The effect of paternal diabetes on pre-diabetic phenotypes in adult offspring

A short running title: Paternal inheritance and pre-diabetic phenotypes

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**Objective:** Paternal and maternal type 2 diabetes (exclusive of gestational diabetes) may influence risk factors in the offspring differently (possible epigenetic effects of parental diabetes) and are difficult to identify without accurate dates of diagnosis. We aimed to examine a metabolic phenotype in three different groups of offspring to see distinct paternal versus maternal effects.

**Research design and Methods:** We examined body composition, insulin action (M), in non-diabetic and insulin secretion (AIR) in normal glucose tolerant full-heritage Pima Indian adults categorized by disparate parental diabetes status: 1) offspring of fathers with early onset diabetes (age < 35 years) and non-diabetic mothers (ODF; n=10), 2) offspring of mothers with early onset diabetes (age < 35 years) (OMED; not exposed to diabetes in utero) and non-diabetic fathers (OMED; n=11) and 3) offspring of parents without diabetes until > 50 years (CON; n=15).

**Results:** ODF were leaner compared to CON and OMED (percent of body fat (%BF): least squared means adjusted for age and sex (95%CI): 27.3 (23.3, 31.3) vs. 35.4 (32.2, 38.5) in CON and 32.4 (28.8, 36.1) % in OMED, p=0.04). ODF were more insulin sensitive (had a higher M) than OMED or CON but not after adjustment for age, sex and %BF. AIR adjusted for M, age, sex and %BF was lower in ODF versus CON and OMED (p<0.05).

**Conclusions:** Adult ODF were leaner and had lower early insulin secretion despite being equally insulin sensitive after adjustment for body fat compared to the other groups indicating a paternal imprinted effect.
Previous studies of heredity established parental history of type 2 diabetes as one of the dominant risk factors for development of type 2 diabetes (1,2). The offspring phenotype may vary depending on which parent is affected and whether the offspring was exposed to diabetes in utero. Low birth weight (LBW), thought to result from in utero maternal undernutrition, is one such phenotypic feature and has been shown to predict development of type 2 diabetes (3,4). However, in Pima Indians, LBW predicts diabetes only when paternal (not maternal) diabetes is present (3). In fact, offspring's lower birth weight predicted subsequent development of diabetes in the fathers. These data indicated a possible paternally transmitted imprinted effect. Gautier et. al. (5) reported that insulin secretion was positively associated with age of diabetes onset in the mother. However, this was in a mixed cohort where either or both parents could have had diabetes.

Recent studies in mice demonstrated that while a low birth weight phenotype, first generated using maternal undernutrition, confers impaired glucose tolerance via both subsequent parental lines, the low birth weight phenotype is passed on via paternal inheritance only (6). As these mice reach maturity, paternal offspring continue to have lower body weight but similar degrees of impairment in glucose tolerance and impaired insulin secretion.

We hypothesized that offspring of individuals with early onset paternal diabetes would have a different metabolic phenotype compared to those with early onset maternal diabetes (exclusive of exposure to diabetes in utero) and controls. To disentangle the separate parental effects in offspring, we carefully selected three groups of individuals: 1) offspring of fathers with early onset of diabetes 2) offspring of mothers with early onset of diabetes and 3) offspring in which neither parent developed diabetes. We compared body composition (percentage of body fat (%BF)), insulin action (M) and acute insulin response (AIR) among these groups.

**RESEARCH DESIGN AND METHODS**

**Subjects: Determination of Parental Diabetes Status** - Parental diabetes status was determined from a longitudinal study of health conducted within the Gila River (Pima) Indian Community in which residents ≥ 5 years old have been invited for research examinations approximately every 2 years consisting of measurement of height and weight and a 75 gram oral glucose tolerance test (OGTT). Diabetes was diagnosed according to WHO 1999 criteria (7) or by review of the medical record if made in a clinical setting.

We defined 3 groups of offspring according to parents diabetes status: 1) offspring of diabetic fathers (ODF) consisting of subjects whose father developed diabetes < 35 years, but whose mother remained non-diabetic until > 35 years of age; 2) offspring of mothers with early onset of diabetes (OMED) consisting of subjects whose mother developed diabetes < 35 years, but after the date of birth (DOB) of the subject documented by a normal OGTT following delivery (mean time between DOB of subject and mothers date of diagnosis; 8.9 ± 4.2 years) and whose fathers remained non-diabetic until > 35 years of age and 3) a control group (CON) consisting of offspring whose parents were known to be non-diabetic until >50 years of age. The third group can be considered as a control group because of previous evidence that offspring of parents who do not develop diabetes until > age 50 years have comparable risk for developing diabetes as offspring whose parents never developed diabetes (8).

For additional characterization of the parents, we determined a diabetic cumulative
incidence score (CI) of each parent in the sample (8). Briefly, the diabetes score was derived by first calculating the specific cumulative incidence of diabetes as a function of age (Cl_a) in the Pima population. If diabetes was present, then the score is calculated by 1-CI_a at the age of first diagnosis of diabetes. If diabetes was not present, then score is calculated as 0-CI_a at the time of the last biennial examination. The score thus contains information on whether an individual developed diabetes and the age of onset of diabetes, being positive if diabetes was ever present and greater if diabetes developed at an earlier age. Conversely, a negative score is calculated if the individual was non-diabetic at the last biennial examination and is most negative in those who remain non-diabetic into old age.

Study Subject - Volunteers also participated in a study of metabolic determinants for the development of type 2 diabetes and obesity. At the time of their participation, all subjects were in good health and without evidence of diabetes (by OGTT), as determined by a comprehensive medical evaluation including medical history, physical examination, and routine laboratory testing. Subjects were admitted for 10–15 days to the Clinical Research Unit of the National Institute of Diabetes and Digestive and Kidney Diseases in Phoenix, Arizona, and were provided a standard weight-maintaining energy needs (EN-WM) diet containing 50% of calories as carbohydrate, 30% as fat, and 20% as protein for at least 3 days before metabolic testing. After at least 3 days, volunteers underwent a 75-g oral glucose tolerance test (after a 12-h overnight fast) to exclude diabetes. Volunteers were then assessed for body composition, insulin action in vivo, and energy expenditure. Only subjects who were full-heritage Pima Indian or related Tohono O'odham were included in the analysis.

Dual-energy X-ray absorptiometry - Body composition was measured by dual-energy X-ray absorptiometry using a total body scanner (DPX-L; Lunar Corp, Madison, WI). Percentage body fat (%BF), fat mass (FM), and fat-free mass (FFM) were calculated as previously described (9).

Measurement of insulin action. Insulin action was assessed at physiologic insulin concentrations during the hyperinsulinemic-euglycemic clamp technique (10). Briefly, after an overnight fast, a primed (30 µCi) continuous (0.3 µCi/min) infusion of [3-3H] glucose infusion was started to determine endogenous glucose production (EGP). At least 2 h after starting the isotope infusion, a primed continuous intravenous insulin infusion was administered for 100 min at a constant rate of 40 mU/m^2/min. Blood samples for measurement of 3-[3H] glucose specific activity were collected at the end of the basal period and every 10 min during the final 40 min of insulin infusion. Under basal (i.e., fasting) conditions, EGP was calculated as the 3-[3H] glucose infusion rate divided by the steady-state plasma 3-[3H] glucose specific activity. The rate of total insulin-stimulated glucose disposal was calculated for the last 40 min of the insulin infusion and was corrected for the rate of EGP calculated from Steele's equation (11). Individual variation in plasma glucose and insulin concentrations during the clamp were taken into account in the calculation of insulin action (M-low) (12). All measurements derived from the clamp were normalized to estimated metabolic body size (EMBS), which is directly derived from FFM but takes into account for the fact that the intercept of the relationship between FFM and resting metabolic rate is not zero (-17.7 kg in our laboratory [i.e. EMBS=FFM + 17.7 kg] (12).

Insulin secretion was measured as the response to a 25-g intravenous glucose tolerance test (IVGTT). The acute insulin response (AIR) was calculated as the average increment in plasma insulin concentration above basal in samples obtained 3, 4, and 5
minutes after the bolus injection of glucose (13).

**Meal test.** At approximately 0730 h, subjects were fed a mixed-meal breakfast (consisting of a bacon-and-egg sandwich on toast accompanied by orange juice) containing 10% of calories from protein, 45% from fat, and 45% from carbohydrates and providing approximately 20% of daily energy requirements for each subject. The meal was consumed within 15 min and subjects rested quietly in bed throughout the study. Blood samples for insulin and glucose were drawn before and 30, 60, 90, 120, 150, and 180 min after initiation of the meal test.

**Analytic measurements.** Plasma glucose concentrations were measured by the glucose oxidize method (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations were measured by the Herbert modification of the method of Yalow and Berson (14), by an automated auto-analyzer (ICN Radiochemicals, Costa Mesa, CA), or by an automated immunoassay (Access, Beckman Instruments). Values from the final two assays were regressed to the original assay. All studies were approved by the Institutional Review Board of the National Institute of Diabetes, Digestive and Kidney Disease. All volunteers gave written and informed consent.

**Statistical analysis:** Statistical analyses were performed by using the procedures of the SAS statistical package (version 8.2; SAS Institute Inc, Cary, NC). AIR, and M-low were log10 transformed to normalize their distributions before parametric analyses. Unless otherwise specified, all data are expressed as means ± SDs. General, anthropometric, and metabolic characteristics in table 1 were evaluated using Students t-test, ANOVA or chi-square analyses for continuous and categorical variables, respectively. Since even mild degrees of glucose intolerance can be associated with impairments in insulin secretion (15) only subjects with normal glucose tolerance were included for further analyses of AIR. The glucose and insulin responses during the OGTT, IVGTT and meal test by group was compared by analysis of covariance using MIXED procedure in SAS. The general linear models were used to adjust %BF for age and sex; M-low (mg.kg⁻¹.min⁻¹) for age, sex, %BF, and AIR for age, sex, %BF, and M-low (10). Adjusted least-squares means (lsmeans) were used to compare groups by parental diabetes status using post hoc t-test, with Tukey adjustment for multiple comparisons. For all groups further analyses were conducted adjusted for the same co-variates using general estimating equations to account for family membership.

**RESULTS**

General, anthropometric, and metabolic characteristics of the study population are shown in Table 1. We found 10 ODF, 8 of 10 fathers from ODF group had diabetes pre-conception and 2 fathers had diagnosis of diabetes 3 and 4 years after the offspring birth. Onset age of diabetes for fathers was lower in the ODF group than for mothers in the OMED group (26.6 ± 4.2 vs. 30.4 ± 3.0 years, p=0.02). This slight difference in age was due to exclusion of mothers in the OMED group who did not have a non-diabetic OGTT following child birth. Consistent with this age difference fathers’ CI score in the ODF group was higher than mothers’ CI score in OMED group (p=0.04, Table 1). There was no significant difference between mothers CI score in ODF group and fathers CI score in OMED group, indicating no difference in relative age of onset of diabetes in the opposite parent. The CI score was comparable between mothers and fathers in the control group (Table 1).

The birth weight and height was not different among the 3 groups (Table 1). ODF had lower mean %body fat than CON or OMED (Table1). After adjustment for age and sex, %BF remained lower in the ODF
group in comparison to CON (lsmeans (95%CI): 27.3 (23.3, 31.3) vs. 35.4 (32.2, 38.5) in CON and 32.4 (28.8, 36.1) % in OMED, p=0.04). The FFM, adjusted for age and sex, tended to be lower in ODF in comparison to CON and OMED, but did not reach statistical significance ((57.9 (50.4, 65.3) kg in ODF vs. 65.8 (60.0, 71.7) kg in CON and 67.2 (60.4, 74.1) kg in OMED, p=0.1).

ODF had lower fasting, but comparable 2 hour plasma glucose concentrations compared with CON or OMED (p=0.01; p=0.1 respectively; ANOVA; Table 1). Despite similar fasting plasma insulin concentration and glucose responses during the OGTT, plasma insulin concentrations at 30 and 120 min during oGTT (INS30, INS120) were lower in the ODF group compared to the CON and OMED groups (F=4.38, p=0.02; F=3.81, p=0.04, respectively, Figure 1). After adjustment for age, sex, 30 min glucose concentration and M-low only INS30 remained lower in the ODF group in comparison to both CON and OMED (least squared means (95%CI): 722 (132, 1582) vs. 2699 (2047, 3352) in CON and 1790 (1367, 2206) pmol/L in OMED, p=0.01).

Intravenous glucose administration resulted in a comparable increase in plasma glucose levels in all 3 groups. The insulin responses were significantly lower in ODF than in CON subjects (effect of diagnosis x time p<0.0001; ANOVA; Figure 1). In addition, log transformed AIR was lower in the ODF than the CON group (Table 1) and it remained significant after adjusted for age, sex, %BF and M-low (lsmeans (95%CI): 2.08 (1.96, 2.20) in ODF vs. 2.55 (2.24, 2.75) in CON and 2.40 (2.13, 2.55) pmol/L in OMED, p<0.05).

During the meal test, there was no difference in glucose responses between groups. The insulin responses during meal test were significantly lower in ODF than in CON subjects (effect of diagnosis x time x sex p=0.02; Figure 1). Plasma insulin concentrations at 30 min of the meal test were slightly lower in the ODF group compared to both the CON and OMED groups, but it did not reach statistical significance prior to (1084 ± 462 in ODF vs. 2090 ± 1081 in CON and 1472 ± 673 pmol/L, p=0.07; Figure 1), or following adjustment for age, sex, 30 min plasma glucose concentration and M-low (lsmeans (95%CI): 1422 (489, 2355) in ODF vs. 2087 (1372, 2803) in CON and 1492 (1059, 1924) pmol/L, p=0.1).

The ODF group were on average leaner (having a lower %BF), therefore the mean log transformed M-lows were higher in ODF group compared to the CON and OMED groups (Table 1), but after adjustment for age, sex, and %BF M-lows were comparable in all 3 groups (lsmeans (95%CI): 2.68 (1.80, 3.55) in ODF vs. 2.34 (1.73, 2.96) in CON, 2.28(1.87, 2.68) mg.kg⁻¹. EMBS.min⁻¹. in OMED, p=0.4). For all analyses, results did not differ after accounting for family membership.

**DISCUSSION**

Our analyses indicate that adult non-diabetic offspring of fathers with early onset of diabetes are leaner than offspring of either mothers with early onset of type 2 diabetes or controls (i.e. neither parent developed type 2 diabetes by age 50 years). ODF had lower insulin secretion demonstrated by multiple tests (during IVGTT, OGTT, and meal test), but after adjusting for body size, comparable insulin action in vivo. Previous studies have shown that the offspring of individuals with early onset type 2 diabetes are at increased risk for developing diabetes (5,8). Some studies report that the risk associated with maternal and paternal early onset of diabetes is approximately additive (16), while others have not (17). The present analysis was undertaken to clarify the separate effects of parental diabetes on the offspring’s phenotype. To our knowledge this is the first
study to show a paternal influence on adult body composition. While previous studies have shown that offspring of diabetic fathers have lower birth weights (3,18), these studies did not explore parameters for body composition at later ages.

The general effect of family history on offspring phenotype has been extensively examined, but attempts to separate different parental effects have been more limited. Lindsay et al (3) found that paternal history of diabetes was associated with lower birth weight. In fact, only those with LBW and paternal history of diabetes had increased diabetes risk, and LBW predicted diabetes risk in the father. Our results are in agreement with a previous study also in Pima Indians (5) in which lower AIR was associated with earlier onset of diabetes in either parent. In that study, early onset was defined as age of onset below the median age for the cohort (age < 41 years for mothers and < 46 years for fathers). In those whose mothers developed diabetes < 35 years of age (exclusive of exposure to diabetes in pregnancy), insulin secretion rate (ISR) was also lower than in those whose parents developed type 2 diabetes at a later age. However, it is not clear to what extent differences in glucose tolerance (i.e. greater impaired glucose tolerance in group of offspring of the mothers with early onset of type 2 diabetes) may have affected these results. Our results extend and add to these findings as we are able to demonstrate in different tests (IVGTT, OGTT and meal tests) done on different days a consistently lower acute insulin response. During the OGTT, the 30 minute insulin remained lower in ODF compared to both OMED and CON, even after adjustment for age, sex, 30 minute glucose, and M-low. In contrast, the 120 minute insulin was no longer significantly lower after the same adjustments. These results indicate that the primary effect of early paternal diabetes is on early insulin secretion, rather than insulin action. This is supported by the relatively lower fasting plasma glucose in ODFs, and the lack of a difference in M-low after adjustment for body size.

Our results imply an imprinted effect identified by early onset paternal diabetes. Imprinting is the expression of only a single copy of a gene depending on parent-of-origin and is commonly found in genes that affect fetal growth. There are at least two distinct mechanisms through which epigenetic information can be inherited: DNA methylation (commonly associated with gene silencing and contributes to X chromosomal inactivation) and histone modification (19). Numerous genes have been found to be imprinted and some of them are involved also in longitudinal and skeletal growth, and may also be involved in organ growth (20). Notable among them are genes such as IGF-2 which are growth hormone signaling genes (21). In human liver, the major production source of circulating IGF2, the IGF2 gene is maternally imprinted during fetal life, whereas postnatally IGF2 is primarily transcribed from the P1 promoter, which is biallelically active in the liver. In other adult tissue (including pancreas), IGF2 is mainly transcribed from the paternally active promoters (22). Animal studies have demonstrated that genes involved directly in pancreatic beta cell development might be imprinted, e.g. the gene for insulin receptor subunit Sur1 (6,21). Another study demonstrates that genes essential to pancreatic development, such as pancreatic homeobox transcription factor (Pdx-1) are susceptible to epigenetic modifications (23). In humans an independent association between paternal insulin resistance and cord insulin concentrations (24) also indicates indirect evidence of imprinting.

Genes related to adipocyte development, specifically preadipocytes factor 1 (Pref1), Necdin, and paternally expressed gene 1 (Peg1) can be imprinted
Pref (an inhibitor of adipogenesis) is expressed from the paternally inherited chromosome and imprinting of Pref1 is under complex control by both maternal and paternal alleles (6). Although a study in mice overexpressing Pref1 showed reduced fat content (25), additional studies are required to elucidate the potential role of epigenetic effects in adipose tissue development in humans.

Because of our stringent group criteria, our sample size is small. However, our findings of a leaner phenotype in ODF are robust, and consistent with the previous findings of lower birth weight in this group. Our results could have been affected by the fact that these individuals were studied in adulthood, and so any individuals who developed type 2 diabetes prior to age 18 years were excluded. However, this would have, if anything, reduced our power to see group differences, particularly in an important diabetes risk factor such as AIR. Although the age of onset of diabetes in the parents was quite young, there was a slight (3.8 ± 4.0 yrs.), but statistically significant difference in the age of onset of diabetes in parents of the ODF vs. OMED group. However, the acute insulin response in ODF was markedly lower across different tests on different days implying that the paternal effect was more important than this relatively small difference in age of diagnosis.

In conclusion, the results of the present analyses indicate that offspring of fathers with early onset diabetes are leaner and have lower early insulin secretion compared to individuals where both parents remained without diabetes up to 50 years of age. Insulin action, after adjustment for body size, was comparable in all 3 groups of offspring. These findings indicate an important role of paternal heritability in body composition and beta cell dysfunction. Paternal transmission patterns for susceptibility to diabetes indicate that epigenetic mechanisms are involved in the predisposition to diabetes. Whether epigenetic markers are associated with parent-of-origin effects needs further investigation.

**Author contributions:** A.P. contributed to discussion, wrote manuscript. J.B. researched data, contributed to discussion, reviewed/edited manuscript. C.B. researched data, contributed to discussion, reviewed/edited manuscript. J.K. researched data, contributed to discussion, reviewed/edited manuscript.

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**Legend to figure:**

**Figure 1.** Plasma glucose and insulin concentrations during OGTT (A, B), during IVGTT (C, D) and during meal test (E, F) in ODF (circles), CON (squares) and inOMED (triangles). Statistical significance as revealed by two-way analysis of variance ANOVA for factors time, group, and their interactions (time x group). OGTT: *post hoc* tests for significant differences in time vs. group interactions (effect of time (p<0.0001 for all models)); effect of group (glucose: p=0.02, insulin: p=0.001); effect of time x groups interaction, (glucose: p=0.3, insulin: p=0.1). IVGTT: *Post hoc* tests for significant differences in time vs. group interactions (effect of time (p<0.0001 for all models)); effect of group (glucose: p=0.02, insulin: p<0.0001); effect of time x groups interaction, (glucose: p=0.3, insulin: p<0.0001). IVGTT data available only in 6 subjects in ODF, 6 in CON and 7 in OMED group. Meal test: *post hoc* tests for significant differences in time vs. group interactions (effect of time (p<0.0001 for all models)); effect of group (glucose: p=0.2, insulin: p<0.0001); effect of time x groups interaction, (glucose: p=0.3, insulin: p=0.002). Meal test data available only in 6 subjects in ODF, 8 in CON and 11 in OMED group.

Data are expressed as mean ± SE. *p<0.05 for specific time intervals
Table 1. General, anthropometric, and body composition parameters of subjects according to the age of diabetes onset in the parents: ODF- offspring of diabetic fathers (onset age of diabetes before 35 years); CON- controls; OMED- offspring of mothers with early onset of diabetes before age 35 years.

<table>
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<tr>
<th></th>
<th>CON</th>
<th>ODF</th>
<th>OMED</th>
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<tbody>
<tr>
<td></td>
<td>(n = 15)</td>
<td>(n = 10)</td>
<td>(n=11)</td>
</tr>
<tr>
<td>Female/Male</td>
<td>6/9</td>
<td>2/8</td>
<td>6/5</td>
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<tr>
<td>Age (years)</td>
<td>28.4 ± 8.2</td>
<td>22.9 ± 6.1</td>
<td>26.4 ± 6.9</td>
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<tr>
<td>Mother's CI</td>
<td>-0.440 ± 0.495</td>
<td>-0.577 ± 0.347</td>
<td>0.851 ± 0.047</td>
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<tr>
<td>Father's CI</td>
<td>-0.637 ± 0.373</td>
<td>0.901 ± 0.059</td>
<td>-0.438 ± 0.185</td>
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<tr>
<td>Birth weight (g)</td>
<td>3367 ± 359</td>
<td>3412 ± 528</td>
<td>3413 ± 325</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>104.5 ± 30</td>
<td>79.6 ± 19.6*</td>
<td>98.4 ± 12.2</td>
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<tr>
<td>Height (cm)</td>
<td>169 ± 7</td>
<td>172 ± 6</td>
<td>167 ± 8</td>
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<tr>
<td>Body fat (%)</td>
<td>35.5 ± 8.2</td>
<td>25.1 ± 9.1*</td>
<td>34.4 ± 7.8</td>
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<tr>
<td>Body mass index (kg/m²)</td>
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<td>27.6 ± 6.9*</td>
<td>35.9 ± 5.2</td>
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<tr>
<td>Fasting plasma glucose (mmol/L)†</td>
<td>5.19 ± 0.40</td>
<td>4.55± 0.55*</td>
<td>5.22 ± 0.43</td>
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<tr>
<td>2-h Plasma glucose (mmol/L)†</td>
<td>6.58 ± 1.28</td>
<td>5.88 ± 1.33</td>
<td>7.33 ± 1.55</td>
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<tr>
<td>NGT/IGT</td>
<td>13/2</td>
<td>9/1</td>
<td>6/5</td>
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<tr>
<td>Log₁₀M-low (mg.kg⁻¹.min⁻¹)</td>
<td>0.27 ± 0.04</td>
<td>0.52 ± 0.15*</td>
<td>0.34 ± 0.06</td>
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<tr>
<td>BGO (mg/kgEMBS·min⁻¹)</td>
<td>2.33 ± 0.33</td>
<td>2.62 ± 0.09</td>
<td>2.43 ± 0.48</td>
</tr>
<tr>
<td>Log₁₀AIR (pmol/l)²</td>
<td>2.39 ± 0.30</td>
<td>2.09 ± 0.28*</td>
<td>2.41 ± 0.15</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD.

1birth weight in ODF group available only in 7 subjects

† in ODF group data available only in 8 subjects

2only NGT subjects

CI- diabetes cumulative incidence score; NGT- normal glucose tolerance; IGT- impaired glucose tolerance; EMBS- estimated metabolic body size (fat-free mass +17.7); BGO- basal glucose output

* p<0.05 ODF vs. CON; (ANOVA; sex differences chi-square analysis).