Adiposity, Dysmetabolic Traits, and Earlier Onset of Female Puberty in Adolescent Offspring of Women With Gestational Diabetes Mellitus: A Clinical Study Within the Danish National Birth Cohort

Grunnet, L. G.; Hansen, S.; Hjort, L.; Madsen, C. M.; Bach Kampmann, Freja; Thuesen, A. C. B.; Granstrømi, C.; Strøm, M.; Maslova, E.; Frikke-Schmidt, R.; Damm, P.; Chavarro, J. E.; Hu, F. B.; Olsen, S. F.; Vaag, A.

Published in:
Diabetes Care

Link to article, DOI:
10.2337/dc17-0514

Publication date:
2017

Document Version
Peer reviewed version

Adiposity, Dysmetabolic Traits and Earlier Onset of Female Puberty in Adolescent Offspring of Women with Gestational Diabetes Mellitus: A clinical study within the Danish National Birth Cohort.

Running title: Gestational diabetes impacts offspring health

Louise G. Grunnet, PhD[1,4], Susanne Hansen, PhD[2], Line Hjort, MSc[1,3,4], Camilla M. Madsen[1]MSc, Freja B. Kampmann, MSc[1,4,5], Anne Cathrine B. Thuesen[1]MD, Charlotta Granstrøm, MSci[2], Marin Strøm, PhD[2,6], Ekaterina Maslova, ScD[2,4,7], Ruth Frikke-Schmidt, PhD[8] , Peter Damm, DMSc [9], Jorge E. Chavarro ScD [10], Frank B. Hu PhD[11], Sjurdur F. Olsen PhD[2], Allan Vaag DMSc[1,12]

[1] Department of Endocrinology - Diabetes and Metabolism, Rigshospitalet, Copenhagen Denmark

[2] Centre for Fetal Programming, Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark

[3]Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark.

[4]The Danish Diabetes Academy, Odense, Denmark.

[5]Division for Diet, Disease Prevention and Toxicology, National Food Institute, Technical University of Denmark, Søborg, Denmark

[6] Faculty of Natural and Health Sciences, University of the Faroe Islands, Tórshavn, Faroe Islands

[7]Department of Primary Care and Public Health, Imperial College, London, UK

[8] Department of Clinical Biochemistry, Rigshospitalet, Copenhagen Denmark

[9]Center for Pregnant Women with Diabetes, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark

[10]Departments of Nutrition and Epidemiology, Harvard TH Chan School of Public Health &Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School

[11] Departments of Nutrition and Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA.

[12] Astrazeneca, Early Clinical Development, Göteborg, Sweden

Key terms: Gestational diabetes, Offspring, Metabolic health, maternal pre-pregnancy BMI, hyperglycemia

Corresponding author: Louise G. Grunnet, Rigshospitalet, Tagensvej 20, 2200 København N, Denmark. Email: louise.groth.grunnet@regionh.dk Phone: +45 35457127 (Reprint requests to the corresponding author)

Word count: Abstract = 257 Text = 3274 Number of tables: 3 Number of figures: 2

Abstract

Offspring of pregnancies affected by gestational diabetes mellitus (GDM) are at increased risk of developing type 2 diabetes. However, the extent to which these dysmetabolic traits may be due to offspring and/or maternal adiposity is unknown.

Objective: We examined body composition and associated cardio-metabolic traits in 561 9-16 year old offspring of GDM and 597 control offspring.

Research Design and Methods: We measured anthropometrics, puberty status, blood pressure, fasting glucose, insulin, C-peptide and lipids levels and dual energy X-ray (DEXA) scan in a subset of the cohort. Differences in the outcomes between GDM offspring and controls were examined using linear and logistic regression models.

Results: After adjustment for age and gender, GDM offspring displayed higher weight, BMI, WHR, systolic blood pressure and resting heart rate and lower height. GDM offspring had higher total and abdominal fat percentages and lower muscle mass percentages, but these differences disappeared after correction for offspring BMI. GDM offspring displayed higher fasting plasma glucose, insulin, C-peptide, HOMA-IR and plasma triglycerides, whereas fasting plasma HDL-cholesterol levels were decreased. Female GDM offspring had an earlier onset of puberty than control offspring. GDM offspring had significantly higher BMI, WHR, fasting glucose and HOMA-IR after adjustment for maternal pre-pregnancy BMI, and glucose and HOMA-IR remained elevated in GDM offspring after correction for both maternal and offspring BMI.

Conclusions: In summary, adolescent offspring of GDM women show increased adiposity, an adverse cardio-metabolic profile and earlier onset of puberty among girls. Increased fasting glucose and HOMA-IR among GDM offspring may be explained by programming effects of hyperglycemia in pregnancy.

Offspring of mothers with gestational diabetes (GDM) are at increased risk for developing obesity, insulin resistance and type 2 diabetes (1-4). Maternal obesity is one of the most important risk factors of both GDM and offspring adiposity, and the extent to which maternal obesity explains offspring adiposity and associated dysmetabolic traits independent of other factors including hyperglycaemia in pregnancy is controversial and the particular impact of hyperglycemia per se on offspring metabolic health is debated (5,6). Whereas intensified glucose lowering treatment of GDM women reduced macrosomia at birth, no beneficial effect on offspring adiposity or associated cardio-metabolic dysfunctions at age 5-10 years was seen (7,8).

Few studies have examined the impact of GDM on adiposity and insulin resistance during adolescence, and the joint influence of these factors on the onset of puberty. Early onset is associated with emotional challenges and can result in short stature, both of which may have influence at the individual and public health level. One study examined the impact of GDM on adiposity and metabolic variables across 5 puberty stages and found no difference in puberty development between GDM offspring and controls, but an increase in body fat percentages during all Tanner stages (9).

Previous studies of GDM offspring have been variable size (n=24-1475 cases) with the largest studies having only few clinical measurements such as height, weight and weight to hip-ratio in the offspring (10,11). In the present study, we report clinical and metabolic characteristics including body composition and puberty status in a large cohort of 9-16 years old offspring of women with and without previous GDM recruited from the Danish National Birth Cohort (DNBC) (12). Additionally, we examine the association of GDM with offspring metabolic disease and puberty development while accounting for offspring and maternal degree of adiposity.

Research design and methods

Subjects

Participants were recruited from the DNBC that enrolled 91,827 primarily Caucasian women collectively contributing more than 100,000 pregnancies between January 1996 and October 2002 as described in detail elsewhere (12). Briefly, data collection included 4 telephone interviews in gestation weeks 12 and 30, and 6 and 18 months postpartum. From the DNBC we included 1350 women with a diagnosis of GDM and 2629 randomly selected controls and invited them and their offspring to participate in a clinical follow-up examination in 2012 March - 2014 April (13). In total, 608 (44%) case mother-offspring pairs and 626 (28%) control mother-offspring pairs participated. Main reason for non-participation was lack of time. We excluded multiple births including 33 twins (31 GDM), 3 triplets (all GDM) and only included the first sibling in our analyses (80 siblings in total, 40 kept in analyses) to avoid correlated measures. This left us with 561 GDM offspring and 597 control offspring in our analyses (Figure 1). The offspring were 9-16 years old at the time of examination. The study was approved by the Regional Scientific Ethics Committee for the municipalities of Copenhagen and Frederiksberg (H-4-2011-045 and H-4-2013-129). Consent from both parents was essential for the participation of the child in the study.

Diagnosis of GDM

Initially, we used two different sources to identify women with a history of GDM. Women were classified with a diagnosis of GDM if they had responded positively to a question about GDM in at least one of the interviews conducted in gestation week 30 or 6 months postpartum, respectively. Furthermore, we used the Danish National Patient Register to extract information about diagnoses of GDM (ICD-10 classification: O244 and O249). Women with a self-reported diagnosis of GDM and/or an ICD-10 diagnosis and/or were classified as having had suspected GDM in our main analyses. Additionally, in sensitivity analyses we used an alternative diagnosis of GDM defined as

"best clinical judgement". This diagnosis was based on a thorough review by two clinicians of hospital records of 96.5% of all GDM cases classified as described above. The 'best clinical judgement" or verified GDM diagnosis was based upon several available data such as blood glucose measurements if available, or on other notes from the doctor indicating GDM. In the sensitivity analyses in the present paper, only those with GDM based upon the "the best clinical judgment" were included (n=332).

In Denmark a risk factor based screening for GDM with a 75 g diagnostic oral glucose tolerance test (OGTT) was recommended during 1996-2002. If results from 75 g 3 hour OGTT was available from the hospital records, GDM was diagnosed if two or more glucose values exceeded the mean +3 SDs on a curve based on a group of 40 Danish healthy, nonobese, nonpregnant women without a family history of diabetes. However, in a few smaller departments the WHO criteria was used. The diagnosis of the GDM women is described in detail in (14).

Clinical examinations:

All participating offspring underwent a clinical examination that included anthropometric, metabolic and body composition measurements. Offspring were weighed without shoes and lightly dressed. We measured waist circumference at the level of umbilicus using a soft tape on standing subjects. Hip circumference was measured over the widest part of the gluteal region. After 10 minutes, the resting blood pressure and heart rate were measured with an Omron blood pressure device in supine position. All measurements were taken twice and if the differences exceeded 0.5 cm or 0.5 kg for the anthropometric measurements, or 5 mmHg for blood pressure measurements, a third measurement was taken. In all analyses, the mean value of the measurements was used.

Offspring metabolic outcomes were obtained from a fasting blood sample that was taken during the clinical examination, and plasma, serum, buffy coat and whole blood (PAXgene) was collected.

Blood samples for glucose measurements were drawn in K-oxalat-Na-fluoride vials and in lithium-heparin vials for insulin, C-peptide and lipid traits. All parameters were measured using standard laboratory methods on the Modular P-module (Roche, Mannheim, Germany). Coefficients of variance were 4 -5 % for glucose, insulin, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides and 8 % for C-peptide. HOMA-IR was calculated as ([(Fasting plasma insulin (pmol/l) * fasting plasma glucose (mmol/l)) /22.5] *0.144) (15).

In order to determine the puberty status of the study participants, clinical evaluations were made including pubertal staging of breast development and pubic hair for the girls according to Tanner's classifications (16,17). Breast stage ≥B2 or girls pubic hair stage ≥PH2 was considered to be a marker of pubertal onset. Among the boys, a testicular volume of ≥4 ml, pubic hair stage ≥PH2 or boys genital stage ≥G2 was considered to be a marker of pubertal onset. A total of 238 GDM offspring and 256 control offspring agreed to have at least one of the Tanner score examinations performed. Finally, body composition outcomes were evaluated in a subset (n=637) of the offspring who had a dual energy x-ray (DEXA) scanning performed (GE Healthcare, Lunar Prodigy Advanced EnCore, including pediatric software).

Statistical analyses

Normally distributed, continuous outcomes were described using mean and standard deviation (SD), whereas median and inter quartile range (IQR) were used for skewed, continuous outcomes. Differences in anthropometric, metabolic, and body composition outcomes between offspring exposed to GDM and control offspring were examined using linear regression models. For normally distributed outcome variables, we calculated β coefficients and 95% confidence intervals (CIs) to estimate mean differences, whereas skewed variables were log-transformed and for these we

calculated % differences and 95% CIs. We used logistic regression models to analyze puberty status and calculated odds ratios (ORs) and 95% CIs.

A priori we decided to first adjust for offspring age at the clinical examination and to analyze the age-adjusted estimates separately for boys and girls to investigate potential sex-specific differences. Because we did not see any marked differences between male and female offspring in the effect estimates, we decided to include offspring sex in our minimally adjusted model as a potential confounder rather than to stratify on sex. To examine whether observed cardio-metabolic differences were explained by the offspring's own degree of adiposity, we included a model with adjusting for offspring BMI and one model with additional adjustment for offspring WHR. For those variables that remained statistically significantly different between GDM and control offspring after adjustment for offspring BMI and WHR, we subsequently conducted analyses in which we adjusted for maternal pre-pregnancy BMI (in categories <18.5 kg/m^2, 18.5-24.99 kg/m^2, 25-29.99 kg/m^2 and >=30 kg/m^2). Maternal pre-pregnancy BMI was obtained from telephone interviews in gestation week 12. We did not adjust for other potential confounding variables, since our main aim was to determine if any observed differences were related to maternal obesity or hyperglycemia in pregnancy, rather than teasing out the contribution of a range of other potential confounding variables.

In addition, we stratified for maternal pre-pregnancy BMI in four groups to examine if there were differences when comparing GDM offspring to controls across groups of maternal pre-pregnancy BMI. Additionally, we performed analyses in a sub-sample of women where GDM (N=332) was defined according to 'best clinical judgement' (14) compared to controls.

Results

Table 1 presents the clinical characteristics of the offspring. There was an even sex distribution among case and control offspring, but control offspring were slightly older than GDM offspring at the time of follow-up, which was due to a time displacement for the examination time for some of the control offspring. When we adjusted for this age difference, GDM offspring were heavier and had a higher BMI, larger waist and hip circumferences, elevated WHR, as well as increased systolic blood pressure and heart rate compared to control offspring (Table 1). Furthermore, GDM offspring had higher fasting whole blood and fasting plasma glucose levels, as well as higher fasting plasma insulin, fasting C-peptide and HOMA-IR levels. In addition, the GDM offspring had an unhealthier plasma lipid profile with higher triglyceride and lower HDL levels. GDM offspring also had higher total fat percentages, more abdominal fat, and lower lean body mass percentages than control offspring. Among the female GDM offspring the odds of having reached puberty based on Tanner stage for breast development was almost doubled compared to control offspring. No differences in puberty development were observed between male GDM versus control offspring (Table 1). After adjustment for puberty status, we still observed significant differences in the insulin resistance markers between GDM and control offspring (data not shown).

Since adiposity is one of the most important risk factors for insulin resistance, type 2 diabetes and cardiovascular disease (CVD) (18), we examined whether the adverse cardio-metabolic profile among GDM offspring was explained by their increased adiposity compared to controls. When adjusted for offspring BMI, GDM offspring still had significantly higher waist circumference, WHR, fasting plasma glucose, fasting insulin level and HOMA-IR levels (Table 2). Blood pressure, fasting C-peptide levels, lipid profile and data on body composition obtained from a DEXA scan were not significantly different between the two groups after adjustment for offspring BMI (Table 2). In addition, the association with earlier onset of puberty among female GDM offspring was no longer significant after adjustment for the offspring's own BMI (Figure 2). Adjusting for offspring

BMI Z-score instead of BMI did not change our results (data not shown). Additionally, GDM offspring displayed significantly higher fasting glucose (1.04 (1.02;1.05) p<0.0001) and HOMA-IR (1.09 (1.02;1.16) p=0.006) after further adjustment for WHR.

As adiposity is highly heritable (19,20) we subsequently examined if the increased BMI, waist circumference and WHR together with the adverse metabolic profile among the GDM offspring could be explained by maternal obesity before pregnancy. After adjustment for maternal pre-pregnancy BMI, GDM offspring still had higher BMI (Table 2). WHR, waist circumference, fasting glucose and HOMA-IR were all significantly higher in GDM offspring after further adjustment for both offspring BMI and maternal pre-pregnancy BMI (Table 2). Interestingly, the difference in the anthropometric, metabolic and body composition outcomes between GDM offspring and controls appeared to be stronger among offspring whose mothers were normal weight in pregnancy (18.5< BMI < 25) compared to overweight (BMI=25-30) or obese (BMI>30) (Table 3). Among obese mothers, no differences were observed between GDM offspring and controls.

Finally, defining the GDM diagnosis using 'best clinical judgement' and only including offspring of hospital records GDM diagnosis resulted in similar or stronger associations, supporting our findings in all cases (supplementary table 1).

Discussion

We found that 9-16 year old offspring of GDM mothers had the following characteristics: 1) higher BMI, WHR, fat percentages and lower lean mass percentages, 2) increased fasting glucose, insulin and C-peptide, HOMA-IR, systolic blood pressure and triglycerides as well as reduced HDL-cholesterol levels, and 3) female offspring had an earlier onset of puberty. Interestingly, after adjustment for the offspring's own BMI and maternal pre-pregnancy BMI, GDM offspring still had

significantly higher fasting glucose, HOMA-IR and WHR, but differences in onset of puberty disappeared.

Previous studies have shown that exposure to GDM was associated with higher BMI, waist circumferences and increased subscapular to triceps skinfold ratio (21), increased fat mass and central adiposity among GDM male offspring (22) and an increased risk of overweight and obesity among GDM offspring (11). In these studies, the associations between maternal hyperglycemia and offspring adiposity were attenuated but still significant after adjustment for maternal pre-pregnancy BMI. In adult offspring born to mothers with GDM, BMI was on average 0.94 kg/m^2 greater than in their brothers born before the mother was diagnosed with diabetes suggesting that it is most likely due to intrauterine mechanisms and not familial confounding (10). A meta-analysis by Philipps *et al*. concluded that maternal diabetes was associated with increased offspring BMI z-score, but that this was no longer significant after adjustment for maternal pre-pregnancy BMI (6). However, only five studies had data available on maternal pre-pregnancy BMI and four of these were based on small sample sizes, and the adjusted analyses were made on all diabetic pregnancies and not only pregnancies affected by GDM. Others studies have found increased total fat percentages and increased lean mass measured by DEXA scans in prepubertal offspring of mothers with GDM; however, in these studies no adjustment for offspring BMI or maternal pre-pregnancy BMI was made (23,24). Taken together, our study highlights the importance of adjusting for maternal pre-pregnancy BMI when analysing the impact of maternal hyperglycemia in pregnancy on offspring's degree of obesity. We a priori chose not to include birth weight in our analyses since we considered birthweight as a mediator of the association between GDM and later risk of obesity among the offspring. However, if we adjusted for birthweight there was still a significant difference between GDM and control offspring with regards to adiposity measurements such as BMI, waist circumference, total fat percentages and gynoid and android fat while birthweight per se

independently was associated with offspring BMI and waist circumference but not with DEXA (data not shown)".

Our finding of higher systolic blood pressure in GDM offspring was also observed in 3 years old offspring, where after adjustment for the offspring's skinfold thicknesses, the association between GDM and increased systolic blood pressure was no longer significant (25). Similar to our findings, a meta-analysis showed that GDM offspring had higher systolic blood pressure, but no difference in diastolic blood pressure as compared with control offspring was observed. However, all these studies had smaller GDM offspring samples, no adjustment for offspring BMI and no significant association between offspring systolic blood pressure and maternal pre-pregnancy BMI was found in five of the studies were data on maternal pre-pregnancy BMI was available (26). In contrast, in 3-17 year old GDM offspring no association between GDM and offspring blood pressure was found (27). However, the latter study included a smaller sample size and the GDM diagnosis was based upon one single question on a questionnaire.

Our findings of multiple early disturbances in the cardio-metabolic system in offspring of GDM pregnancies are in line with previous smaller studies. However, not all previous studies adjusted the disturbances in the cardio-metabolic traits for the offspring's own degree of adiposity (1,3,28). Similar to our results other studies adjusted for offspring BMI and reported an attenuation, but still significant association, of the impact of GDM on offspring insulin insensitivity and risk of future type 2 diabetes (29,30). One study showed that adult offspring born to women with GDM had reduced insulin sensitivity compared to offspring from the background population, also after adjusting for sex and overweight (2). Holder *et al*. followed obese adolescents for an average of 2.8 years and found that insulin sensitivity at follow-up were significantly lower in the group that had been exposed to GDM (n=45) in utero after adjusting for offspring BMI (31). Additionally, others

have found that greater maternal glucose concentration in pregnancy was associated with reduced insulin sensitivity and greater static beta cell response after a meal tolerance test in 21 pre-pubertal children, independent of the children's own fat percentages measured by DXA scanning (32). In the present study we found that the C-peptide levels were no longer significantly different between GDM and control offspring after adjustment for offspring BMI, whereas fasting insulin levels remained significant. We speculate that the difference in levels of statistical significance represents variations of the assays used rather than biologically important differences in insulin and C-peptide kinetics. Indeed, the confidence interval for C-peptide was substantially larger than the confidence interval for insulin. Alternatively, the relatively increased plasma insulin compared with plasma C-peptide levels among GDM offspring could reflect lower insulin clearance as a result of insulin resistance. C-peptide is cleared by the kidneys and therefore not influenced by insulin resistance (33).

We found that the probability of having reached puberty assessed by breast development, considered the golden standard for evaluating puberty onset and development among girls (34), was doubled among offspring of GDM mothers. In contrast, others showed no differences in Tanner stages among GDM and non-GDM offspring in analyses when boys and girls were analyzed together (35). One study showed that GDM was associated with a two months earlier transition to Tanner stage >2 examined by pubic hair development among boys (36). However, these studies did not adjust for offspring BMI. There is a general agreement that a higher fat mass or higher BMI among girls is associated with an earlier onset of puberty (37,38), which supports our results that the earlier onset of puberty among GDM female offspring is mainly driven by the offspring's BMI and emphasizing the importance of adjusting for offspring adiposity when addressing the impact of hyperglycemia on puberty development.

Our results stratifying on maternal pre-pregnancy BMI, suggest that hyperglycemia may be more relevant in the absence of severe maternal adiposity, i.e. the impact of hyperglycemia on offspring's body composition may be overruled by severe obesity in the mother. This is in accordance to a recent study, also based on the DNBC, showing that the effect of maternal fasting plasma glucose in pregnancy on offspring obesity at 7 year, appeared more pronounced among non-obese GDM women compared with obese GDM women (39). However, more studies are needed to understand the separate role and the combined potential superimposing effect of maternal hyperglycemia and of maternal obesity during pregnancy, on their offspring metabolic health.

Besides maternal obesity and hyperglycemia, other factors such as paternal obesity influence the offspring's level of obesity and adiposity. The strengths of this study included a large sample of GDM pregnancies and good statistical power to examine long-term consequences of intrauterine hyperglycemia in this longitudinal study with more than ten years of follow-up. Detailed data were available on body composition, cardio-metabolic factors, and clinical assessments of puberty onset in the offspring, allowing for more precise phenotypical characterization. Since puberty is, among other factors, characterized by insulin resistance (40), it is an enormous strength in the present study that we can take the stage of pubertal development into account.

The present study had some limitations. The GDM group contained both confirmed and suspected cases and may therefore have included women without GDM. Nonetheless, a sensitivity analysis of groupings based on clinician's best judgement in a subsample of the women did not alter the results. Another limitation is that a few smaller departments used the WHO criteria and not the commonly used Danish criteria for GDM. While detailed clinical data was available for the offspring, comparable information was not available for mothers or fathers for the relevant time period. For example, no detailed measures of maternal body composition were available for the pre-pregnancy period, and no data on paternal health was available to account for any genetic predisposition.

However, the explained genetic variance for most complex diseases is ≤10% and thus the impact on our results may not have been substantial. Misclassification of reported maternal BMI is likely to have been more prevalent at higher BMI values, and may have underestimated the frequencies in overweight and obese categories. This would have led to residual confounding in the models adjusting for maternal BMI. We also lacked data on postnatal environment, and possible shared social and familial obesogenic risk factors such as diet. Dietary information for both parents and offspring was not available until teen years. Although parental and offspring diets during this time period were only weakly correlated (Bjerregaard A.A, preliminary data), we cannot exclude that stronger correlations existed earlier in childhood. Our data on puberty may also be subjective to selection bias, since the boys and girls that did not want to participate in the puberty examination, were often also those that were older and had attained puberty. Since the DEXA scanning was only available at the Copenhagen University Hospital, it was only offspring examined at this location that was offered a DEXA scan. This may have caused some bias, since the mothers of offspring with a DXA scan were older, had higher socio economic status and lower pre-pregnancy BMI. However, these differences were similar for the two exposure groups, which suggest that any present bias may be minor. Furthermore, we cannot exclude that our results to some extent may be due to other confounding factors such as socio-economic status or breastfeeding duration, rather than hyperglycemia and maternal obesity per se.

In conclusion, we demonstrated that 9-16 year old offspring of GDM mothers had higher BMI, WHR and higher fat percentage with more abdominal obesity, higher systolic blood pressure, fasting glucose, insulin and C-peptide levels and higher HOMA-IR and an earlier onset of puberty among girls. The association to higher blood pressure, higher fasting C-peptide levels, adverse lipid profile and earlier onset of puberty seemed driven by the offspring's own BMI, whereas GDM

offspring still had higher WHR, fasting glucose and HOMA-IR even after adjustment for both the offspring's own BMI and maternal pre-pregnancy BMI. This supports an independent role of hyperglycemia in pregnancy programming body composition as well as insulin resistance among adolescent offspring.

Acknowledgement

A special appreciation to all the mother-child dyads that participated in this study.

The authors' contributions were as follows – LGG, AV, SFO, JC, FBH: study concept and design; SFO: Contributed to establishing DNBC and proposed the basic design of the present study; LGG, SH, LH, CMM, FBK, ACBT, MS, RFS: took part in the planning and conductance of the clinical examinations; SH, LGG, CG: conducted the statistical analyses. LGG drafted the manuscript; SH, LH, CMM, FBK, ACBT, MS, EM, PD, JC, FBH, SFO, AV: contributed critical advice and revisions of the manuscript; LGG and AV are the guarantors of this work, had full access to all the data in the study and takes responsibility for the contents and integrity of the data in this article. All authors read and approved the final manuscript.

Conflict of interest: Dr. Damm reports: Participate in a multicenter, multinational study by Novonordisk A/S as investigator on the use of insulins in pregnancy in women with type 1 and type 2 diabetes . All other authors have nothing to disclose

Funding:

The Innovation Fund Denmark (09-067124 and 11-115923), The Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National

Institutes of Health (contract #HHSN275201000020C), Rigshospitalet, Copenhagen University Hospital,

Reference List

1. Boerschmann H, Pfluger M, Henneberger L, Ziegler AG, Hummel S: Prevalence and predictors of overweight and insulin resistance in offspring of mothers with gestational diabetes mellitus. *Diabetes care* 33:1845-1849, 2010

2. Kelstrup L, Damm P, Mathiesen ER, Hansen T, Vaag AA, Pedersen O, Clausen TD: Insulin resistance and impaired pancreatic beta-cell function in adult offspring of women with diabetes in pregnancy. *J Clin Endocrinol Metab* 98:3793-3801, 2013

3. Egeland GM, Meltzer SJ: Following in mother's footsteps? Mother-daughter risks for insulin resistance and cardiovascular disease 15 years after gestational diabetes. *Diabet Med* 27:257-265, 2010

4. Metzger BE: Long-term outcomes in mothers diagnosed with gestational diabetes mellitus and their offspring. *Clin Obstet Gynecol* 50:972-979, 2007

5. Donovan LE, Cundy T: Does exposure to hyperglycaemia in utero increase the risk of obesity and diabetes in the offspring? A critical reappraisal. *Diabet Med* 32:295-304, 2015

6. Philipps LH, Santhakumaran S, Gale C, Prior E, Logan KM, Hyde MJ, Modi N: The diabetic pregnancy and offspring BMI in childhood: a systematic review and meta-analysis. *Diabetologia* 54:1957-1966, 2011

7. Gillman MW, Oakey H, Baghurst PA, Volkmer RE, Robinson JS, Crowther CA: Effect of treatment of gestational diabetes mellitus on obesity in the next generation. *Diabetes care* 33:964-968, 2010

8. Landon MB, Rice MM, Varner MW, Casey BM, Reddy UM, Wapner RJ, Rouse DJ, Biggio JR, Jr., Thorp JM, Chien EK, Saade G, Peaceman AM, Blackwell SC, VanDorsten JP: Mild gestational diabetes mellitus and long-term child health. *Diabetes care* 38:445-452, 2015

9. Davis JN, Gunderson EP, Gyllenhammer LE, Goran MI: Impact of gestational diabetes mellitus on pubertal changes in adiposity and metabolic profiles in Latino offspring. *J Pediatr* 162:741-745, 2013

10. Lawlor DA, Lichtenstein P, Langstrom N: Association of maternal diabetes mellitus in pregnancy with offspring adiposity into early adulthood: sibling study in a prospective cohort of 280,866 men from 248,293 families. *Circulation* 123:258-265, 2011

11. Nehring I, Chmitorz A, Reulen H, von KR, Ensenauer R: Gestational diabetes predicts the risk of childhood overweight and abdominal circumference independent of maternal obesity. *Diabet Med* 30:1449-1456, 2013

12. Olsen J, Melbye M, Olsen SF, Sorensen TI, Aaby P, Andersen AM, Taxbol D, Hansen KD, Juhl M, Schow TB, Sorensen HT, Andresen J, Mortensen EL, Olesen AW, Sondergaard C: The Danish National Birth Cohort--its background, structure and aim. *Scand J Public Health* 29:300-307, 2001

13. Zhang C, Hu FB, Olsen SF, Vaag A, Gore-Langton R, Chavarro JE, Bao W, Yeung E, Bowers K, Grunnet LG, Sherman S, Kiely M, Strom M, Hansen S, Liu A, Mills J, Fan R: Rationale, design, and method of the Diabetes & Women's Health study--a study of long-term health implications of glucose intolerance in pregnancy and their determinants. *Acta Obstet Gynecol Scand* 93:1123-1130, 2014

14. Olsen SF, Houshmand-Oeregaard A, Granstrom C, Langhoff-Roos J, Damm P, Bech BH, Vaag AA, Zhang C: Diagnosing gestational diabetes mellitus in the Danish National Birth Cohort. *Acta Obstet Gynecol Scand* 2016

15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412-419, 1985

16. Marshall WA, Tanner JM: Variations in pattern of pubertal changes in girls. *Arch Dis Child* 44:291-303, 1969

17. Marshall WA, Tanner JM: Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 45:13-23, 1970

18. Reaven G, Abbasi F, McLaughlin T: Obesity, insulin resistance, and cardiovascular disease. *Recent Prog Horm Res* 59:207-223, 2004

19. Allison DB, Kaprio J, Korkeila M, Koskenvuo M, Neale MC, Hayakawa K: The heritability of body mass index among an international sample of monozygotic twins reared apart. *Int J Obes Relat Metab Disord* 20:501-506, 1996

20. Malis C, Rasmussen EL, Poulsen P, Petersen I, Christensen K, Beck-Nielsen H, Astrup A, Vaag AA: Total and regional fat distribution is strongly influenced by genetic factors in young and elderly twins. *Obes Res* 13:2139-2145, 2005

21. Crume TL, Ogden L, West NA, Vehik KS, Scherzinger A, Daniels S, McDuffie R, Bischoff K, Hamman RF, Norris JM, Dabelea D: Association of exposure to diabetes in utero with adiposity and fat distribution in a multiethnic population of youth: the Exploring Perinatal Outcomes among Children (EPOCH) Study. *Diabetologia* 54:87-92, 2011

22. Regnault N, Gillman MW, Rifas-Shiman SL, Eggleston E, Oken E: Sex-specific associations of gestational glucose tolerance with childhood body composition. *Diabetes care* 36:3045-3053, 2013

23. Chandler-Laney PC, Bush NC, Rouse DJ, Mancuso MS, Gower BA: Maternal glucose concentration during pregnancy predicts fat and lean mass of prepubertal offspring. *Diabetes care* 34:741-745, 2011

24. Chandler-Laney PC, Bush NC, Granger WM, Rouse DJ, Mancuso MS, Gower BA: Overweight status and intrauterine exposure to gestational diabetes are associated with children's metabolic health. *Pediatr Obes* 7:44-52, 2012

25. Wright CS, Rifas-Shiman SL, Rich-Edwards JW, Taveras EM, Gillman MW, Oken E: Intrauterine exposure to gestational diabetes, child adiposity, and blood pressure. *Am J Hypertens* 22:215-220, 2009

26. Aceti A, Santhakumaran S, Logan KM, Philipps LH, Prior E, Gale C, Hyde MJ, Modi N: The diabetic pregnancy and offspring blood pressure in childhood: a systematic review and meta-analysis. *Diabetologia* 55:3114-3127, 2012

27. Beyerlein A, Nehring I, Rosario AS, von KR: Gestational diabetes and cardiovascular risk factors in the offspring: results from a cross-sectional study. *Diabet Med* 29:378-384, 2012

28. Krishnaveni GV, Veena SR, Hill JC, Kehoe S, Karat SC, Fall CH: Intrauterine exposure to maternal diabetes is associated with higher adiposity and insulin resistance and clustering of cardiovascular risk markers in Indian children. *Diabetes care* 33:402-404, 2010

29. Clausen TD, Mathiesen ER, Hansen T, Pedersen O, Jensen DM, Lauenborg J, Damm P: High prevalence of type 2 diabetes and pre-diabetes in adult offspring of women with gestational diabetes mellitus or type 1 diabetes: the role of intrauterine hyperglycemia. *Diabetes care* 31:340-346, 2008

30. Vaarasmaki M, Pouta A, Elliot P, Tapanainen P, Sovio U, Ruokonen A, Hartikainen AL, McCarthy M, Jarvelin MR: Adolescent manifestations of metabolic syndrome among children born to women with gestational diabetes in a general-population birth cohort. *Am J Epidemiol* 169:1209-1215, 2009

31. Holder T, Giannini C, Santoro N, Pierpont B, Shaw M, Duran E, Caprio S, Weiss R: A low disposition index in adolescent offspring of mothers with gestational diabetes: a risk marker for the development of impaired glucose tolerance in youth. *Diabetologia* 57:2413-2420, 2014

32. Bush NC, Chandler-Laney PC, Rouse DJ, Granger WM, Oster RA, Gower BA: Higher maternal gestational glucose concentration is associated with lower offspring insulin sensitivity and altered beta-cell function. *J Clin Endocrinol Metab* 96:E803-E809, 2011

33. Lee CC, Haffner SM, Wagenknecht LE, Lorenzo C, Norris JM, Bergman RN, Stefanovski D, Anderson AM, Rotter JI, Goodarzi MO, Hanley AJ: Insulin clearance and the incidence of type 2 diabetes in Hispanics and African Americans: the IRAS Family Study. *Diabetes care* 36:901-907, 2013

34. TANNER JM, WHITEHOUSE RH: Standards for subcutaneous fat in British children. Percentiles for thickness of skinfolds over triceps and below scapula. *Br Med J* 1:446-450, 1962

35. Davis JN, Gunderson EP, Gyllenhammer LE, Goran MI: Impact of gestational diabetes mellitus on pubertal changes in adiposity and metabolic profiles in Latino offspring. *J Pediatr* 162:741-745, 2013

36. Monteilh C, Kieszak S, Flanders WD, Maisonet M, Rubin C, Holmes AK, Heron J, Golding J, McGeehin MA, Marcus M: Timing of maturation and predictors of Tanner stage transitions in boys enrolled in a contemporary British cohort. *Paediatr Perinat Epidemiol* 25:75-87, 2011

37. Aksglaede L, Juul A, Olsen LW, Sorensen TI: Age at puberty and the emerging obesity epidemic. *PLoS One* 4:e8450, 2009

38. Crocker MK, Stern EA, Sedaka NM, Shomaker LB, Brady SM, Ali AH, Shawker TH, Hubbard VS, Yanovski JA: Sexual dimorphisms in the associations of BMI and body fat with indices of pubertal development in girls and boys. *J Clin Endocrinol Metab* 99:E1519-E1529, 2014

39. Zhu Y, Olsen SF, Mendola P, Yeung EH, Vaag A, Bowers K, Liu A, Bao W, Li S, Madsen C, Grunnet LG, Granstrom C, Hansen S, Martin K, Chavarro JE, Hu FB, Langhoff-Roos J, Damm P, Zhang C: Growth and obesity through the first 7 y of life in association with levels of maternal glycemia during pregnancy: a prospective cohort study. *Am J Clin Nutr* 2016

40. Kelly LA, Lane CJ, Weigensberg MJ, Toledo-Corral CM, Goran MI: Pubertal changes of insulin sensitivity, acute insulin response, and beta-cell function in overweight Latino youth. *J Pediatr* 158:442-446, 2011

Figure legends

Figure 1.

Flow chart of enrolment and examination of women and children in the Diabetes and Women's Health Study.

Figure 2.

Differences in offspring puberty status comparing GDM offspring to control offspring. Odds ratios are adjusted for offspring BMI

Table 1. Anthropometric, metabolic, body composition, and puberty characteristics of GDM offspring and controls at age 9-16 years.

	Crude measurements			Age and sex adjusted estimates	
	GDM offspring	**Control offspring**	**P[a]**	**Mean difference or % difference* (95% CI)**	**P[b]**
Anthropometric characteristics	N=546-561	N=590-597			
Age (years)	12.1 (1.5)	12.8 (1.5)	<0.001		
Sex (boys)	295 (52.6%)	301 (50.4%)	0.68		
Weight (kg)	48.5 (12.7)	47.2 (12.1)	0.08	4.66 (3.48, 5.84)	<0.001
Height (cm)	156.8 (11.4)	159.5 (11.4)	<0.001	1.15 (0.27, 2.03)	0.01
BMI (kg/m2)*	18.8 (4.2)	17.9 (3.4)	<0.001	9% (7-11%)	<0.001
Waist (cm)	73.3 (10.6)	69.9 (8.4)	<0.001	4.92 (3.87, 5.98)	<0.001
Hip circumference (cm)	83.8 (9.6)	82.7 (9.3)	0.07	3.47 (2.55, 4.39)	<0.001
Waist hip ratio (WHR)	0.87 (0.06)	0.85 (0.05)	<0.001	0.02 (0.01;0.03)	<0.001
Head circumference (cm)	55.8 (2.1)	55.5 (2.2)	0.18	0.51 (0.28, 0.75)	<0.001
Systolic blood pressure (mmHg)	109.7 (8.6)	109.5 (8.6)	0.75	1.04 (0.06, 2.01)	0.04
Diastolic blood pressure (mmHg)	62.5 (6.0)	62.6 (6.1)	0.84	-0.20 (-0.92, 0.51)	0.58
Heart rate (BPM)	69.7 (2.1)	68.1 (10.0)	0.001	0.81 (-0.34, 1.96)	0.17
Metabolic characteristics	N=468-522	N=508-559			
Whole blood fasting glucose (mmol/l)*	4.7 (0.8)	4.5 (0.7)	<0.001	3% (1-5%)	0.001
Fasting plasma glucose (mmol/l)*	5.0 (0.8)	4.8 (0.6)	<0.001	4% (3-6%)	<0.001
Fasting insulin (pmol/l)*	69.4 (47.3)	61.3 (34.7)	0.001	17% (10-24%)	<0.001
Fasting C-peptide (pmol/l)	596 (211)	575 (189)	0.05	51 (28,74)	<0.001
HOMA-IR*	2.2 (1.6)	1.9 (1.1)	<0.001	21% (14-30%)	<0.001
Triglycerides (mmol/l)*	0.73 (0.4)	0.70 (0.4)	0.58	5% (1-10%)	0.04
HDL cholesterol (mmol/l)	1.5 (0.4)	1.6 (0.4)	0.11	-0.07 (-0.11, -0.02)	0.004
LDL cholesterol (mmol/l)	2.4 (0.7)	2.3 (0.6)	0.08	0.06 (-0.01, 0.14)	0.11
Total cholesterol (mmol/l)	4.3 (0.7)	4.2 (0.7)	0.22	0.03 (-0.05, 0.12)	0.47
Body composition measured by DXA	N=191	N=446			
Total fat %	31.2 (8.1)	27.0 (7.0)	<0.001	3.45 (2.22, 4.69)	<0.001
Total lean mass %	66.2 (7.5)	70.1 (6.5)	<0.001	-3.21 (-4.37, -2.07)	<0.001
Total android tissue % fat*	25.6 (20.1)	19.4 (13.8)	<0.001	22% (12-33#)	<0.001
Total gynoid tissue % fat	35.3 (8,3)	31.2 (7.8)	<0.001	2.98 (1.71, 4.25)	<0.001
Fat distribution (android/gynoid ratio)	0.73 (0.2)	0.66 (0.18)	<0.001	0.08 (0.05, 0.12)	<0.001
Total bone mass density (mg/cm^2)	0.9 (0.1)	1.0 (0.1)	<0.001	0.02 (0.001, 0.03)	0.04
Puberty status	N=192-238[Y]	N=176-256 [Y]			
Girls breast stage (n yes ≥B2, %)	141 (59.2%)	169 (66.0%)	0.18	1.99 (1.18, 3.34)	0.01
Girls pubic hair (n yes ≥ PH2, %)	99 (44.8%)	133 (56.1%)	0.06	1.51 (0.90, 2.55)	0.12
Boys testis size (n yes ≥ 4 ml, %)	143 (74.5%)	156 (85.7%)	0.02	0.77 (0.42, 1.41)	0.40
Boys pubic hair (n yes≥ PH2, %)	50 (24.3%)	60 (29.6%)	0.02	1.74 (0.92, 3.28)	0.09
Boys genital stage (n yes≥ G2, %)	63 (32.6%)	66 (37.5%)	0.07	1.24 (0.72, 2.14)	0.45

For the crude estimate data are presented as mean (SD) or median***** (IQR) for normally and non-normally distributed variables, respectively.

When data area adjusted for age and sex they are presented as either mean difference, when the residuals are normal distributed, and as % difference when data are log transformed.

[a]P-values calculated using Student's T-test, Kruskal Wallis test*, or Chi-square tests.

[Y] Values are n (%) and adjusted estimates are odds ratios (95% CI) and are only adjusted for age.

P[b]: age and sex adjusted measurements comparing GDM to control offspring

Android tissue % fat: Located in the abdominal area

Gynoid tissue fat %: Located around the hips

Table 2. Differences in offspring anthropometric and metabolic characteristics comparing GDM offspring to controls after adjustment for offspring age, sex and BMI and maternal pre-pregnancy BMI

Offspring outcomes	**Mean difference or % difference* (95% CI)**	P[c]	**Mean difference or % difference* (95% CI)**	P[d]
Anthropometric characteristics				
BMI (kg/m^2)[#]	-	-	4% (2-6%)	<.0001
Waist circumference (cm)	0.83 (0.30;1.35)	0.002	0.52 (-0.06;1.08)	0.08
Hip circumference (cm)	0.01 (-0.48;0.49)	0.97	-	-
Waist hip ratio (WHR)	0.009 (0.003;0.02)	0.002	0.009 (0.002;0.02)	0.01
Head circumference (cm)	0.17 (-0.05;0.40)	0.13	-	-
Systolic blood pressure (mmHg)	0.30 (-0.70;1.30)	0.55	-	-
Diastolic blood pressure (mmHg)	-0.63 (-1.26; 0.11)	0.09	-	-
Heart rate (BPM)	0.82 (-0.37;2.02)	0.17	-	-
Metabolic characteristics				
Whole blood fasting glucose (mmol/l)*	2% (1-4%)	0.02	2% (1-4%)	0.02
Fasting plasma glucose (mmol/l)*	4% (1-5%)	<.0001	4% (2-5%)	<.0001
Fasting Insulin (pmol/l)*	7% (1-13%)	0.04	4% (-2-11%)	0.18
C-peptide (pmol/L)	8.7 (-13;30)	0.43	-	-
HOMA-IR*	11% (4-18%)	0.002	8% (1-16%)	0.02
Triglycerides (mmol/l)*	0% (-5-5%)	0.92	-	-
HDL cholesterol (mmol/l)	-0.02 (-0.07;0.02)	0.30	-	-
LDL cholesterol (mmol/l)	0.003 (-0.07;0.08)	0.93	-	-
Total cholesterol (mmol/l)	-0.002 (-0.09;0.09)	0.96	-	-
Body composition measured by DXA				
Total fat %	0.72 (-0.17;1.61)	0.11	-	-
Total lean mass %	-0.70 (-1.54;0.14)	0.10	-	-
Total android tissue % fat*	2% (-4-8%)	0.50	-	-
Total gynoid tissue % fat	0.56 (-0.48;1.59)	0.29	-	-
Fat distribution (android/gynoid ratio)	0.005 (-0.02;0.03)	0.66	-	-
Total bone mass density (mg/cm^2)	-0.007 (-0.02;0.007)	0.30	-	-

*non-normally distributed variables

P[c] adjusted for age sex and offspring BMIP[d] adjusted for age sex and offspring BMI and maternal pre-pregnancy BMI [#] adjusted for age sex and maternal pre-pregnancy BMI Only variables that were significant after adjustment for offspring BMI were further adjusted for maternal pre-pregnancy BMI

Table 3. Age and sex-adjusted mean differences or % differences* (95 % CI) for offspring characteristics across groups of maternal pre-pregnancy BMI comparing GDM offspring to controls.

	N	Maternal pre-pregnancy BMI <18.5 Mean difference or % difference * (95% CI)	p	N	Maternal pre-pregnancy BMI 18.5-<25 Mean difference or % difference * (95% CI)	p	N	Maternal pre-pregnancy BMI 25-<30 Mean difference or % difference * (95% CI)	p	N	Maternal pre-pregnancy BMI ≥30 Mean difference or % difference * (95% CI)	p
Anthropometric characteristics												
Weight (kg)	44	-2.04 (-7.64, 3.55)	0.46	646	3.24 (1.79, 4.70)	<0.001	233	1.34 (-1.63, 4.31)	0.37	180	1.43 (-2.86, 5.73)	0.51
Height (cm)	44	-2.63 (-9.01, 3.75)	0.41	646	1.56 (0.36, 2.76)	0.01	234	-0.63 (-2.78, 1.52)	0.57	180	-0.08 (-2.97, 2.80)	0.95
BMI (kg/m^2)*	44	-2% (-12-9%)	0.72	646	5% (3-7%)	<0.001	234	4% (-1-9%)	0.12	180	3% (-3-10%)	0.34
Waist (cm)	44	1.34 (-4.32, 7.00)	0.63	645	2.66 (1.51, 3.82)	<0.001	232	3.00 (0.22, 5.78)	0.03	180	2.25 (-1.86, 6.37)	0.28
Hip circumference (cm)	44	-1.74 (-5.90, 2.41)	0.40	646	1.80 (0.66, 2.94)	0.002	233	1.18 (-1.07, 3.43)	0.30	179	1.68 (-1.61, 4.97)	0.32
Head circumference (cm)	44	-1.14 (-2.43, 0.15)	0.08	644	0.42 (0.08, 0.76)	0.01	230	0.53 (0.03, 1.03)	0.04	179	-0.22 (-0.98, 0.55)	0.58
Systolic blood pressure (mmHg)	44	-0.29 (-7.62, 7.04)	0.94	645	1.56 (0.16, 2.97)	0.03	231	-0.54 (-2.74, 1.65)	0.63	178	-0.75 (-3.80, 2.30)	0.63
Diastolic blood pressure (mmHg)	44	0.68 (-4.00, 5.36)	0.77	644	-0.34 (-1.37, 0.70)	0.53	231	0.06 (-1.50, 1.62)	0.94	178	-1.22 (-3.54, 1.09)	0.30
Heart rate (BPM)	44	8.87 (-1.48, 19.22)	0.09	640	0.33 (-1.27, 1.93)	0.69	227	1.94 (-0.74, 4.62)	0.16	174	-1.26 (-4.87, 2.34)	0.49
Metabolic characteristics												
Whole blood fasting glucose (mmol/l)*	44	2% (-2-16%)	0.79	609	2% (1-5%)	0.07	222	4% (1-8%)	0.02	165	1% (-3-6%)	0.57
Plasma glucose (mmol/l)*	41	-3% (-12-6%)	0.46	574	4% (2-6%)	0.0001	210	4% (1-7%)	0.01	156	1% (-3-5%)	0.64
C-peptide (pmol/l)	40	155 (-4, 313)	0.06	572	26 (-5, 57)	0.10	213	-5 (-62, 52)	0.87	154	43 (-33, 119)	0.26
Insulin (pmol/l)*	39	10% (-14-59%)	0.60	559	11% (2-21%)	0.02	204	-1% (-13-13%)	0.99	150	19% (-1, 43%)	0.06
Triglycerides (mmol/l)*	40	5% (-17-51%)	0.79	573	5% (-1-11%)	0.12	214	-6% (-17-6%)	0.29	154	8% (-8-27%)	0.36
HOMA-IR*	39	9% (-27-62%)	0.68	551	15% (5-26%)	0.002	201	3% (-11-19%)	0.68	148	22% (-1-50%)	0.06
HDL cholesterol (mmol/l)	40	-0.10 (-0.41, 0.20)	0.50	573	-0.03 (-0.10, 0.03)	0.30	214	-0.03 (-0.14, 0.08)	0.57	154	-0.01 (-0.15, 0.12)	0.84
LDL cholesterol (mmol/l)	40	0.02 (-0.51, 0.55)	0.94	573	0.02 (-0.09, 0.12)	0.76	214	0.13 (-0.07, 0.33)	0.19	154	0.03 (-0.23, 0.28)	0.84
Total cholesterol (mmol/l)	40	-0.01 (-0.64, 0.61)	0.97	573	0.001 (-0.12, 0.12)	0.98	214	0.15 (-0.07, 0.36)	0.18	154	-0.01 (-0.29, 0.26)	0.93
Body composition												
Total fat %	36	1.11 (-7.29, 9.50)	0.79	393	2.03 (0.42, 3.64)	0.01	113	1.34 (-1.63, 4.30)	0.37	69	0.43 (-3.58, 4.43)	0.83
Lean mass %	36	-0.98 (-8.73, 6.77)	0.80	393	-1.91 (-3.40, -0.41)	0.01	113	-1.26 (-4.01, 1.50)	0.37	69	-0.47 (-4.19, 3.25)	0.80
Total android tissue % fat*	36	6% (-43-97%)	0.85	393	12% (1-26%)	0.06	113	3% (-14-24%)	0.74	69	1% (-19-26%)	0.94
Total gynoid tissue % fat	36	0.69 (-8.02, 9.41)	0.87	393	1.69 (-0.07, 3.45)	0.06	113	0.72 (-2.19, 3.63)	0.63	69	0.20 (-3.50, 3.90)	0.92
Fat distribution (android/gynoid ratio)	36	0.06 (-0.17, 0.28)	0.60	393	0.05 (-0.004, 0.09)	0.05	113	0.02 (-0.06, 0.10)	0.57	69	0.004 (-0.09, 0.10)	0.94
Total bone mass density (mg/cm^2)	36	-0.04 (-0.14, 0.06)	0.44	393	0.01 (-0.01, 0.03)	0.45	113	0.001 (-0.03, 0.04)	0.96	69	0.01 (-0.04, 0.05)	0.79