Low Birth Weight and Zygosity Status Is Associated With Defective Muscle Glycogen and Glycogen Synthase Regulation in Elderly Twins

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OBJECTIVE—An adverse intrauterine environment indicated by both low birth weight and monozygosity is associated with an age- or time-dependent reduction in glucose disposal and nonoxidative glucose metabolism in twins, suggesting impaired regulation of muscle glycogen synthesis.

RESEARCH DESIGN AND METHODS—We measured the activities of glycogen synthase (GS), GS kinase (GSK)3α, GS phosphorylation, and glycogen levels in muscle biopsies obtained from 184 young and elderly twins before and after a euglycemic-hyperinsulinemic clamp.

RESULTS—Elderly monozygotic twins had significantly lower fractional GS activity amidst higher glycogen and GS protein levels compared with dizygotic twins. In addition, we demonstrated strong nongenetic associations between birth weight and defect muscle glycogen metabolism in elderly—but not in younger—twins. Thus, for every 100 g increase in birth weight within pairs, GS fractional activity, GS protein level, and glycogen content was increased by 4.2, 8.7, and 4.5%, respectively, in elderly twins. Similarly, for every 100 g increase in birth weight, GSK3α activity and GS phosphorylation at the sites 2, 2+2a, and 3a+3b were decreased by 3.1, 9.0, 10.1, and 9.5%, respectively.

CONCLUSIONS—The age- or time-dependent nongenetic impact of birth weight on insulin action in twins may partly be explained by reduced insulin activation of muscle GS, mediated through increased GSK3α activity and GS phosphorylation. Reduced GS activity and negative feedback inhibition of glycogen metabolism by glycogen per se may contribute to the insulin resistance in elderly monozygotic compared with dizygotic twins. ***Diabetes*** **56:2710–2714, 2007**

Adverse fetal environment as indicated by low birth weight is associated with an increased risk of type 2 diabetes and insulin resistance in adult life (1,2). We have previously reported a nongenetic influence of birth weight on the development of type 2 diabetes (3) and the underlying pathophysiological mechanisms, including peripheral insulin resistance among elderly twins (4). In addition, monozygosity is associated with a reduced glucose disposal and nonoxidative glucose metabolism (NOGM) in elderly twins, independent of birth weight (5). Monozygotic twins are exposed to a different and possibly more adverse fetal environment compared with dizygotic twins due to the vascular, placental, and nutritive circumstances characterizing monozygotic pregnancies (6).

Glycogen storage accounts for the quantitatively largest proportion of muscle glucose metabolism during insulin infusion in the physiological range in normal subjects and similarly represents a major defect of muscle glucose metabolism in insulin-resistant states, including first-degree relatives of patients with type 2 diabetes (7–9). Insulin activates muscle glycogen synthase (GS) enzyme activity by reducing its phosphorylation state, particularly at the site 3a+3b (10,11). A key insulin-regulated enzyme responsible for the phosphorylation (and thereby inactivation) of GS is GS kinase (GSK)3α (12,13).

Using the classical twin approach, we recently demonstrated a predominant environmental influence on muscle GS activity (14). Defective GS activity or regulation may in theory represent a molecular mechanism linking adverse fetal environment and insulin resistance in skeletal muscle. The present report represents an expansion of our twin study, focusing explicitly on the impact of zygosity status and birth weight on skeletal muscle GS activation and regulation in young and elderly monozygotic and dizygotic twins. In particular, studying twins takes advantage of the genetic similarity within monozygotic twins and is therefore an instrumental tool in discriminating between associations of genetic and nongenetic origin.

RESEARCH DESIGN AND METHODS

Young (aged 22–31 years) and elderly (aged 57–66 years) same-sex monozygotic and dizygotic twin pairs were identified through the Danish Twin Register as described (4,5,14). Twins with available original midwife records, including birth weight, were enrolled in the clinical examination. All twins were born at term (±3 weeks) and without known diabetes, serious disease, or medication influencing glucose or lipid metabolism that could not be withdrawn. The influence of heredity, birth weight, twin, and zygosity status on insulin secretion and peripheral and hepatic insulin sensitivity has previously been reported (5,14,15). Measures of protein and enzyme activity in skeletal muscle were achieved for a total of 184 young (monozygotic $n = 63$; dizygotic $n = 38$) and elderly (monozygotic $n = 40$; dizygotic $n = 43$) twins. Zygosity was determined by polymorphic genetic markers (15). The study was approved by the regional ethical committee and conducted according to the principles of the Declaration of Helsinki.

Clinical examination. Subjects underwent a 2-h (40 mU/m^2 per min) hyperinsulinemic-euglycemic clamp including indirect calorimetry as previously described (5,14,15). Muscle biopsies were taken from the vastus lateralis muscle during the basal and insulin-stimulated steady-state periods, which were defined as the last 30 min of the basal and clamp periods, respectively.

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GS, glycogen synthase; GSK, GS kinase; NOGM, nonoxidative glucose metabolism.

TABLE 1
Clinical characteristics in young and elderly monozygotic and dizygotic twins

	Young			Elderly		
	Monozygotic	Dizygotic	*P*	Monozygotic	Dizygotic	*P*
n	63	38		40	43	
Age (years)	28.2 ± 0.29	27.6 ± 0.3	0.10	61.7 ± 0.4	62.1 ± 0.3	0.61
Birth weight (g)	2,632 ± 68	2,571 ± 76	0.57	2,658 ± 61	2,722 ± 80	0.73
BMI (kg/m^2)	24.3 ± 0.4	23.8 ± 0.5	0.43	26.4 ± 0.7	26.1 ± 0.9	0.83
WHR	0.85 ± 0.01	0.82 ± 0.01	0.17	0.90 ± 0.02	0.86 ± 0.02	0.20
Body fat percentage (%)	22.5 ± 0.9	21.8 ± 1.1	0.62	27.9 ± 2.0	28.4 ± 1.8	0.87
R_d (mg · kg^{-1} · min^{-1})	12.1 ± 0.5	11.1 ± 0.5	0.14	8.9 ± 0.6	10.8 ± 0.6	0.02
NOGM (mg · kg^{-1} · min^{-1})	7.4 ± 0.4	6.5 ± 0.4	0.10	4.9 ± 0.5	6.7 ± 0.5	0.03

Data are means ± SE. WHR, waist-to-hip ratio.

The biopsy specimens were quickly blotted on filter paper and frozen in liquid nitrogen. The biopsies were stored at −80°C until further processed.

In vitro experiments. Methods to determine muscle glycogen content, GS protein expression, total and fractional GS, and GSK3α activities have previously been described in detail (14).

Statistical methods. Comparisons between monozygotic and dizygotic twins were performed with an ANOVA using PROC MIXED of the SAS/STAT system. Monozygotic twins share their entire genome and dizygotic half of their genome, which is why it may be questioned whether twins are independent observations. Accordingly, we have adjusted for this intra–twin-pair relationship. The full ANOVA model included a random-effect term for twin-pair membership and fixed-effect terms for zygosity (5).

The linear regression analyses using monozygotic twin intrapair differences in birth weight and measures of GS activity and regulation were performed using SigmaStat 3.0.

RESULTS

Insulin action and relation to GS activity and regulation. Birth and adult anthropometry was similar in monozygotic and dizygotic twins in both age-groups. Insulin action was similar in young monozygotic and dizygotic twins, whereas elderly monozygotic twins had significantly lower glucose disposal (R_d) and NOGM compared with dizygotic twins, as previously reported (4) (Table 1).

R_d and NOGM were significantly associated with GS fractional activity (R_d: $r = 0.36$, $P < 0.00001$; NOGM: $r = 0.38$, $P < 0.00001$) and GS phosphorylation at site 2+2a (R_d: $r = -0.17$, $P = 0.02$; NOGM: $r = -0.15$, $P = 0.045$) (Fig. 1). Glycogen and GS protein content, total GS, and GSK3α activity—together with GS phosphorylation at sites 2 and 3a+3b—were not associated with in vivo measures of insulin action (data not shown).

Impact of zygosity on GS activity and regulation. All measures of GS activity and regulation were similar in younger monozygotic and dizygotic twins (Table 2). Elderly monozygotic twins had significantly higher glycogen content and GS protein expression during both steady-state periods but lower insulin-stimulated fractional GS activity compared with dizygotic twins (Table 2).

Nongenetic influence of birth weight. Regression analyses using intra–twin-pair differences were performed in monozygotic twins to determine the quantitative magnitude of the nongenetic influence of birth weight on muscle glycogen metabolism. In elderly twins, a 100 g increase in birth weight within a pair was associated with substantial increments in basal GS protein and glycogen contents (mean ± SE 8.7 ± 3.2 and 4.5 ± 1.0%, $P = 0.01$ and $P < 0.001$, respectively), as well as in basal and insulin-stimulated GS fractional activities (5.3 ± 2.0 and 4.2 ± 2.0%, $P = 0.01$ and $P = 0.046$, respectively). Similarly, for each 100 g increase in intrapair birth weight, marked reductions of insulin-stimulated GSK3α activity (3.1 ± 0.8%, $P < 0.001$) and the degree of GS phosphorylation at sites 2 (9.0 ± 2.9%, $P = 0.002$), 2+2a (10.1 ± 3.8%, $P = 0.007$), and 3a+3b (9.5 ± 4.3%, $P = 0.02$) in the basal state, as well as site 3a+3b during insulin infusion (5.5 ± 2.8%, $P = 0.046$), were seen (Fig. 2).

In the younger monozygotic twins, a 100 g increase in birth weight was associated with a 2.1 ± 1.0% ($P = 0.044$) decrease in basal GS protein content and a 9.9 ± 3.0% ($P = 0.0002$) increase in GS phosphorylation at site 3a+3b.

DISCUSSION

Performing analyses with intrapair differences allows adjustment for common environmental and maternal factors

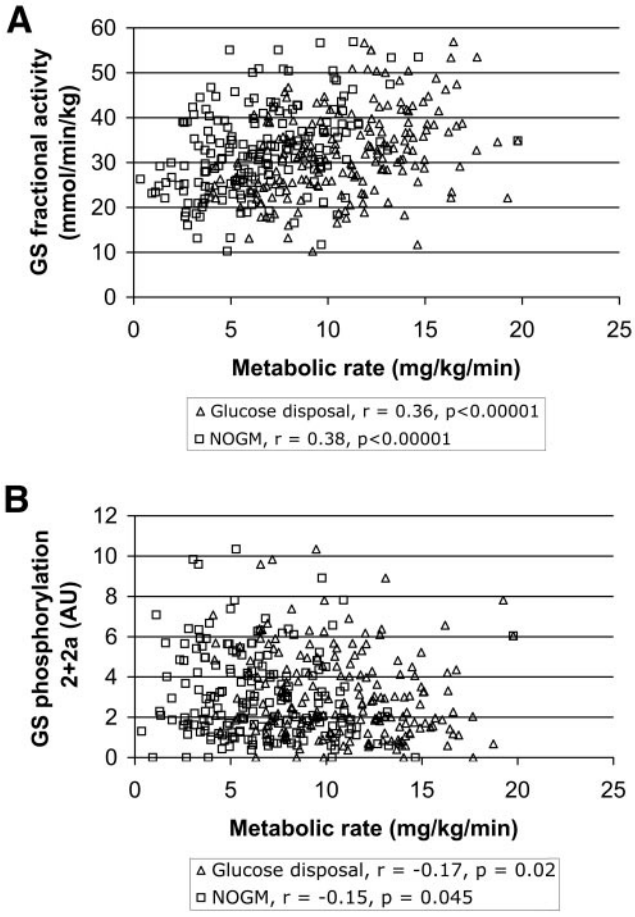

FIG. 1. Association between metabolic rates (glucose disposal and NOGM) and GS fractional activity (*A*) and GS phosphorylation at site 2+2a (*B*), respectively, in young and elderly twins ($n = 184$).

TABLE 2
Measures of GS activity and regulation during the basal and insulin-stimulated steady-state periods in young and elderly monozygotic and dizygotic twins

	Young			Elderly		
	Monozygotic	Dizygotic	*P*	Monozygotic	Dizygotic	*P*
n	63	38		40	43	
Basal						
Glycogen (mmol/kg ww muscle)	92.0 ± 3.7	86.0 ± 3.7	0.25	94.8 ± 4.6	81.0 ± 4.2	0.033
GS total protein (AU)	656.3 ± 43.2	659.3 ± 51.3	0.96	703.8 ± 69.2	522.1 ± 56.8	0.049
GS total activity (mmol · min^{-1} · kg^{-1})	4.81 ± 0.13	4.99 ± 0.18	0.41	4.20 ± 0.19	4.02 ± 0.21	0.55
GS fractional activity (% FV)	17.25 ± 0.96	15.5 ± 0.98	0.21	15.5 ± 1.05	17.5 ± 1.05	0.19
pGS site 3a+3b (AU)	0.29 ± 0.03	0.24 ± 0.03	0.25	0.23 ± 0.03	0.26 ± 0.03	0.32
pGS site 2 (AU)	1.01 ± 0.08	1.11 ± 0.09	0.46	1.01 ± 0.11	1.10 ± 0.09	0.51
pGS site 2+2a (AU)	3.75 ± 0.35	4.32 ± 0.44	0.33	3.96 ± 0.64	4.09 ± 0.50	0.88
GSK3α activity (nmol · min^{-1} · mg^{-1})	5.11 ± 0.19	4.82 ± 0.16	0.24	5.20 ± 0.25	5.18 ± 0.19	0.94
Insulin						
Glycogen (mmol/kg ww muscle)	93.4 ± 3.5	87.3 ± 3.7	0.24	92.1 ± 3.6	80.7 ± 4.1	0.04
GS total protein (AU)	582.8 ± 40.0	574.5 ± 58.3	0.91	617.1 ± 75.9	401.1 ± 48.2	0.022
GS total activity (mmol · min^{-1} · kg^{-1})	5.01 ± 0.16	5.00 ± 0.20	0.97	4.42 ± 0.19	3.91 ± 0.20	0.07
GS fractional activity (% FV)	33.7 ± 1.4	31.0 ± 1.4	0.17	29.4 ± 1.7	34.8 ± 1.6	0.028
pGS site 3a+3b (AU)	0.17 ± 0.02	0.17 ± 0.02	0.99	0.15 ± 0.02	0.15 ± 0.01	0.88
pGS site 2 (AU)	1.16 ± 0.10	1.30 ± 0.09	0.32	1.15 ± 0.10	1.24 ± 0.13	0.55
pGS site 2+2a (AU)	2.79 ± 0.24	3.36 ± 0.37	0.21	3.30 ± 0.44	3.39 ± 0.41	0.87
GSK3α activity (nmol · min^{-1} · mg^{-1})	3.50 ± 0.14	3.46 ± 0.13	0.82	3.76 ± 0.16	3.52 ± 0.17	0.31

Data are means ± SE. AU, arbitrary units; FV, fractional velocity.

in both monozygotic and dizygotic twins. In addition, genetic factors can be eliminated in monozygotic twin pairs, permitting distinction between associations of genetic and nongenetic origin. Using this design, we previously demonstrated a nongenetic positive association between birth weight and insulin sensitivity in elderly twins (5). In younger twins, we observed a trend toward a paradoxical inverse association between birth weight and insulin sensitivity, suggesting that the relationship may be age or time dependent. In the present study, we unmasked a quantitatively significant nongenetic impact of birth weight on GS activity and regulation among elderly monozygotic twin pairs. Skeletal muscle GS fractional activity correlates closely with both glucose disposal and NOGM and is therefore likely to represent a molecular mechanism explaining or contributing to the link between birth weight and insulin sensitivity in elderly twins. For every 100 g difference in birth weight within a twin pair, the heavier twin exhibited increments in insulin-stimulated muscle fractional GS activity, GS protein, and glycogen contents of between 4 and 9%, with concomitant 3–10% reductions in GSK3α activity and degree of GS phosphorylation at sites 2, 2+2a, and 3a+3b. This pattern of reduced glycogen synthesis in elderly low–birth weight twins agrees with our previously reported association between birth weight and defects of NOGM in elderly twins (5). Although seldom within twin pairs, a birth weight difference of 1 kg—equivalent to the difference in birth weight between "low birth weight" (<2,500 g) subjects and the average birth weight of 3,500 g in Caucasian newborns—had a quantitative impact on insulin-stimulated fractional GS activity in elderly twins comparable with that previously observed in both patients with overt type 2 diabetes and their first-degree relatives of ~20–40% (8,9,16,17).

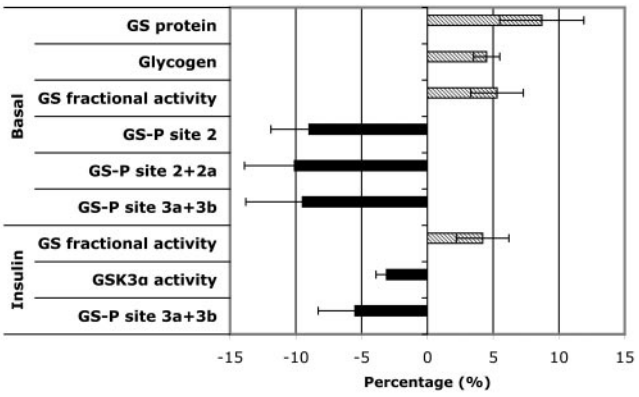

FIG. 2. Percentage change in GS activity and regulation (■, decrease; ▨, increase) for each 100 g increase in within-pair birth weight in elderly monozygotic twins. Data are percent (SE).

In the younger monozygotic twins, low birth weight was associated with an increase in GS protein content and a decrease in GS phosphorylation at site 3a+3b. Although neither of these variables were associated with in vivo measures of glucose uptake and/or storage, the findings are altogether consistent with the notion of an age- or time-dependent nongenetic effect of birth weight on both NOGM (5) and muscle GS activation in twins. Whether these divergent effects of birth weight on in vivo glucose metabolism in young and elderly twins are truly age related (i.e., advanced age unmasks the adverse metabolic defects associated with low birth weight) or a result of different life and nutritional conditions during the periods of birth (i.e., a time or cohort effect) remains to be clarified.

Previous studies have failed to demonstrate any influence of birth weight on postprandial GS activity (18) or on basal or insulin-stimulated GS gene expression levels (19) in relatively young human subjects. The significant positive association between birth weight and muscle glycogen content in elderly twins in the present study is consistent with studies reporting reduced muscle and liver glycogen contents in rats exposed to protein restriction during fetal

life (20,21). It is likely that the lack of association between birth weight and GS activity and regulation in previous human studies may be explained by a relatively small number of study subjects, differences in age, and perhaps most importantly by the failure to adjust for genetic influence (18,19). The defect in GS activity associated with low birth weight is mediated by increased GSK3α activity and GS phosphorylation and may represent an independent mechanism linking fetal environment and insulin resistance or may operate in concert with additional underlying defects of insulin signaling. Indeed, we have previously demonstrated an inverse influence of birth weight on GLUT4 (22) and transcriptional coactivator peroxisome proliferator–activated γ coactivator-1α (23) in twins, as well as on expression levels of specific proteins involved in insulin signaling in young low–birth weight subjects compared with control subjects (24).

Monozygotic twins are exposed to a different and possibly more adverse fetal environment compared with dizygotic twins due to the fact that they often share the same placenta (6). Consistent with the thrifty phenotype hypothesis, this may explain our previous finding that there is insulin resistance in elderly monozygotic compared with dizygotic twins (4,5) and, in the present context, as well as decreased fractional GS activity in elderly monozygotic compared with dizygotic twins. Accordingly, the lower glucose disposal and NOGM in the elderly monozygotic twins may be explained by reduced fractional GS activity. Interestingly, elderly monozygotic twins had a somewhat surprisingly increased GS protein expression and glycogen content compared with elderly dizygotic twins. While the increased GS protein expression may represent a compensatory mechanism, the elevated muscle glycogen content could at least partly be responsible for the reduced GS fractional activity due to negative feedback inhibition by increased glycogen content. Although type 2 diabetes in most studies is characterized by reduced glycogen content, increased glycogen content induced by overfeeding and inactivity may contribute to reduced insulin action (25,26). In addition, it was recently demonstrated that an increased glycogen–to–oxidative enzyme activity relationship may contribute to insulin resistance (26). In support of this view, the muscle glycogen level was associated in a negative manner with GS fractional activity ($r = -0.33$, $P < 0.00001$), which in turn was associated to insulin action.

Our findings suggest that programming in utero influences GS activity and glycogen synthesis in elderly twins in different ways. Importantly, the effect of zygosity is independent of and goes beyond the effect of birth weight, and we believe that our findings represent an illustrative example of the diverse and sometimes apparently opposing effects of two surrogate measures of the fetal environment (i.e., zygosity and birth weight) on adult phenotype.

In conclusion, we provide evidence for a major role of the fetal environment in the regulation of GS activity and glycogen metabolism in skeletal muscle. The age- or time-dependent nongenetic impact of birth weight on insulin action and NOGM in twins may partly be explained by reduced insulin activation of GS activity. This effect is mediated through increased GSK3α activity and phosphorylation of GS at sites 2, 2+2a, and 3a+3b. Reduced GS activity and negative feedback inhibition of glycogen metabolism by glycogen may contribute to the insulin resistance in elderly monozygotic compared with dizygotic twins.

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