Original Article

The Intrauterine Environment as Reflected by Birth Size and Twin and Zygosity Status Influences Insulin Action and Intracellular Glucose Metabolism in an Age- or Time-Dependent Manner

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According to the "fetal origins hypothesis," monozygotic (MZ) twins may be more prone to develop various metabolic abnormalities compared with dizygotic (DZ) twins, and twins all together may be more predisposed to metabolic defects compared with singletons. To determine the impact of twin and zygosity status as well as birth size on in vivo measures of glucose metabolism, we examined 123 young (aged 22–31 years) and 103 elderly (aged 57–66 years) MZ and DZ twins and age-matched singleton control subjects. All participants were born at term with available birth records. Peripheral and hepatic insulin action and intracellular glucose partitioning was determined by a euglycemic-hyperinsulinemic clamp using tritiated glucose combined with indirect calorimetry. In elderly subjects, zygosity status influenced nonoxidative glucose metabolism, while twin status per se was associated with elevated hepatic glucose production during both steady-state periods. Birth weight was associated with nonoxidative glucose metabolism in a nongenetic manner within twins and with a high glucose and low lipid oxidation in singletons. In younger subjects, twin status influenced glucose and lipid oxidation rates. We demonstrate a complex age- or time-dependent relationship between independent markers of fetal environment and glucose homeostasis in twins. The documented differential programming effects associated with either low birth weight and twin or zygosity status all represent known defects of glucose homeostasis in type 2 diabetes. *Diabetes* 55:1819–1825, 2006

Fetal growth restriction is more likely to occur in twins compared with singletons because of their shared uterine environment. Monozygotic (MZ) twins are often monochorionic and share the same placenta and nutritive source and may consequently have a different and potentially more adverse intrauterine environment compared with dizygotic (DZ) and dichorionic MZ twins having separate placentas (1). According to the "fetal origins hypothesis," MZ twins may therefore be more prone to develop various metabolic abnormalities compared with DZ twins, and twins all together may be more predisposed to metabolic impairments compared with singletons.

It has been questioned whether the factors regulating intrauterine growth in twin and singleton pregnancies are similar (2). Likewise, it is unknown whether the putative effect of intrauterine growth restriction on the development of adult diseases differs between twins and singletons (3). A Danish twin study (4) demonstrated similar longevity/death rates in a selected group of MZ and DZ twin pairs both surviving the age of 6 years compared with singletons. Other studies (5–7), however, have shown differences in prevalence of cancer among MZ and DZ twins versus singletons. We recently demonstrated reduced insulin sensitivity in elderly MZ compared with DZ twins (8,9). Conversely, young MZ twins were slightly more insulin sensitive than young DZ twins (9), indicating an age- or time-dependent effect of the fetal environment on glucose homeostasis in twins.

The association between birth weight and metabolic abnormalities including type 2 diabetes (10–11) could theoretically be explained by a common genotype leading to reduced fetal growth and reduced insulin secretion and/or action (12). Twin studies allow for control of the influence of genetic factors. MZ twins are genetically identical, and when performing correlations between within–twin pair differences in birth weight and other phenotypic variables, any associations found are of nongenetic origin. Furthermore, this type of analysis allows for control of common environmental and maternal factors with a putative influence on the outcome in both MZ and DZ twins, which ensure a higher sensitivity and power to detect associations between the intrauterine environment and adult phenotype.

Today, type 2 diabetes is recognized as an age-dependent, multiple-organ disease involving muscle (peripheral insulin resistance of oxidative and in particular nonoxidative metabolism), liver (increased hepatic glucose production), adipose tissue (increased lipolysis and abnormal release of several hormonal substances influencing glucose homeostasis including adiponectin), β-cell (abnormal insulin secretion), and possibly other organs and tissues such as the kidneys (increased glucose production), gut (abnormal release of gut incretin hormones, e.g., glucogon-like peptide-1), and brain (abnormal sensing of glucose, free fatty acids, and other nutrients) (13,14). In the present study, we investigated the impact of zygosity

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DZ, dizygotic; LBW, low birth weight; MZ, monozygotic; PI, ponderal index.

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TABLE 1
Birth and adult anthropometry among young MZ and DZ twins and singletons

	MZ	DZ	Singletons
n (male/female)	66 (36/30)	44 (24/20)	13 (8/5)
Age (years)	28.2 ± 1.8	27.5 ± 1.9	28.8 ± 4.9
Birth weight (g)	2,627.3 ± 532.4	2,554.0 ± 431.2	3,425 ± 626*
PI (kg/m^3)	22.8 ± 2.1	22.7 ± 2.5	24.5 ± 2.3†
Height (cm)	176.0 ± 9.4	175.8 ± 10.7	176.1 ± 9.1
Lean body mass (kg)	54.9 ± 1.8	54.7 ± 2.5	56.7 ± 3.2
Total fat mass (kg)	16.7 ± 1.0	15.6 ± 1.0	19.8 ± 2.8
Total fat percentage (%)	22.5 ± 1.2	21.4 ± 1.3	24.6 ± 2.8
Trunk fat percentage (%)	19.1 ± 1.1	17.5 ± 1.0	21.6 ± 3.1

Data are means ± SD. *$P < 0.0001$, †$P = 0.02$ MZ and DZ twins vs. singletons.

and twin status and birth weight on some of the most important defects of glucose metabolism in type 2 diabetes including in vivo peripheral and hepatic insulin action as well as intracellular glucose partitioning based upon gold standard methods in young and elderly twins and age-matched singleton control subjects. Based on the strong age dependency of type 2 diabetes, results from previous animal studies (15), and our own data in twins (9), we hypothesized that advanced age might unmask metabolic defects linking impaired growth in utero with an increased risk of developing type 2 diabetes.

RESEARCH DESIGN AND METHODS

Twin subjects were identified through the Danish Twin Register as previously described (9). A random sample of same-sex MZ and DZ twin pairs aged 57–66 and 22–31 years were included in the study. All subjects were healthy without known diabetes and born full term (≥37 weeks) with available birth records. The age-matched singleton control subjects were identified among spouses to the participating twins. Zygosity was determined by polymorphic genetic markers (16).

A total of 98 twin pairs were enrolled in the clinical examination. Upon a standard oral glucose tolerance test, 4.8% of the elderly MZ and 2.3% of the elderly DZ twins had previously unknown type 2 diabetes according to the current World Health Organization criteria. None of the younger twins had type 2 diabetes, and in the singletons only one young subject was diagnosed with type 2 diabetes. Importantly, there was no significant difference in glucose tolerance status between MZ and DZ twins and singletons within each age-group. All twin and control subjects were included in the study regardless of glucose tolerance status. Results according to zygosity differences and heritability estimates of insulin secretion and action (9,17), as well as heritability estimates of muscle gene expressions (18) and glycogen synthase activation (19) in subsets of this twin population, were previously published. The twin-singleton comparisons as well as analyses of the impact of zygosity status and birth weights on indirect calorimetry-derived glucose and fat turnover rates, including oxidative and nonoxidative glucose metabolism, have of course not been previously published. The study was approved by the regional ethical committees, and the study was conducted according to the principles of the Helsinki Declaration.

Clinical examination. The subjects were instructed to abstain from strenuous physical activity for 24 h and to perform a 10- to 12-h overnight fast before both examination days. Anthropometric measures (i.e., BMI and waist-to-hip ratio) were performed as previously described (9). Body composition was measured by a dual-energy X-ray absorptiometry scanner (Hologic). The subjects underwent a 2-h hyperinsulinemic-euglycemic clamp. A primed constant continuous infusion of [3-^{3}H]-tritiated glucose (bolus 22 μCi, 0.22 μCi/min) was initiated at 0 min and continued throughout the clinical investigation (basal period (120 min) and clamp period (120 min). During the clamp period, a continuous insulin infusion (40 mU/m^2 per min) was initiated and continued for 120 min. A variable infusion of glucose (180g/l) enriched with tritiated glucose (HOT-GINF) (100 μCi/500 ml) maintained euglycemia during insulin infusion. Steady state was defined as the last 30 min of the basal and clamp periods when tracer equilibrium (i.e., constant specific activity) was anticipated. Indirect calorimetry was performed during the basal and clamp steady-state periods in order to calculate rates of glucose oxidation and lipid oxidation. The methods are previously described in detail (17).

Analytical methods. Plasma glucose and plasma insulin concentrations were analyzed as previously described (9).

Basal and insulin-stimulated glucose turnover rates. Glucose disposal rates and hepatic glucose production rates were calculated at 10-min intervals during the steady-state periods using Steele's non–steady state equations (20). In these calculations, distribution volume of glucose was 200 ml/kg body wt and the pool fraction was 0.65. Hepatic glucose production was calculated by subtracting the rate of glucose infusion from the rate of appearance of glucose assessed by tritiated glucose measurements. Nonoxidative glucose metabolism was calculated as glucose disposal minus glucose oxidation as determined by indirect calorimetry. Glucose turnover rates are expressed as milligrams per kilogram lean body mass per min and presented as mean values of the 30-min steady-state periods.

Statistical methods. The variables were near-normal distributed, and for comparisons between MZ and DZ twins and singletons within each age-group an ANOVA was performed using PROC MIXED of the SAS/STAT system (Version 9.1; SAS Institute). MZ twins share their entire genome, while DZ twins on average share half of their segregating genes. Therefore, observations in neither MZ nor DZ twins pairs can be regarded as independent observations. Accordingly, we adjusted for the intra–twin pair relationship in the analyses. Data are presented as means ± SD. Phenotypic correlations and correlations of within-pair differences were performed using Pearsons correlation.

RESULTS

Clinical characteristics. In both age-groups, MZ and DZ twins and singletons had similar age and adult anthropometry (Tables 1 and 2). Measures of birth weight and ponderal indexes (PIs) were similar in MZ and DZ twins, whereas singletons had significantly higher birth weights and PIs compared with both MZ and DZ twins.

Metabolites during the euglycemic-hyperinsulinemic clamp. Plasma glucose, insulin, and C-peptide (data not shown) were similar in young and elderly subjects during the basal and insulin-stimulated steady-state periods (Fig. 1*A* and *B* and Fig. 2*A* and *B*).

Constant specific activity of glucose tracer was reached in young and elderly subjects during both steady-state periods. The level of specific activity was slightly, though significantly, different within the younger subjects during the basal period (singletons vs. twins, $P = 0.048$) and within the elderly subjects during the insulin-stimulated state (DZ vs. MZ and singletons, $P = 0.046$) (Fig. 3*A* and *B*).

Basal and insulin-stimulated glucose turnover rates. In the younger subjects, MZ and DZ twins had similar glucose and lipid oxidation rates, whereas twins all together had a higher basal ($P = 0.027$) and insulin-stimulated glucose oxidation ($P = 0.009$) compared with singletons (Table 3). Furthermore, twins had a nearly significantly lower lipid oxidation compared with singletons ($P = 0.053$). The remaining metabolic variables, including rates of glucose disposal, hepatic glucose production, and nonoxidative glucose production, were similar among younger MZ and DZ twins as well as singletons (Table 3).

In addition to the previously reported lower insulin-

TABLE 2
Birth and adult anthropometry among elderly MZ and DZ twins and singletons.

	MZ	DZ	Singletons
n (male/female)	42 (22/20)	44 (16/28)	17 (7/10)
Age (years)	61.6 ± 2.6	62.0 ± 2.0)	59.6 ± 6.3
Birth weight (g)	2,664 ± 377	2,703 ± 533	3,905 ± 130*
PI (kg/m^3)	24.0 ± 3.2	24.3 ± 3.1	27.9 ± 4.5†
Height (cm)	166.8 ± 8.5	168.6 ± 10.8	167.0 ± 9.7
Lean body mass (kg)	51.1 ± 2.7	49.4 ± 2.4	50.5 ± 2.1
Total fat mass (kg)	20.5 ± 1.7	20.6 ± 1.6	23.6 ± 2.1
Total fat percentage (%)	27.7 ± 2.1	28.1 ± 1.8	30.3 ± 2.1
Trunk fat percentage (%)	25.4 ± 2.3	24.7 ± 1.8	27.6 ± 2.2

Data are means ± SD. *$P < 0.0001$, †$P = 0.05$ MZ and DZ twins vs. singletons.

stimulated glucose disposal and nonoxidative glucose metabolism in elderly MZ twins compared with DZ twins (17), elderly MZ twins also had a significantly lower insulin-stimulated glucose disposal and nonoxidative glucose metabolism compared with singletons (Table 4). In contrast, basal glucose disposal and both basal and insulin-stimulated rates of hepatic glucose production were significantly higher in both MZ and DZ twins compared with singletons. Nonoxidative glucose metabolism during the basal state as well as glucose and lipid oxidation during both steady-state periods were similar among elderly twins and singletons.

Intratwin pair correlations. When correlating within-pair differences and thereby correcting for a genetic impact among elderly MZ twins, significant positive correlations were seen between birth weight and insulin-stimulated glucose disposal ($r = 0.47$, $P = 0.002$) and nonoxidative glucose metabolism ($r = 0.46$, $P = 0.002$), respectively, indicating a nongenetic association. No significant associations were observed between birth weight and metabolic variables in the younger twins. Apart from a significant negative correlation between the PI and basal hepatic glucose production ($r = -0.26$, $P = 0.04$) in young MZ twins, no significant within pair correlations were seen between the PI and metabolic rates.

Phenotypic correlations in singletons. Among the elderly singletons, there was a positive correlation between birth weight and basal glucose oxidation ($r = 0.53$, $P = 0.04$) and negative correlations between birth weight and lipid oxidation during the basal ($r = -0.72$, $P = 0.002$) and clamp ($r = -0.59$, $P = 0.02$) periods. Among the young control subjects, no significant correlations were seen

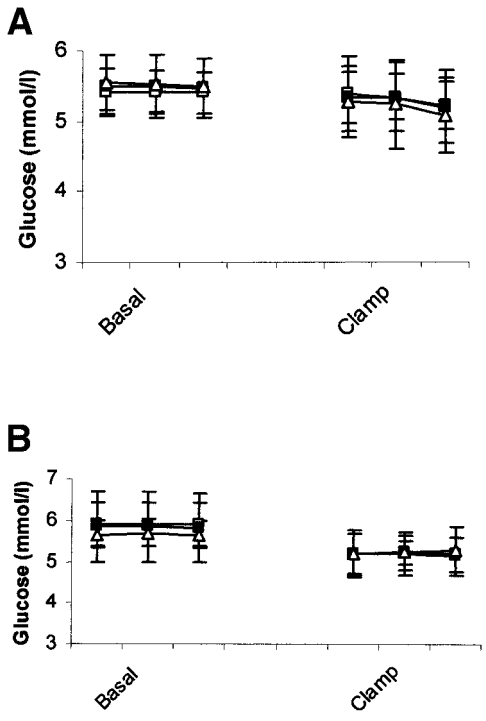

FIG. 1. Plasma glucose concentrations (mmol/l) during the basal (90–120 min after initiation of triated glucose infusion; see RESEARCH DESIGN AND METHODS) and clamp (90–120 min after initiation of insulin; see RESEARCH DESIGN AND METHODS) steady-state periods among young (*A*) and elderly (*B*) MZ and DZ twins and singletons. Data are means ± SD. △, control; ■, DZ; □, MZ.

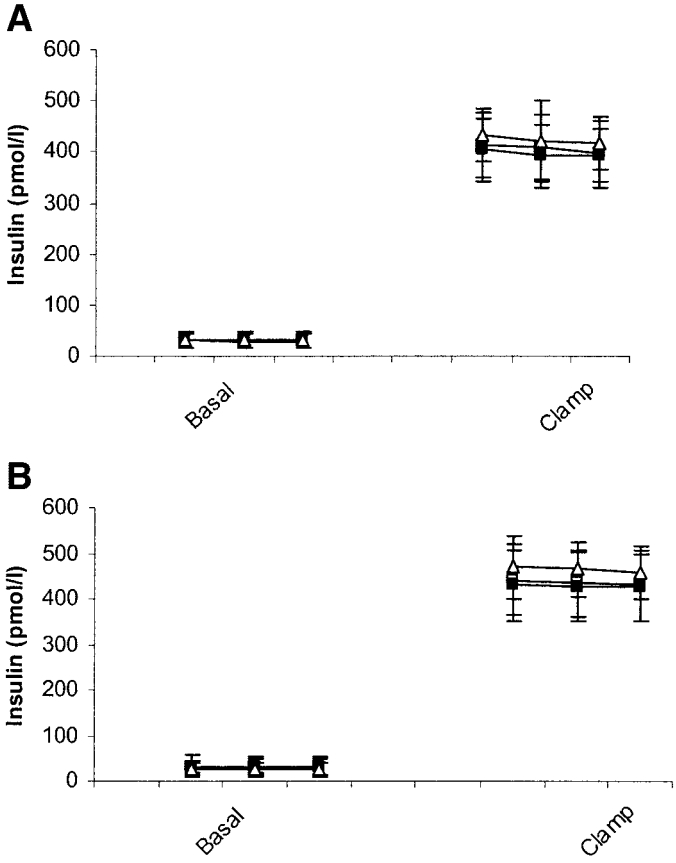

FIG. 2. Plasma insulin concentrations (pmol/l) during the basal (90–120 min after initiation of triated glucose infusion; see RESEARCH DESIGN AND METHODS) and clamp (90–120 min after initiation of insulin; see RESEARCH DESIGN AND METHODS) steady-state periods among young (*A*) and elderly (*B*) MZ and DZ twins and singletons. Data are means ± SD. △, control; ■, DZ; □, MZ.

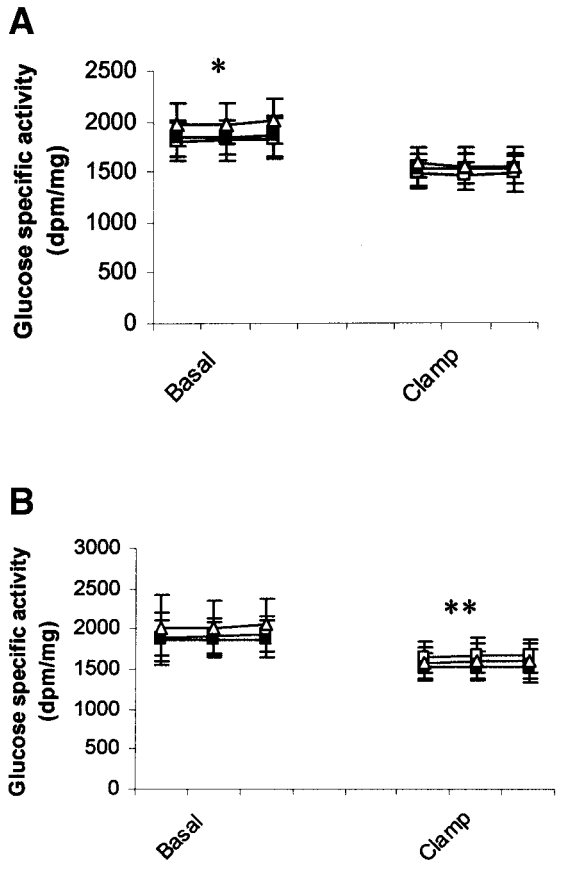

FIG. 3. Glucose-specific activity (dpm/mg) during the basal (90–120 min after initiation of triated glucose infusion; see RESEARCH DESIGN AND METHODS) and clamp (90–120 min after initiation of insulin; see RESEARCH DESIGN AND METHODS) steady–state periods among young (*A*) and elderly (*B*) MZ and DZ twins and singletons. Data are means ± SD. **P* = 0.048 singletons vs. twins; ***P* = 0.046 DZ vs. MZ and singletons. △, control; ■, DZ; □, MZ.

between birth weight and measures of glucose and lipid metabolism. In contrast, the PI was significantly negatively correlated to basal hepatic glucose production ($r = -0.69$, $P = 0.02$) during insulin stimulation in the young twins.

DISCUSSION

Fetal environment as reflected by birth weight, PI, and twin and zygosity status has profound influences on glucose and lipid metabolism. The effects seem to be age- or time-dependent and most interestingly seem to have different metabolic targets. *1*) Zygosity status has an impact on nonoxidative glucose metabolism, with elderly MZ twins having a significantly lower nonoxidative glucose metabolism compared with DZ twins (17). *2*) Twin status has an influence on glucose and lipid oxidation in the young group, with twins having higher glucose and lower lipid oxidation rates compared with singletons. In addition, twin status has an influence on basal and insulin-stimulated hepatic glucose production, with elderly MZ and DZ twins having a higher basal hepatic glucose production and a reduced insulin-stimulated suppression of hepatic glucose production compared with singletons. *3*) Birth weight has a nongenetic influence on nonoxidative glucose metabolism among elderly twins, and low birth weight is associated with low glucose oxidation and high lipid oxidation rates in elderly singletons. *4*) Finally, the PI is associated with basal hepatic glucose production in young twins in a nongenetic manner, and the PI is furthermore correlated with hepatic glucose production in the basal state among young singletons.

We have previously found an influence of zygosity on insulin-stimulated glucose uptake, with elderly MZ twins being more insulin resistant compared with DZ twins (9,17). The present study takes a step further in documenting significantly lower glucose storage exclusively in elderly MZ twins. Interestingly, elderly DZ twins and singletons have similar glucose storage rates. Thus, zygosity status, but not twin status per se, influences the pathway of nonoxidative glucose metabolism. Interestingly, the metabolic differences in elderly MZ compared with DZ twins (and singletons) were independent of birth weight and adult anthropometry. There were no significant phenotypic correlations between absolute birth weight determinations and metabolic parameters in twins. However, when performing correlations of within-pair differences in the twins allowing correction for common environmental and maternal factors, in particular among MZ twins for the influence of genotype, we detected a

TABLE 3
Basal and insulin-stimulated metabolic turnover rates among young MZ and DZ twins and singletons

	MZ	DZ	Singletons	ANOVA *P*	Twins versus singletons
Hepatic glucose production					
Basal	3.10 ± 0.56	3.04 ± 0.46	2.97 ± 0.52	0.72	0.47
Clamp	1.49 ± 0.49	1.49 ± 0.36	1.52 ± 0.46	0.98	0.86
Glucose disposal					
Basal	3.10 ± 0.53	3.01 ± 0.41	2.98 ± 0.49	0.62	0.56
Clamp	12.25 ± 3.38	10.95 ± 2.84	10.99 ± 2.85	0.19	0.42
Glucose oxidation					
Basal	2.17 ± 1.11	2.02 ± 0.98	1.54 ± 0.73	0.06	0.03
Clamp	4.72 ± 1.27	4.54 ± 1.46	3.73 ± 0.96	0.02	0.01
Nonoxidative glucose metabolism					
Basal	0.94 ± 0.97	0.99 ± 0.90	1.44 ± 0.77	0.15	0.44
Clamp	7.53 ± 2.98	6.41 ± 2.46	7.26 ± 2.42	0.21	0.82
Lipid oxidation					
Basal	1.26 ± 0.49	1.32 ± 0.48	1.42 ± 0.46	0.51	0.32
Clamp	0.32 ± 0.42	0.38 ± 0.49	0.61 ± 0.42	0.10	0.05

Data are means ± SD. Turnover rates are presented as mg · kg LBM^{-1} · min^{-1}.

TABLE 4
Basal and insulin-stimulated metabolic turnover rates among elderly MZ and DZ twins and singletons

	MZ	DZ	Singletons	ANOVA *P*	Twins versus singletons
Hepatic glucose production					
Basal	3.02 ± 0.39	3.10 ± 0.42	2.77 ± 0.44	0.05	0.03
Clamp	1.57 ± 0.52	1.70 ± 0.83	1.15 ± 0.58	0.02	0.01
Glucose disposal					
Basal	3.05 ± 0.37	3.14 ± 0.40	2.78 ± 0.42	0.03	0.003
Clamp	8.86 ± 3.06*	10.84 ± 3.34	10.53 ± 3.32	0.05	0.48
Glucose oxidation					
Basal	1.82 ± 1.12	1.75 ± 0.82	1.62 ± 0.87	0.77	0.48
Clamp	3.88 ± 1.32	4.26 ± 1.25	3.86 ± 0.98	0.36	0.46
Nonoxidative glucose metabolism					
Basal	1.23 ± 1.03	1.42 ± 0.81	1.16 ± 1.04	0.51	0.52
Clamp	4.99 ± 2.83†	6.67 ± 3.29	6.67 ± 3.23	0.26	0.36
Lipid oxidation					
Basal	1.36 ± 0.54	1.52 ± 0.47	1.36 ± 0.44	0.27	0.49
Clamp	0.56 ± 0.49	0.58 ± 0.51	0.60 ± 0.43	0.93	0.99

Data are means ± SD. Turnover rates are presented as mg · kg LBM^{-1} · min^{-1}. *P = 0.02; †P = 0.03 MZ vs. DZ twins.

nongenetic influence of birth weight per se on insulin-stimulated glucose disposal and nonoxidative glucose metabolism among elderly twins. This provides further evidence for an age-dependent influence of the fetal environment on nonoxidative glucose metabolism. Only few studies (21–23) have used gold standard methods in the investigation of the influence of the fetal environment on insulin sensitivity. Jaquet et al. (22) demonstrated reduced insulin-stimulated whole-body and nonoxidative glucose disposal among young men with low birth weight (LBW) compared with control subjects (22,24), whereas a study (23) from our group found normal whole-body and nonoxidative glucose disposal rates but reduced muscle glucose uptake in young men with LBW (25). Consistent with these findings, we demonstrated no influence of twin or zygosity status on whole-body glucose uptake or nonoxidative glucose metabolism among the younger subjects, indicating that increasing age (or time) may be an important factor unmasking the effect of fetal environment on glucose metabolism. The underlying molecular mechanisms are unknown, but recent studies have demonstrated reduced GLUT4 (24) and insulin signaling proteins and/or gene expression in skeletal muscle from rats (26) and humans (27) experiencing an adverse fetal environment. Furthermore, impaired mitochondrial function and oxidative phosphorylation in skeletal muscle has been shown in rats experiencing growth retardation in utero (28). In humans, the postprandial activity of glycogen synthase in skeletal muscle, which is the rate-limiting step in muscle glycogen synthesis, was not related to intrauterine growth as evidenced by birth weight (29).

We found a clear positive association between birth weight and glucose oxidation in elderly singletons, whereas glucose oxidation was similar in elderly singletons and twins. In the young age-group, we found a significantly higher insulin-stimulated glucose oxidation, together with a near-significantly lower lipid oxidation, in twins compared with singletons. These latter findings (i.e., a switch in the glucose–to–lipid oxidation ratio in subjects exposed to an adverse intrauterine environment) are to some extent in line with the idea of a defective mitochondrial function and lipid oxidation as potential underlying pathophysiological mechanisms in insulin resistance (30). The previous finding of a reduced glycolytic flux rate (23) and glucose oxidation (24) in young men with LBW may seem somewhat contradictory to our present results but could be explained by differences in twin versus singleton fetal programming, number of subjects, and in particular the age of the study subjects.

Elderly MZ and DZ twins had a significantly higher hepatic glucose production during the basal period compared with singletons. Additionally, the suppression of hepatic glucose production during insulin stimulation was reduced in twins compared with singletons. Despite a similar basal glucose turnover in young twins and singletons, we demonstrated a negative association between basal glucose turnover and PI in both young twins and singletons. Vuguin et al. (31) reported an elevated basal hepatic glucose production in normoglycemic intrauterine growth-retarded rats compared with control rats, indicating that uteroplacental insufficiency causes a primary defect in hepatic metabolism before the onset of overt hyperglycemia. Another study in animals demonstrated that an adverse intrauterine environment induced by dexamethasone resulted in overexpression of hepatic PEPCK and increased gluconeogenesis (32). Furthermore, maternal hypoxia (33) and protein deprivation (34) during pregnancy resulted in overexpression of PEPCK and downregulation of glucokinase, with the net effect of inappropriately elevated hepatic glucose production. In contrast to the present results, our study among young men with LBW demonstrated an enhanced suppression of endogenous glucose production during insulin stimulation compared with control subjects (23), which was proposed to represent a compensatory abnormality of metabolism in LBW subjects, supporting the idea of a role for age in unmasking some of the adverse effects of intrauterine programming on glucose homeostasis.

All metabolic differences in young and elderly twins, as well as in singletons, studied and reported in this article represent key metabolic defects in type 2 diabetes (i.e., hepatic glucose production and skeletal muscle insulin resistance). Many metabolic parameters have been tested, but, importantly, all parameters are derived from our specific a priori hypothesis of differences in metabolic pathways associated with insulin resistance and glucose intolerance in young and elderly twins and singletons. In particular, we hypothesized that advanced age might un-

mask some of the metabolic defects associated with impaired growth in utero. Consequently, we did not expect similar associations between intrauterine growth and adult glucose or fat metabolism in younger versus elderly twins.

It has been speculated whether twins are comparable with singletons regarding fetal growth (2,3) and in particular whether growth restriction has the same significance to adult glucose metabolism in twins and singletons. Twins have similar growth velocities compared with singletons until the 28th to 30th week of gestation (2), after which there is a divergence of twin growth to singleton growth that progressively widens toward the end of pregnancy. Importantly, the differences in birth weight and PI between twins and singletons in the present study (~800–900 g) are not due to prematurity because all examined subjects (as defined a priori in the protocol) were born within 3 weeks of term. Likewise, prematurity cannot account for the metabolic differences between MZ and DZ twins and singletons in this study (35).

The twins in the present study were not only smaller at birth according to birth weight, but they were also disproportionate in size (i.e., lower PI) compared with singletons. Interestingly, different expressions of the fetal environment were associated with different defects in distinct pathways of glucose and fat metabolism in young and elderly subjects. In the elderly subjects, birth weight was associated with nonoxidative glucose metabolism within twins and glucose and lipid oxidation within singletons. No associations were seen between the PI and measures of glucose and fat metabolism in elderly twins. In contrast, in the young subjects, the PI was associated with hepatic glucose production in both twins and singletons, whereas birth weight was not correlated to any metabolic pathways. Furthermore, the measures of metabolism associated with twin status (i.e., glucose oxidation together with basal glucose turnover and hepatic glucose production) were correlated to the PI indicating that the influence of twin status per se may be mediated by or related to the lower PI (i.e., disproportionate fetal growth) among twins compared with singletons. In contrast, many of the metabolic parameters associated with zygosity status (i.e., glucose disposal and storage) were also correlated to birth weight, suggesting that birth weight and zygosity status partly independent of each other (i.e., same birth weight in MZ and DZ twins) may influence adult metabolism through similar or parallel programming mechanisms.

Interestingly, despite similar birth weights, the elderly subjects were ~10 cm shorter than the younger subjects (Tables 1 and 2). Adult height has previously been demonstrated to be influenced by the fetal environment (36), and this difference is likely to be explained by the well-known improved prenatal and perhaps especially early postnatal nutritional and other life conditions in Western countries during the period from before 1940 and until now. Indeed, this period in the last century may be one of the periods in the history of mankind when the most significant and condensed qualitative and quantitative improvements of both early and adult life conditions have taken place for entire populations in the Western world. With that in mind, it could be speculated that the different metabolic abnormalities demonstrated in twins compared with singletons in the elderly and younger subjects, respectively, and perhaps those associated with low birth weight and PI in the elderly but not in younger singletons and vice versa, may have been unmasked or precipitated by the generally different life and nutritional conditions at the time when they were born. In other words, the presumed age effects could as well be due to a cohort effect mediated by the different prenatal and early postnatal circumstances in the two age-groups.

The classical twin model is based upon the key assumption that both prenatal and postnatal environmental covariance is the same for MZ and DZ twin pairs and that twins in general resemble singletons according to the phenotype in question. Accordingly, the documented metabolic differences between twins and singletons, as well as the differences between MZ and DZ twins, do indeed challenge these major assumptions, questioning the validity of the classical twin approach in studies of phenotypic traits related to diabetes and glucose metabolism. We have recently reported heritability estimates based upon biometric modeling (17–19). Heritability estimates were only calculated for data fulfilling the criteria for application of the model that is equal means and variances across twin pairs and/or zygosity. This approach does to some extent justify and validate the results but still leaves the question open of whether reports from twin studies can be generalized to the background population mainly comprising singletons.

In conclusion, our results point toward a clear, though complex, relationship between an adverse intrauterine environment evidenced by birth weight and twin and zygosity status on one side and glucose metabolism on the other side. The idea of a differential age-dependent fetal programming of distinct metabolic pathways in different organs may offer an explanation for the complexity of the overt diabetic state characterized by different degrees of abnormalities in the tissues involved in regulation of glucose metabolism. We have documented that programming of twins in utero involves an age-dependent differential programming of some of the major metabolic defects in type 2 diabetes, including hepatic glucose production, peripheral insulin resistance, and the intracellular partitioning of glucose fluxes. These findings may add significantly to our understanding of the mechanisms linking an adverse intrauterine environment to type 2 diabetes.

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REFERENCES

1. Langman J: Fetal membranes and placenta. In *Medical Embryology.* London, Williams & Wilkins, 1981, p. 83–102
2. Hall JG: Twinning. *Lancet* 362:735–743, 2003
3. Phillips DI: Twin studies in medical research: can they tell us whether diseases are genetically determined? *Lancet* 341:1008–1009, 1993
4. Christensen K, Vaupel JW, Holm NV, Yashin AI: Mortality among twins after age 6: fetal origins hypothesis versus twin method. *BMJ* 310:432–436, 1995
5. Swerdlow AJ, De Stavola BL, Swanwick MA, Maconochie NES: Risks of breast and testicular cancers in young adult twins in England and Wales: evidence on prenatal and genetic aetiology. *Lancet* 350:1723–1728, 1997
6. Braun MM, Ahlbom A, Floderus B, Brinton LA, Hoover RN: Effect of

twinship on incidence of cancer of the testis, breast and other sites (Sweden). *Cancer Causes Control* 6:519–524, 1995
7. Verkasalo PK, Kaprio J, Pukkala E, Koskenvuo M: Breast cancer risk in monozygotic and dizygotic female twins: a 20-year population-based cohort study in Finland from 1976 to 1995. *Cancer Epidemiol Biomarkers Prev* 8:271–274, 1999
8. Poulsen P, Vaag A, Beck-Nielsen H: Does zygosity influence the metabolic profile in twins? A population based cross sectional study. *BMJ* 319:151–154, 1999
9. Poulsen P, Levin K, Beck-Nielsen H, Vaag A: Age-dependent impact of zygosity and birth weight on insulin secretion and insulin action in twins. *Diabetologia* 45:1649–1657, 2002
10. Hales CN, Barker DJP, Clark PMS, Cox LJ, Osmond C, Winter PD: Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ* 303: 1019–1022, 1991
11. Vaag A, Poulsen P: Twins in diabetes research. *Diabetes Annual* 13:159–174, 2000
12. Poulsen P, Vaag AA, Kyvik KO, Jensen DM, Beck-Nielsen H: Low birth weight is associated with NIDDM in discordant monozygotic and dizygotic twin pairs. *Diabetologia* 40:439–446, 1997
13. Vaag A, Alford F, Henriksen FL, Christopher M, Beck-Nielsen H: Multiple defects of both hepatic and peripheral intracellular glucose processing contribute to the hyperglycaemia of NIDDM. *Diabetologia* 38:326–336, 1995
14. Vaag A: On the pathophysiology of late onset non-insulin dependent diabetes mellitus: current controversies and new insights. *Dan Med Bull* 46:197–234, 1999
15. Ozanne SE, Wang CL, Coleman N, Smith GD: Altered muscle insulin sensitivity in the male offspring of protein-malnourished rats. *Am J Physiol* 271:E1128–E1134, 1996
16. Morling N: [Forensic genetics]. *Ugeskr Laeger* 165:922–925, 2003 (in Danish)
17. Poulsen P, Levin K, Petersen I, Christensen K, Beck-Nielsen H, Vaag A: Heritability of insulin secretion, peripheral and hepatic insulin action, and intracellular glucose partitioning in young and old Danish twins. *Diabetes* 54:275–283, 2005
18. Ling C, Poulsen P, Carlsson E, Ridderstråle M, Beck-Nielsen H, Groop L, Vaag A: PGC-1 expression in human skeletal muscle is influenced by insulin and age. *J Clin Invest* 114:1518–1526, 2004
19. Poulsen P, Wojtaszewski J, Richter E, Beck-Nielsen H, Vaag A: Etiology of muscle glycogen synthase activity and regulation: a twin study. *Diabetes* 54:1289–1296, 2005
20. Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann N Y Acad Sci* 82:420–430, 1959
21. McKeigue PM, Lithell HO, Leon DA: Glucose tolerance and resistance to insulin-stimulated glucose uptake in men aged 70 years in relation to size at birth. *Diabetologia* 41:1133–1138, 1998
22. Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C: Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. *J Clin Endocrinol Metab* 85:1401–1406, 2000
23. Jensen CB, Storgaard H, Dela F, Holst JJ, Madsbad S, Vaag AA: Early differential defects of insulin secretion and action in 19-year-old caucasian men who had low birth weight. *Diabetes* 51:1271–1280, 2002
24. Jaquet D, Vidal H, Hankard R, Czernichow P, Levy-Marchal C: Impaired regulation of glucose transporter 4 gene expression in insulin resistance associated with in utero undernutrition. *J Clin Endocrinol Metab* 86:3266–3271, 2001
25. Hermann TS, Rask-Madsen C, Ihlemann N, Dominguez H, Jensen CB, Storgaard H, Vaag AA, Kober L, Torp-Pedersen C: Normal insulin-stimulated endothelial function and impaired insulin-stimulated muscle glucose uptake in young adults with low birth weight. *J Clin Endocrinol Metab* 88:1252–1257, 2003
26. Fernandez-Twinn DS, Wayman A, Ekizoglou S, Martin MS, Hales CN, Ozanne SE: Maternal protein restriction leads to hyperinsulinemia and reduced insulin-signaling protein expression in 21-mo-old female rat offspring. *Am J Physiol Regul Integr Comp Physiol* 288:R368–R373, 2005
27. Ozanne SE, Jensen CB, Tingey KB, Storgaard H, Madsbad S, Vaag A: Low birthweight is associated with specific changes in muscle insulin-signalling protein expression. *Diabetologia* 48:547–552, 2005 Mar.
28. Selak MA, Storey BT, Peterside I, Simmons RA: Impaired oxidative phosphorylation in skeletal muscle of intrauterine growth-retarded rats. *Am J Physiol Endocrinol Metab* 285:E130–E137, 2003
29. Phillips DI, Borthwick AC, Stein C, Taylor R: Fetal growth and insulin resistance in adult life: relationship between glycogen synthase activity in adult skeletal muscle and birthweight. *Diabet Med* 13:325–329, 1996
30. Kelley DE, He J, Menshikova EV, Ritov VB: Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 51:2944–2950, 2002
31. Vuguin P, Raab E, Liu B, Barzilai N, Simmons R: Hepatic insulin resistance precedes the development of diabetes in a model of intrauterine growth retardation. *Diabetes* 53:2617–2622, 2004
32. Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR: Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest* 101:2174–2181, 1998
33. Parimi PS, Croniger CM, Leahy P, Hanson RW, Kalhan SC: Effect of reduced maternal inspired oxygen on hepatic glucose metabolism in the rat fetus. *Pediatr Res* 53:325–332, 2003
34. Ozanne SE, Smith GD, Tikerpae J, Hales CN: Altered regulation of hepatic glucose output in the male offspring of protein-malnourished rat dams. *Am J Physiol* 270:E559–E564, 1996
35. Hofman PL, Regan F, Jackson WE, Jefferies C, Knight DB, Robinson EM, Cutfield WS: Premature birth and later insulin resistance. *N Engl J Med* 351:2179–2186, 2004
36. Jaquet D, Collin D, Levy-Marchal C, Czernichow P: Adult height distribution in subjects born small for gestational age. *Horm Res* 62:92–96, 2004