 were pregnant and 31 declined to participate. Thus an OGTT was performed on 513 non-pregnant mothers: 4 vomited and 509 completed the test (94% of eligible). Fourteen mothers were classified as IFG, 20 as IGT and 8 as DM; together they are referred to as hyperglycemic and the rest as normoglycemic (n=467) (Figure 1).

Women who did not have an OGTT during pregnancy (n=173) and those who were not followed up did not differ in age, BMI and SES compared to those who were studied.

*Characteristics of the women at 6-year follow-up:* (Table 1)

Hyperglycemic women were of similar age, socio-economic status and parity compared to normoglycemic women. None of these women reported a first-degree relative with diabetes. Hyperglycemic women were shorter, heavier, had higher BMI, higher waist and hip circumferences and waist-hip

ratio, thicker skinfolds and higher percentage body fat. Their shorter height was mostly accounted for by shorter legs. Hyperglycemic women had gained more weight over the period of the study and had higher blood pressure, fasting plasma triglyceride concentrations and TLC. Fasting plasma insulin concentrations and HOMA-R were similar in the two groups. Fifteen percent of the hyperglycaemic and 1% of the normoglycemic women had the 'metabolic syndrome'.

*Pre-pregnancy and pregnancy characteristics:* (Table 2)

Hyperglycemic women reported a larger number of adverse fetal outcomes (mostly abortions) but had similar parity. They had larger skinfolds and marginally higher body fat percent and waist circumference.

At 28 weeks gestation, the subsequently hyperglycaemic women had higher 2-hour plasma glucose concentration (OGTT) and higher blood pressure, other biochemical and haematological measurements were similar in the two groups. Macronutrient intake, frequencies of intake of green leafy vegetables, fruit and milk, and circulating concentrations of vitamin B12, folate, vitamin C, and homocysteine were similar in the two groups (data not shown).

As a group these women had high levels of physical activity. Domestic work included cooking, washing clothes and utensils, fetching water and firewood. Farming activities included taking care of animals, milking of animals, and farm labor. The hyperglycaemic women had a lower physical activity score compared to the normoglycemic women; this was due to lower domestic activity.

None of the PMNS women smoked. Ten percent (n=4) of hyperglycemic women and 28% of normo-glycemic women either chewed tobacco or used it as tooth powder (Mishri).

Birth weight and other anthropometric measurements of the newborn were similar in the two groups of women, as were the rates of LBW, SGA, LGA and pre-term delivery.

*Multivariate associations of 2-hour plasma glucose concentration and incident hyperglycaemia at 6 years:* (Tables 3A and 3B)

We analysed the associations of glycemic status at follow up by 2 methods: 1) using 2h plasma glucose concentration as a continuous variable, and 2) incident hyperglycemia (IFG + IGT + DM) as a categorical variable.

Multiple linear regression analysis showed that higher two hour plasma glucose concentration was associated with shorter legs and higher pre-pregnant fat mass, higher 2-hour plasma glucose concentration, TLC and systolic blood pressure during pregnancy, and higher weight gain over 6 years. Age, SES, parity, pre-pregnant waist circumference, physical activity and plasma insulin concentrations during pregnancy, and birth weight of the child were not significantly associated in this analysis. Replacing pre-pregnant fat mass by BMI revealed that BMI was not related.

To assess the relative significance of the association of independent variables with 2h plasma glucose concentration we performed STEPWISE regression analysis where the program serially selects independent variable most correlated with the dependent variable controlling for those already selected. This process is repeated till the newly added variable fails to make a significant contribution to variance.. The results are shown in online only Appendix Table 3B (available at <http://care.diabetesjournals.org>). The contribution was 2.3% for weight gain, followed by pre pregnant fat mass (2.6%) and leucocyte count (1.4%), 2-hour glucose (1.5%) and systolic blood pressure (0.9%) at 28 weeks gestation. Age, socio-economic score, parity, pre-pregnant leg length and waist circumference, physical activity and

fasting insulin at 28 weeks gestation, birth weight of child were not contributory.

Logistic regression analysis showed that incident hyperglycemia was predicted by shorter leg length and lower physical activity during pregnancy. Age, SES, parity, pre-pregnant waist circumference, fat mass, 2-hour glucose concentration and plasma insulin concentrations, TLC and blood pressure during pregnancy, and birth weight of the child were not significant predictors in this analysis. The results were very similar if the incident hyperglycemia was restricted to IGT+DM.

### **Discussion:**

This is the first community-based study in India to report on incident hyperglycemia in rural women. Eight percent of these young women had developed fasting or post-glucose hyperglycemia between 21-28 years of age. Hyperglycemic women were shorter (especially in the legs) and more adipose from before pregnancy and at the time of diagnosis had higher BMI, waist circumference, WHR and body fat percent compared to the normoglycemic women. However, by international standards many of these women were underweight and thin: 26 (62%) hyperglycemic women had a BMI <18.5 kg/m<sup>2</sup> before pregnancy and 14 (33%) at the time of diagnosis of hyperglycemia. Our results support the previous finding that the thin-fat phenotype predisposes to type 2 diabetes (20). It is also clear that the international cutpoints for obesity and central obesity are inappropriate in young rural Indian women. The recently suggested cutpoint of BMI for 'obesity' related public health action in Asians (23 kg/m<sup>2</sup>) (21) also seems far too high for these rural Indian women.

In our study, shortness and fatness independently predicted higher glycemia, and the short and fat women were the most hyperglycemic. Short stature reflects a deficit in linear growth in early life,

contributed both by genetic and nutritional factors; it is a risk factor for GDM, type 2 diabetes, hypertension and coronary heart disease (8,22,23). Short legs represent a caudal diminution of growth and may represent intrauterine programming of body proportions. Increasing adiposity in a short individual reflects a rapid nutritional transition and increases risk of type 2 diabetes, even in the young rural Indian women.

A number of measurements during pregnancy were predictive of later hyperglycemia. These included higher post-glucose glycemia, higher blood pressure and higher TLC (all within the 'normal' range). The plasma glucose concentration during pregnancy of subsequently hyperglycemic women was far below currently used cut-point for diagnosis of gestational diabetes, suggesting that the gradient of risk may be very steep in susceptible populations. Higher glycemia was associated with higher TLC in pregnancy and at follow up, suggesting an association with inflammation. TLC and other inflammatory markers are predictive of incident diabetes in Pima Indians and also in the ARIC study (24,25). 'Inflammation' could reflect heightened 'innate' immunity (6). However, in pregnancy, it could be a maternal response to the conceptus (26), and the placenta secretes a number of proinflammatory cytokines (IL6, TNF- $\alpha$ , leptin etc) (27). Association between higher blood pressure and hyperglycemia could reflect endothelial dysfunction which also contributes to type 2 diabetes susceptibility (7). Thus, our results suggest that metabolic, inflammatory and vascular changes in pregnancy predict future hyperglycemia and support the concept that pregnancy is a window on future health and disease.

Incident hyperglycemia (IFG+IGT+DM) was predicted by short legs, lower physical activity during pregnancy and higher weight gain. In this farming community, physical activity during pregnancy is a surrogate of the habitual physical activity which is quite high.

The typical daily activity of the hyperglycemic women far exceeds the current recommendations for physical activity to prevent diabetes (150 minutes walking per week) (28). Finally, weight gain predicted incident hyperglycemia. This is too well known and operates through increased insulin resistance.

Our study has much strength. We made a wide range of measurements, specifically designed to answer relevant hypotheses. The community participation and follow-up rates are high (>90%). The rural women represent 70% of India's population who will contribute increasingly to the burgeoning epidemic of diabetes in India. Our findings, therefore, provide useful public health information for the policy makers. One weakness is that leg length was measured only at follow-up; however it is fairly stable in young adults.

Our findings suggest possible interventions to reduce the incidence of diabetes in Indians. There is a need to take a 'life-course' approach and include measures to promote skeletal and lean growth in early life, promote physical activity, and eliminate sources of chronic

inflammation. In practical terms, active prevention of weight gain in people who are 'thin' by the currently accepted norms will be a challenging task.

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**Table 1: Anthropometric and biochemical characteristics of the mothers at follow-up (6 years after the index pregnancy) according to current glycaemic status (WHO 1999)**

	Normoglycemic (n=467)	Hyperglycemic (n=42)	p	p1
Age (years)	27.9 (3.5)	28.6 (4.5)	0.258	--
Completed secondary school N (%)	330 (53.6)	39 (68.4)	0.128	--
Upper SES (upper quartile of score) N (%)	180 (31.4)	18 (36.7)	0.694	--
Deliveries after index delivery	1.6 (0.9)	1.7 (0.9)	0.671	0.525
Weight (kg)	44.4 (6.9)	47.7 (8.9)	0.004	0.026
Height (cm)	152.1 (5.0)	150.4 (4.4)	0.040	0.063
Leg length (cm)	73.5 (3.9)	71.4 (4.5)	0.001	0.001
BMI (kg/m <sup>2</sup> )	18.9 (2.6)	20.9 (3.8)	0.0001	0.0001
Waist (cm)	65.6 (6.9)	70.3 (11.1)	0.0001	0.002
Hip (cm)	85.4 (6.8)	88.6 (10.1)	0.012	0.028
Waist/Hip Ratio	0.77 (0.06)	0.79 (0.09)	0.010	0.043
Sum of 4 skinfolds (mm)	37.1 (17.6)	49.4 (25.1)	0.0001	0.0001
Body fat % (calculated from 4 skinfolds)	22.0 (5.8)	25.2 (7.3)	0.001	0.003
Weight gain pre-pregnancy to 6-year follow-up (kg)	2.7 (5.1)	6.0 (6.8)	0.0001	0.0001
Fat mass gain pre-pregnancy to 6-year follow-up (kg)	1.2 (3.5)	2.9 (4.5)	0.004	0.007
Fasting plasma glucose (mmol/L)	5.1 (0.5)	6.2 (1.8)	0.0001	0.0001
2-hour plasma glucose (OGTT) (mmol/L)	5.2 (1.1)	8.8 (3.4)	0.0001	0.0001
Fasting plasma insulin (pmol/L)	35.4 (34.0)	37.5 (24.3)	0.701	0.620
Insulin resistance (HOMA-R)	0.65 (0.56)	0.77 (0.52)	0.129	0.150
Blood pressure (mmHg)	107/64 (9/7)	115/68 (10/7)	0.0001	0.0001
Plasma total cholesterol (mmol/L)	3.6 (0.7)	3.8 (0.7)	0.168	0.402
Plasma HDL cholesterol (mmol/L)	1.2 (0.3)	1.2 (0.3)	0.363	0.385
Plasma triglycerides (mmol/L)	0.7 (0.3)	1.0 (1.4)	0.0001	0.0001
Total leucocyte count (x10 <sup>9</sup> /L)	7.2 (1.6)	7.8 (2.1)	0.045	0.053
Metabolic Syndrome (IDF 2005) N (%)	7 (1.2)	8 (15.4)	0.0001	--

Values are mean (SD). p1 indicates statistical significance after adjusting for maternal age and socio-economic status at the time of the measurements, by ANOVA.

**Table 2: Maternal characteristics before and during the index pregnancy according to glycemic status at 6year follow up**

	Normoglycaemic (n=467)	Hyperglycemic (n=42)	p	p1
<b><u>Pre-pregnancy</u></b>				
Adverse fetal outcome N (%)	24 (5.2)	8 (19.0)	0.0001	--
Parity	1.2 (1.2)	1.3 (1.3)	0.630	0.837
Tobacco use (%)	132 (28.3)	4 (9.5)	--	--
Weight (kg)	41.7 (4.9)	41.6 (6.9)	0.950	0.991
BMI (kg/m <sup>2</sup> )	18.0 (1.8)	18.4 (2.8)	0.232	0.261
Waist (cm)	60.5 (5.4)	62.2 (8.5)	0.069	0.075
Hip (cm)	81.4 (4.9)	82.0 (6.5)	0.465	0.514
Waist/Hip ratio	0.74 (0.06)	0.76 (0.08)	0.180	0.179
Sum of 4 skinfolds (mm)	33.8 (12.3)	38.7 (18.1)	0.018	0.028
Body fat % (calculated from 4 skinfolds)	20.9 (4.2)	22.4 (5.4)	0.044	0.071
<b><u>28 wks gestation</u></b>				
Hemoglobin (g/L)	112.0 (15.0)	114.0 (12.0)	0.363	0.279
Fasting plasma glucose (mmol/L)	3.9 (0.6)	4.1 (0.8)	0.180	0.178
2-hour plasma glucose (OGTT) (mmol/L)	4.4 (1.0)	4.8 (1.4)	0.008	0.009
Fasting plasma insulin (pmol/L)	23.5 (37.9)	20.6 (14.1)	0.635	0.531
Pro-insulin (pmol/L)	2.5 (2.1)	2.5 (1.9)	0.983	0.992
32-33 Split pro-insulin (pmol/L)	3.4 (4.4)	3.2 (2.7)	0.812	0.885
Pro-insulin to insulin ratio	0.21 (0.33)	0.15 (0.12)	0.273	0.288
Insulin resistance (HOMA-R)	0.78 (1.1)	0.73 (0.57)	0.770	0.641
Blood pressure (mmHg)	112/62 (9/8)	115/64 (9/3)	0.055	0.078
Plasma total cholesterol (mmol/L)	4.8 (0.9)	4.9 (0.8)	0.713	0.652
Plasma HDL cholesterol (mmol/L)	1.1 (0.3)	1.1 (0.3)	0.224	0.190
Plasma triglycerides (mmol/L)	1.5 (0.5)	1.6 (0.7)	0.282	0.323
Total leucocyte count (x10 <sup>9</sup> /L)	9.1 (1.9)	9.5 (2.5)	0.286	0.282

<b>Macro nutrient intake/day</b>				
Total energy (kcal)	1690.8 (498.3)	1623.4 (441.8)	0.408	0.753
Protein (g)	44.1 (13.9)	42.9 (12.5)	0.598	0.991
Fat (g)	32.6 (13.8)	31.9 (11.9)	0.747	0.914
Carbohydrate (g)	304.9 (89.8)	290.0 (80.5)	0.311	0.591
<b>Activity scores</b>				
Total	65.7 (25.3)	51.8 (24.6)	0.001	0.003
Domestic	28.7 (13.2)	23.3 (17.6)	0.019	0.031
Farming	27.2 (15.7)	23.3 (16.5)	0.295	0.348
<b><u>At delivery</u></b>				
Preterm delivery N (%)	60 (9.7)	5 (8.6)	0.507	--
Caesarean section N (%)	21 (4.5)	2 (4.8)	0.986	--
Birth weight (g)*	2646 (363)	2628 (425)	0.775	0.709
Birth weight <2500g N (%)	172 (38.0)	13 (33.0)	0.519	--
Birth weight >90 <sup>th</sup> Centile -LGA (%)	35 (7.9)	4 (10.3)	0.599	--
Birth weight <10 <sup>th</sup> Centile-SGA (%)	44 (9.9)	5 (12.8)	0.560	--

Values are mean (SD). p1 indicates statistical significance after adjusting for maternal age and socio-economic status at the time of the measurements, by ANOVA.

\* Birth weight adjusted for gestation and gender.

**Table 3A: Multiple linear regression analysis of predictors of 2-hour plasma glucose concentrations during the OGTT 6 years after delivery, and multiple logistic regression analysis of predictors of incident hyperglycemia (IFG+IGT+DM, WHO 1999) 6 years after delivery**

Predictors of 2h glucose				Predictors of incident hyperglycemia			
	$\beta$	95% CI	p		OR	95% CI of OR	p
Age (years)	0.037	0.0272 - 0.0468	0.554	Age (years)	0.911	0.776-1.071	0.259
Socio-economic score	0.046	0.0421 - 0.0499	0.360	Socio-economic score	0.967	0.909-1.029	0.295
Parity	-0.010	-0.0394 - 0.0194	0.877	Parity	1.321	0.822-2.125	0.250
<b>Pre-pregnant</b>				<b>Pre-pregnant</b>			
Leg length (cm)	-0.120	-0.1259 - (-) 0.1141	0.019	Leg length (cm)	0.875	0.802-0.954	0.003
Waist circumference (cm)	0.038	-0.0321 - 0.0439	0.534	Waist circumference (cm)	1.014	0.926-1.110	0.764
Fat mass (kg)	0.161	0.1473 - 0.1747	0.014	Fat mass (kg)	1.140	0.955-1.361	0.148
<b>At 28 weeks gestation</b>				<b>At 28 weeks gestation</b>			
Physical activity	-0.030	-0.0319 - (-) 0.0280	0.561	Physical activity	0.974	0.954-0.995	0.014
2-hour glucose (mmol/L)	0.112	0.1100 - 0.1139	0.023	2-hour glucose (mmol/L)	1.011	0.991-1.031	0.296
Fasting insulin (pmol/L)	0.036	0.0340 - 0.0379	0.454	Fasting insulin (pmol/L)	0.991	0.966-1.016	0.469
Leucocyte count ( $\times 10^9/L$ )	0.112	0.0983 - 0.1257	0.021	Leucocyte count ( $\times 10^9/L$ )	1.083	0.907-1.294	0.378
Systolic blood pressure (mmHg)	0.098	0.0960 - 0.0999	0.047	Systolic blood pressure (mmHg)	1.025	0.982-1.069	0.261
<b>At delivery</b>				<b>At delivery</b>			
Birth weight of child (g) (Gestation and sex-adjusted)	-0.066	-0.0954 - (-) 0.0366	0.188	Birth weight of child (g) (Gestation and sex-adjusted)	0.738	0.482-1.131	0.163
Weight gain pre-pregnancy to follow-up (kg)	0.160	0.1541 - 0.1659	0.001	Weight gain pre-pregnancy to follow-up (kg)	1.064	0.998-1.134	0.057

**Figure 1: Study subjects**

