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Context: Low birth weight is associated with type 2 diabetes, which to some extent may be mediated via abdominal adiposity and insulin resistance. Fetal growth velocity is high during the third trimester, constituting a potential critical window for organ programming. Intra-pair differences among monozygotic twins are instrumental in determining nongenetic associations between early environment and adult metabolic phenotype.

Objective: Our objective was to investigate the relationship between size at birth and third-trimester growth velocity on adult body composition and glucose metabolism using intra-pair differences in young healthy twins.

Methods: Fifty-eight healthy twins (42 monozygotic/16 dizygotic) aged 18–24 yr participated. Insulin sensitivity was assessed using hyperinsulinemic-euglycemic clamps. Whole-body fat was assessed by dual-energy x-ray absorptiometry scan, whereas abdominal visceral and sc fat (L1–L4) were assessed by magnetic resonance imaging. Third-trimester growth velocity was determined by repeated ultrasound examinations.

Results: Size at birth was nongenetically inversely associated with adult visceral and sc fat accumulation but unrelated to adult insulin action. In contrast, fetal growth velocity during third trimester was not associated with adult visceral or sc fat accumulation. Interestingly, third-trimester growth was associated with insulin action in a paradoxical inverse manner.

Conclusions: Abdominal adiposity including accumulation of both sc and visceral fat may constitute primary nongenetic factors associated with low birth weight and reduced fetal growth before the third trimester. Reduced fetal growth during *vs.* before the third trimester may define distinct adult trajectories of metabolic and anthropometric characteristics influencing risk of developing type 2 diabetes. (*J Clin Endocrinol Metab* 96: 2835–2843, 2011)

There is a well-established association between low birth weight (LBW) and risk of developing insulin resistance and type 2 diabetes (T2D) (1) as well as cardiovascular disease (2, 3). LBW represents the composite end-point of growth velocities and trajectories during gestation including first, second, and third trimesters. Fetal growth velocity (FGV) is at its maximum in the third trimester, constituting a potentially important critical win-

dow for organ programming. Indeed, third-trimester exposure to famine during the Dutch hunger winter of 1944–1945 was linked to decreased glucose tolerance in adult life (4).

There is compelling evidence for detrimental effects of abdominal obesity on all components in the metabolic syndrome (5, 6). LBW was associated with central obesity in some (7) but not all (8) studies. The inconsistency in the literature may arise from many different factors including age-dependent impact of birth weight on adult body composition, genetic admixture, and the nature and timing of fetal insults as well as the use of indirect measures of abdominal obesity in most studies.

Visceral and sc fat has not previously been assessed directly using the multi-slice magnetic resonance (MR) scanning technology in human study cohorts addressing the developmental origin of obesity and T2D. Furthermore, only few studies in the area included detailed ultrasound measurements of growth velocities throughout third trimester (9).

At least two of the currently more than 40 identified T2D gene variants are associated with reduced birth weight, providing proof of principle for a genetic link between LBW and T2D (10–12). Nevertheless, the vast majority of T2D genes are not associated with weight at birth, and studies of genetically identically monozygotic twins have revealed a significant nongenetic impact of size at birth on T2D and associated metabolic changes in adult life (13, 14). Indeed, intra-pair correlations among genetically identical twins has been documented to represent a very sensitive and powerful tool to detect nongenetic associations between markers of the fetal environment including birth weight on one side and metabolic and anthropometric outcome variables relevant to T2D pathophysiology on the other.

We aimed to investigate the nongenetic relationship between size at birth and third-trimester FGV, respectively, and state of the art measures of adult body composition and glucose metabolism in a unique cohort of young adult twins with extensive ultrasound determinations of FGV during the third trimester.

Subjects and Methods

Subjects

Participants were offspring of a cohort of women with twin pregnancies and admitted to Herlev Hospital, Denmark, in the period 1983–1996 (n = 457). Gestational age was determined by ultrasound at wk 18 of gestation. Additional ultrasound examinations were performed with 2-wk intervals from wk 28 of gestation until delivery. Fetal weight was calculated from standard biometric ultrasound examinations (15). Coefficients of variation of ultrasound determinations have in singleton preg-

nancies been reported to be in the range of 6.5% and the difference between estimated fetal weight and actual birth weight to be $-2.2 \pm 8.7\%$ (16). Weight estimates were transformed to SD scores (SDS) using an age- and sex-specific reference of singleton fetal growth (16). FGV in the third trimester was determined by linear regression using a minimum of three examinations (range, 3–8) and was expressed as ΔSDS per 28 d. Birth weight SDS was calculated using a large Swedish singleton growth reference (17).

A random sample of 58 young healthy twins (30 male and 28 female) participated, of which a subgroup of eight participants did not participate in the clamp protocol. None received medication known to interfere with glucose homeostasis. Zygosity was determined by comparison of 10 microsatellite DNA polymorphisms showing very high heterozygosity in the population (18). The protocol was approved by the regional ethics committee, and procedures were performed according to the principles of the Helsinki Declaration. After thorough written and oral explanation of the study, all participants gave their written consent.

Study design

Hyperinsulinemic-euglycemic clamp combined with stable isotope infusion, iv glucose tolerance test (IVGTT), and indirect calorimetry

The study procedure was performed as previously described (19). Female participants who did not use oral contraceptives were examined on d 2–5 of their menstrual period. Participants were instructed to consume a diet rich in carbohydrate for 2 d and to abstain from strenuous exercise activity for 24 h before the examination. After an overnight fast, the participants underwent standard blood testing, anthropometric measurements, blood pressure, and a dual-energy x-ray absorptiometry (DXA) scan. At 0730 h, a polyethylene catheter was placed in each antecubital vein for blood sampling and test infusions. One hand was kept in a heated plexiglas box to ensure arterialization of the venous blood. Immediately after taking the background samples, a primed constant infusion of $[6,6\text{-}^2\text{H}5]$glucose (priming bolus of 20 μmol/kg; continuous infusion rate of 0.220 μmol/min $\cdot$ kg) was started (time 0 min) and maintained for 150 min to determine glucose kinetics in the basal state. Blood samples for measuring plasma glucose enrichments were drawn at baseline (time 0 min) and in the basal steady-state period (time 120–150 min) when the tracer equilibria of $[^2\text{H}5]$glucose was expected. Isotopes were purchased from Cambridge Isotope Laboratories (Andover, MA). A 30-min IVGTT (time 150–180 min) was performed for the assessment of β-cell function. A glucose bolus of 0.3 g/kg was infused over 1 min, and blood samples for glucose, insulin, and C-peptide were collected at 0, 2, 4, 6, 8, 10, 15, 20, and 30 min. After the IVGTT, a primed-continuous insulin infusion was initiated and fixed at 80 mU/m$^2 \cdot$ min through the 180-min clamp (time 180–360 min). The insulin-stimulated steady-state period was defined as the last 30 min of the insulin clamp period. A variable infusion of unlabeled glucose (180 g/liter) was used to maintain euglycemia at 5 mM. Plasma glucose concentration was monitored every 5 min during clamp using a OneTouch (LifeScan, Milpitas, CA) blood glucose meter. Oxygen consumption (V_{O2}) and carbon dioxide production (V_{CO2}) were measured during steady-state using indirect calorimetry with a flow-through canopy gas analyzer system (Deltatrac; Datex, Helsinki, Finland) as previously described (20).

Biochemical and tracer analyses

Blood samples were centrifuged immediately at 4 C, and plasma samples were stored at −80 C. Plasma insulin and C-peptide concentrations were determined by AutoDELPHIA time-resolved fluoroimmunoassay (PerkinElmer Wallac Oy, Turku, Finland). Stable isotope enrichments were measured as previously described (21).

Magnetic resonance

T1 weighted MR images were acquired with a 3-T Philips Achieva whole-body scanner. For each patient, between 15 and 26 slices were used to cover the abdominal region bounded by L1 and L4 (pixel spacing 0.8984×0.8984 mm^2, slice thickness 7 mm, gap 1 mm). The images were automatically processed to quantify the abdominal adiposity using image analysis techniques (22, 23). The applied method distinguished between superficial sc, deep sc, and visceral fat, reporting the quantities as percentages of total abdominal volume.

Calculations

IVGTT

The area under the curve was calculated using a trapezoidal method for insulin during the first-phase insulin response (FPIR), 0–10 min of the IVGTT.

Hyperinsulinemic-euglycemic clamp

For stable isotope tracer calculations, the total rate of glucose appearance was calculated as $R_{a \text{ (endogenous)}} = R_d = F_{total}/E_{glucose}$, where R_a and R_d are the respective rates of appearance and disappearance (micromoles per kilogram fat-free mass per minute), and F_{total} is the total infusion rate of glucose tracer (micromoles per kilogram fat-free mass per minute). $E_{glucose}$ is the enrichment of glucose in plasma expressed as tracer-to-tracee ratio. The R_a of glucose is a measure of endogenous glucose production and represents hepatic glucose production in the basal state (24). To measure insulin action, M-value was calculated as the mean glucose infusion rate during the insulin-stimulated steady-state period (milligrams per kilogram fat-free mass per minute). During the predefined clamp steady-state periods, the coefficients of variation of plasma insulin and glucose levels were 0.11 and 0.06, respectively. Basal and insulin-stimulated glucose and lipid oxidation rates were calculated according to the methods of Frayn (25). The β-cell function was assessed by calculating the disposition index (D_i) expressing the inverse hyperbolic relationship between insulin secretion and insulin action and calculated as FPIR × M-value.

Statistics

The comparison of males and females (see Tables 2 and 3) were performed using mixed ANOVA (PROC MIXED procedure; SAS Institute, Cary, NC). monozygotic twins share their entire genome, whereas dizygotic twins on average share half of their segregating genes. Therefore, observations in neither monozygotic nor dizygotic twin pairs can be regarded as independent observations. Accordingly, we adjusted for the intra-twin pair relationship in the analyses by including a random-effect term for twin pair membership and fixed-effect terms for zygosity.

Percent-wise impact of birth weight, birth weight SDS and third-trimester FGV (per 1 SD) on adult measures of metabolism and body composition were studied in a similar PROC MIXED procedure adjusted for gender. The analyses on MR-derived measures of abdominal obesity were adjusted for current BMI (see Table 4).

To quantify the nongenetic impact of birth weight, birth weight SDS, and third-trimester FGV on adult body composition and glucose homeostasis, multivariate linear regression analyses were made using intra-pair differences (twin A − twin B) exclusively in the 42 monozygotic twins. This approach adjusts for common environmental factors (such as the maternal, placental, and common postnatal environmental effects) and most importantly genetic effects. Consequently, any observed association using this approach is of environmental origin. The multivariate linear regression analyses were made with intra-pair differences of adult anthropometry and body composition as response variable and intra-pair differences of birth weight, birth weight SDS, and third-trimester FGV as explanatory variable. All analyses were adjusted for sex.

The designation of a member in a twin pair is arbitrary; *i.e.* there is no consistency in which of the twins in a pair is assigned A and which is assigned B. The correlation coefficient may differ dependent on the assignment of the twins, so intra-pair differences were calculated using 2n, as previously recommended (26). All analyses were carried out in SAS (version 9.1; SAS Institute); $P < 0.05$ was considered significant.

Results

Clinical characteristics

A total of 42 monozygotic (21 pairs; 26 males and 16 females) and 16 dizygotic twins (eight pairs; four males and 12 females) were included. Subject characteristics stratified by gender and zygosity is provided in Table 1. The median gestational age was 263 d (range, 208–279 d).

Gender differences

Females had a higher total fat percentage and a 2-fold increase of the deep and superficial sc fat contents compared with males (Table 2). However, females and males had similar volume percentage of visceral fat (Table 2). Females had lower fasting plasma glucose but a higher level of free fatty acids compared with males (Table 3). During the hyperinsulinemic-euglycemic clamps, females had a higher rate of glucose appearance from the liver and a higher glucose oxidation rate compared with males (Table 3).

Impact of birth weight, birth weight SDS, and FGV on body composition (Table 4)

The quantitative impact of 1 SD increase in birth weight SDS on visceral, deep sc, and superficial sc adipose tissue was −12.5, −32.3, and −8.1%, respectively, when accounting for current BMI. In comparison, birth weight *per se* was inversely associated with visceral [−14.7% (95% confidence interval = −26.3 to −1.4)] but not deep or superficial sc adipose tissue. Third-trimester FGV was not associated with visceral or sc adipose tissue accumulation.

In supplementary analysis, BMI was replaced as explanatory variable by body fat percentage, whole-body fat

TABLE 1. Subject characteristics

	Males		Females	
	MZ	**DZ**	**MZ**	**DZ**
Pre- and perinatal growth and condition				
n	26	4	16	12
Gestational age (d)	257 (253–267)	268 (266–270)	265 (263–266)	248 (237–259)
Fetus				
FGV	−0.88 (−1.56 to −0.55)	−0.76 (−1.01 to −0.42)	−0.35 (−0.62–0.12)	−0.46 (−1.31–0.41)
Birth				
Birth weight (g)	2450 ± 442	3389 ± 331	2502 ± 317	2185 ± 390
Birth weight Z-score	−1.30 ± 1.24	0.24 ± 0.88	−1.65 ± 1.01	−0.97 ± 1.24
Birth length (cm)	48.0 (46.5–48.5)	51.0 (49.0–52.5)	47.8 (45.5–49.0)	46.0 (44.0–47.5)
Birth length Z-score	−0.50 ± 0.99	−0.50 ± 1.11	−0.61 ± 1.35	−0.35 ± 1.08
Ponderal index	22.8 ± 2.1	26.0 ± 2.2	23.5 ± 2.2	22.8 ± 2.1
Head circumference (cm)	32.8 ± 1.9	34.4 ± 0.95	32.9 ± 1.3	32.0 ± 1.3
APGAR 1	9 (8–10)	9 (7–10)	10 (10–10)	10 (9–10)
APGAR 5	10 (10–10)	10 (10–10)	10 (10–10)	10 (10–10)
Adulthood				
Age (yr)	21.7 ± 2.1	18.9 ± 0.2	21.6 ± 1.4	23.2 ± 1.7
Height (m)	1.79 ± 0.05	1.84 ± 0.03	1.66 ± 0.05	1.69 ± 0.03
Weight (kg)	71.5 ± 8.2	75.4 ± 2.8	54.9 ± 9.2	58.4 ± 8.6
BMI (kg/m²)	22.4 ± 2.3	22.4 ± 1.3	20.0 ± 2.8	20.6 ± 3.0
Waist circumference (cm)	79.1 ± 5.8	80.3 ± 2.1	68.4 ± 6.4	69.2 ± 7.9
Hip circumference (cm)	94.0 ± 6.1	96.5 ± 1.7	91.7 ± 6.8	95.4 ± 7.1
Waist/hip ratio	0.84 ± 0.05	0.83 ± 0.02	0.75 ± 0.04	0.72 ± 0.04
Total cholesterol	3.8 ± 0.6	3.2 ± 0.5	3.8 ± 0.6	4.3 ± 0.72
HDL	1.11 ± 0.25	1.32 ± 0.23	1.72 ± 0.42	1.58 ± 0.26
LDL	2.26 ± 0.57	1.48 ± 0.62	1.71 ± 0.38	2.28 ± 0.68
Triglycerides	0.88 (0.65–1.06)	0.71 (0.59–0.95)	0.67 (0.51–0.94)	0.82 (0.71–1.24)
Systolic blood pressure	123 ± 9	135 ± 5	118 ± 9	111 ± 7
Diastolic blood pressure	63 ± 8	73 ± 7	69 ± 6	64 ± 7

Data are provided as mean ± SD or when not normally distributed as median (interquartile range). DZ, Dizygotic; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MZ, monozygotic.

content (kilograms), or lean body mass (kilograms) (data not shown). However, substitution of BMI with DXA-derived measures of body composition made only very subtle changes to the results presented.

Impact of birth weight, birth weight SDS, and FGV on insulin action and insulin secretion

Birth weight and birth weight SDS were not related to insulin action or insulin secretion. In contrast, FGV was inversely associated with M-value but not related to other measures of glucose metabolism (Table 4).

Nongenetic impact of birth weight, birth weight SDS, and FGV on body composition

Intra-pair differences in birth weight, birth weight SDS, and FGV were correlated with intra-pair differences in adult body composition and glucose metabolism (Table 5). The quantitative nongenetic impact of 1 SD increase in birth weight within a twin pair was 2.0 cm in adult height and 1370 g in adult lean body mass (Table 5). Similar nongenetic relationships were seen for 1 SD increase in intra-twin pair birth weight SDS; however, birth weight SDS was additionally associated with decreased visceral, deep sc, and superficial sc fat accumulation of 1.9, 0.5, and 0.9 volume percent per 1 SD (Table 5). Third-trimester FGV was

associated with adult height but not with measures of abdominal obesity.

Discussion

This study identifies novel and apparently independent differential contributions from third-trimester FGV and birth weight SDS, respectively, on abdominal obesity and insulin action. Birth weight SDS was inversely associated with both sc and visceral obesity in a nongenetic manner but not related to insulin action and insulin secretion. In contrast, third-trimester FGV was inversely associated with insulin action but not significantly with visceral or sc fat accumulation.

Previous studies have reported somewhat conflicting positive, inverse, or no associations between birth weight and different measures of adult obesity. Some of the diversity in the literature may arise from age-dependent associations (8), ethnic differences, and the nature and timing of growth restriction (27) as well as methodological differences related to the definition of obesity. Epidemiological studies have reported a positive association be-

TABLE 2. Adult anthropometrics determined by DXA and MR scan

	Males	Females
DXA		
Total fat (%)	14.2 (11.5–16.6)	26.2 (21.7–28.8)[c]
Total fat (kg)	10.71 (8.38–13.58)	14.95 (11.38–17.24)[b]
Total lean body mass (kg)	62.1 (57.7–65.4)	41.9 (37.7–45.0)[c]
Trunk fat (kg)	3.94 (3.30–5.31)	5.21 (3.53–6.60)
Trunk fat (%)	11.5 (9.75–15.2)	19.4 (15.5–24.1)[c]
Leg fat (kg)	2.06 (1.51–2.56)	2.93 (2.64–3.80)[c]
Leg fat (%)	16.2 (12.7–18.4)	32.0 (27.7–35.2)[c]
Trunk to leg ratio (kg)	2.02 (0.74–2.20)	1.39 (1.25–1.81)[b]
Trunk to leg ratio (%)	0.76 (0.68–0.86)	0.60 (0.53–0.69)[b]
Trunk to total fat ratio (kg)	0.38 (0.36–0.40)	0.34 (0.31–0.39)[a]
Leg to total fat (kg)	0.38 (0.36–0.41)	0.47 (0.42–0.49)[c]
MR		
Superficial sc fat (vol%)	8.48 (7.06–10.79)	15.5 (12.0–20.1)[c]
Deep sc fat (vol%)	1.08 (0.70–2.10)	2.86 (1.77–3.43)[b]
Visceral fat (vol%)	5.62 (4.43–6.75)	4.95 (3.56–6.48)
Total sc (vol%)	10.3 (8.5–12.1)	18.7 (14.0–23.1)[c]
Abdominal fat (vol%)	15.9 (13.2–19.5)	22.6 (17.6–28.8)[b]

Data from the MR scan are provided in volume percent (vol%) (95% confidence interval).

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

TABLE 3. Hyperinsulinemic-euglycemic clamp

	Males	Females
Plasma glucose (mmol/liter)		
Basal	5.28 ± 0.21	4.82 ± 0.36[c]
Serum insulin (pmol/liter)		
Basal	34.9 ± 12.6	33.2 ± 13.7
Free fatty acids (μ mol/liter)		
Basal	269 (217–383)	420 (351–511)[a]
Insulin stimulated	6.0 (4.0–10.0)	9.0 (6.5–12.0)
Glucose oxidation (mg/min · kg FFM)		
Insulin stimulated	4.45 ± 0.56	5.21 ± 0.73[b]
Fat oxidation (mg/min · kg FFM)		
Insulin stimulated	0.10 ± 0.22	−0.02 ± 0.24
Non-Ox GM (mg/min · kg FFM)	7.8 (6.9–9.6)	8.3 (7.2–9.3)
R_a glucose (mg/min · kg FFM)	2.52 ± 0.54	3.23 ± 0.85[b]
M-value (mg/min · kg FFM)	12.2 (11.5–13.8)	13.0 (11.8–15.0)
$D_{i\ peripheral}$ (10^{-3} pmol/liter · min × mg/min · kg FFM)	19.5 (13.6–32.2)	24.4 (19.1–30.7)

Data are shown as mean ± SD or median (interquartile range) when data not normally distributed. FFM, Fat-free mass; R_a, rate of appearance. Non-Ox, Non-oxidative glucose metabolism; GM, glucose metabolism; D_i, disposition index.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

tween birth weight and BMI in young (28), middle-aged (29), and elderly individuals (30). However, a smaller study, using DXA scans, reported that the positive association of birth weight with adult BMI was explained by its association with lean body mass (31). A study in 32 elderly men from England reported higher fat mass and higher trunk-to-limb fat ratio after adjusting for total fat mass in men born with a low birth weight (32). Similarly, we have previously observed an elevated trunk-to-leg fat ratio among young healthy singletons born with low birth weight (7). The present study used state-of-art multi-slice MR imaging (MRI) and found birth weight SDS to be associated with both visceral and sc fat deposition but not with whole-body fat percentage, BMI, or waist circumference.

Visceral fat accumulation is considered to be detrimental for insulin action (33), which potentially can be explained by a greater release of free fatty acids from the visceral than from the sc adipose tissue (34). Others have suggested that the quantitatively higher abundance of sc fat may represent the most critical adipose tissue compart-

ment adversely influencing insulin action (35, 36). A recent study in obese Pima Indians assessed abdominal obesity by MRI and found both ip and deep sc fat to predict peripheral and hepatic insulin action (37). Likewise, both visceral and sc adipose tissue were associated with blood pressure, diabetes, and the metabolic syndrome in the Framingham Heart Study (38). Finally, a prospective study among Japanese Americans found intraabdominal fat to predict T2D independent of BMI and sc fat area (39). The significant inverse intra-twin pair associations between birth weight SDS on one side, and both visceral as well as abdominal sc fat as determined using MRI on the other, demonstrates that an adverse intrauterine environment predisposes to abdominal obesity by influencing both visceral and sc abdominal fat accumulation with no impact on total-body fat content in young adult life. Importantly, the significant associations observed among the genetically identical monozygotic twins provide strong evidence in favor of a nongenetic component contributing to the association between low birth weight and abdominal fat accumulation.

In contrast to birth weight SDS scores, third-trimester FGV was not associated with visceral or sc fat volume

TABLE 4. Percent-wise impact of size and birth and FGV per 1 sd on measures of metabolism and body composition

	Birth weight	Birth weight SDS	FGV
MR			
Superficial sc fat (vol%)[a]	−2.9 (−8.8–3.3)	−8.1 (−12.7 to −3.3)[c]	−3.6 (−8.9–2.0)
Deep sc fat (vol%)[a]	−7.52 (−25.3–14.4)	−32.3 (−43.2 to −21.7)[d]	7.0 (−14.8–34.4)
Visceral fat (vol%)[a]	−14.7 (−26.3 to −1.4)[b]	−12.5 (−23.4 to −0.0)[b]	3.3 (−10.6–19.4)
Total sc (vol%)[a]	−4.2 (−10.5–2.6)	−10.3 (−15.3 to −5.00)[d]	−3.2 (−9.5–3.4)
Abdominal fat (vol%)[a]	−7.3 (−13.8 to −0.2)[b]	−12.0 (−17.1 to −6.5)[d]	−2.5 (−9.3–4.8)
DXA			
Total fat (%)	−3.76 (−8.59–1.33)	0.07 (−4.55–4.90)	−1.69 (−6.39–3.24)
Total fat (kg)	−3.08 (−10.31–4.69)	2.77 (−4.06–10.1)	−1.17 (−7.79–5.93)
Total lean body mass (kg)	1.66 (−0.47–3.91)	1.82 (−0.10–3.78)	−0.98 (−2.68–0.76)
Trunk to leg ratio (kg)	−1.47 (−6.79–4.15)	−0.56 (−5.44–4.56)	3.65 (−1.55–9.13)
Trunk to total fat ratio (kg)	−1.20 (−4.11–1.79)	−0.00 (−2.67–2.73)	1.68 (−1.15–4.59)
Hyperinsulinemic-euglycemic clamp			
Fasting plasma glucose (mmol/liter)	0.54 (1.21–1.02)	−0.37 (1.97–1.25)	0.65 (−1.05–2.37)
Fasting serum insulin (pmol/liter)	7.38 (−3.80–19.9)	4.17 (−5.97–15.4)	1.15 (−8.97–12.4)
Free fatty acids (μ mol/liter)			
Fasting	1.71 (−9.35–14.1)	6.29 (−5.08–19.0)	11.54 (−1.84–26.7)
Insulin stimulated	2.23 (−19.6–18.9)	−8.36 (−23.9–10.4)	6.90 (−11.6–29.2)
Glucose oxidation (mg/min · kg FFM)			
Insulin stimulated	0.25 (−3.77–4.45)	−1.79 (−5.52–2.08)	−3.30 (−7.51–1.10)
Fat oxidation (mg/min · kg FFM)			
Insulin stimulated	0.34 (−32.8–49.9)	−4.86 (35.4–40.1)	4.79 (−26.8–50.0)
Non-Ox GM (mg/min · kg FFM)	2.38 (−5.18–10.54)	−4.30 (−10.1–1.88)	−6.66 (−13.2–0.46)
FPIR	−0.84 (−16.1; 24.3)	6.48 (−8.76; 24.3)	7.97 (−7.43; 25.9)
R_a glucose (mg/min · kg FFM)	0.02 (−6.71–7.14)	−3.59 (−9.48–2.68)	−3.84 (−9.69–2.39)
M-value (mg/min · kg FFM)	1.92 (−3.85–8.03)	−3.24 (−8.28–2.09)	−5.41 (−9.99 to −0.66)[b]
$D_{i\ peripheral}$ (10^{-3} pmol/liter · min × mg/min · kg FFM)	0.41 (−13.3–16.3)	3.24 (−9.79–18.2)	−0.24 (−13.0–14.4)

Mean change (95% confidence interval) in variables per 1 sd change in birth weight (473 g), birth weight z-score (1.23), or FGV (1.14 Δ z-score). All data were adjusted for sex. FFM, Fat-free mass; R_a, rate of appearance; vol%, volume percent.

[a] Data were additionally adjusted for current BMI.

[b] $P < 0.05$.

[c] $P < 0.01$.

[d] $P < 0.001$.

percent, indicating that central fat accumulation represents a consequence of fetal programming before the third trimester. In accordance, a study in 805 human embryos and fetuses suggested the critical window for adipose tissue development to be the period from the 14th to 23rd week of gestation (40). Additionally, maternal undernutrition during the first and second but not third trimester was related to offspring obesity at age 19 in the Dutch Hunger Winter Study (27). We have previously investigated the impact of FGV *vs.* birth weight SDS on body composition assessed by DXA in a cohort of singletons recruited from the same Danish pregnancy clinic (9). In our previous study, birth weight SDS among singletons was inversely associated with DXA fat mass as well as trunk fat percentage, the latter finding being consistent with the current data. In further support of the current twin data, we did not observe any significant association between FGV and body composition among singletons (9). Taken together, the current and previous data support a key role of the intrauterine environment during the first or second trimester in the origin of abdominal obesity. The

minor difference between this and our previous study with regard to the associations between birth weight and total fat mass may be explained by the use of intra-pair differences among twins *vs.* singletons as well as the use of DXA *vs.* MRI in the two studies to assess regional and total fat mass. In terms of methodologies, it may be emphasized that intra-pair correlations among in particular monozygotic twins as well as the use of MRI to assess regional fat mass may represent more distinct, sensitive, and powerful assessments of the association between fetal growth and abdominal obesity as compared with the use of singletons and DXA scans. Finally, comparison between twins and singletons may be complicated by the different intrauterine challenges and shorter gestation or the higher risk of insulin resistance and T2D in twins *per se* (41).

We observed no associations between birth weight SDS and insulin action in this cohort of young healthy twins. In contrast, FGV during the third trimester was associated with insulin action in a somewhat paradoxical inverse manner with a high third-trimester FGV being associated with reduced insulin action. In our previous study of

TABLE 5. Intra-twin pair regression analyses of size at birth and FGV on measures of metabolism and body composition in 42 monozygotic twins

	Δ Birth weight	Δ Birth weight SDS	Δ FGV
Weight (kg)	2.06 (0.47–3.65)[a]	1.60 (−0.05–3.24)	0.66 (−1.22–2.55)
Height (cm)	2.0 (1.3–2.6)[c]	1.91 (1.22–2.60)[c]	1.17 (0.21–2.14)[a]
BMI (kg/m^2)	0.24 (−0.29–0.78)	0.08 (−0.46–0.62)	−0.06 (−0.066–0.54)
Waist (cm)	1.73 (0.35–3.11)[a]	1.13 (−0.31–2.57)	0.03 (−1.65–1.70)
Hip (cm)	1.46 (−0.13–3.04)	1.18 (−0.43–2.79)	−0.31 (−2.15–1.53)
Waist/hip ratio	0.01 (−0.00–0.01)	0.00 (−0.01–0.01)	0.00 (−0.01–0.01)
Total fat (g)	104 (−628–836)	274 (−452–999)	103 (−706–911)
Total fat (%)	−0.57 (−1.34–0.21)	0.02 (−0.77–0.82)	−0.09 (−0.94–0.76)
Lean body mass (g)	1370 (624–2117)[c]	923 (117–1730)[a]	653 (−222–1527)
Trunk to leg ratio	0.00 (−0.08–0.08)	0.02 (−0.06–0.10)	0.04 (−0.06–0.13)
Trunk to total fat ratio	−0.00 (−0.01–0.01)	0.00 (−0.00–0.01)	0.00 (−01–0.01)
Superficial sc fat (vol%)	0.03 (−0.71–0.76)	−0.88 (−1.56 to −0.20)[a]	−0.44 (−1.24–0.35)
Deep sc fat (vol%)	−0.39 (−0.65 to −0.13)[b]	−0.46 (−0.70 to −0.21)[c]	−0.10 (−0.41–0.21)
Visceral fat (vol%)	−1.17 (−2.44–0.10)	−1.89 (−3.06 to −0.71)[b]	−0.65 (−2.08–0.78)
Total sc (vol%)	−0.36 (−1.25–0.53)	−1.34 (−2.13 to −0.55)[c]	−0.54 (−1.52–0.43)
Abdominal fat (vol%)	−1.53 (−3.39–0.33)	−3.22 (−4.84–1.60)[c]	−1.19 (−3.27–0.88)
FPIR (pmol/liter · min)	1.5 (−326–329)	50 (−137–237)	1.5 (−200–203)
R_a glucose (mg/min · kg FFM)	−0.12 (−0.29–0.04)	−0.06 (−0.23–0.11)	−0.12 (−0.29–0.05)
M-value (mg/min · kg FFM)	−0.06 (−1.07–0.94)	−0.28 (−0.96–0.10)	−0.67 (−1.36–0.01)
D$_i$ peripheral (10^{-3} pmol/liter · min × mg/min · kg FFM)	33 (−1835–1900)	188 (−1660–2036)	−1104 (−3080–873)

Intra-twin pair change (95% confidence interval) per 1 SD change in intra-twin pair birth weight (406 g), birth weight SDS (1.24), and FGV (1.33). Analyses were adjusted for sex. FFM, Fat-free mass; R_a, rate of appearance; vol%, volume percent.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

young healthy singletons using different methodologies, we found no association of FGV on insulin action (9). The inverse relationship between FGV and insulin action is in accordance with our previous report of an age-dependent nongenetic influence of birth weight on *in vivo* clamp insulin action with a negative association among young twins and a positive association among elderly twins (42). The notion of a time- or age-dependent effect of birth weight on glucose tolerance and insulin action is supported by studies of offspring of protein-malnourished rats (43, 44). At 3 months of age, the young offspring of protein-restricted mothers had an improved glucose tolerance; however, at 15 month of age, they were less glucose tolerant compared with controls (44).

Following the line of considering distinct trajectories of metabolic and anthropometric alterations associated with impaired fetal growth, we speculate that abdominal obesity proceeds, and may subsequently contribute to, the development of overt insulin resistance.

Caution is warranted in extrapolating whole-body insulin action determinations to represent accurate measurements of insulin action in the primary target tissues of insulin including muscle, liver, and fat. Thus, data are accumulating that insulin action may differ significantly within each of these tissues with hepatic insulin action being distinct from peripheral insulin action (19, 45, 46)

as well as adipose tissue insulin action being distinct from muscle insulin action as recently documented in bed-rest experiments from our group (47) . Indeed, we have previously shown that young LBW men exhibit significant defects of muscle insulin action despite normal whole-body insulin action (48, 49). Although the notion of organ-specific trajectories of insulin action is in line with the idea of impaired muscle insulin action preceding overt whole-body insulin resistance among young LBW men, more knowledge is required to dissect the distinct effects of third-trimester growth velocity, as opposed to growth before or after the third trimester, on both whole-body as well as organ-specific insulin action. All together, we are facing a hitherto unrecognized complexity of the associations as well as underlying mechanisms linking the intrauterine environment to the development of overt whole-body and organ-specific insulin resistance, which needs to be established to understand, and subsequently to prevent, the development of T2D in subjects experiencing impaired fetal growth.

The twin approach is unique in regard to determination of nongenetic contributions to phenotypic traits. We found solid evidence for a nongenetic inverse association between birth weight SDS and visceral and sc adipose tissue. Family studies have suggested that 42–56% of the variability in visceral and sc fat may be due to genetic

causes (50, 51). In addition, recent genome-wide association studies have identified common variants in the *FTO* gene to be associated with obesity and provided further evidence for a genetic influence on common obesity (52). Altogether, central obesity appears to be determined by both genetic and nongenetic factors.

In conclusion, abdominal adiposity including both sc and visceral fat may constitute primary nongenetic defects associated with reduced fetal growth before the third trimester, preceding overt defects of insulin secretion and action. Reduced FGV during *vs.* before the third trimester may define distinct adult trajectories of metabolic and anthropometric characteristics influencing risk of developing T2D.

Acknowledgments

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