Early Differential Defects of Insulin Secretion and Action in 19-Year-Old Caucasian Men Who Had Low Birth Weight

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Several studies have linked low birth weight (LBW) and type 2 diabetes. We investigated hepatic and peripheral insulin action including intracellular glucose metabolism in 40 19-year-old men (20 LBW, 20 matched control subjects), using the hyperinsulinemic-euglycemic clamp technique at two physiological insulin levels (10 and 40 mU/m² per min), indirect calorimetry, and [3-³H]glucose. Insulin secretion was examined during an oral and intravenous glucose tolerance test. Fasting p-glucose was higher in the LBW group (5.6 ± 0.1 vs. 5.4 ± 0.1; $P < 0.05$). Basal plasma glycerol concentrations were significantly lower in the LBW group. Insulin-stimulated glycolytic flux was significantly reduced, and suppression of endogenous glucose production was enhanced in the LBW group. Nevertheless, basal and insulin-stimulated rates of whole-body peripheral glucose disposal, glucose oxidation, lipid oxidation, exogenous glucose storage, and nonoxidative glucose metabolism were similar in the two groups. Insulin secretion was reduced by 30% in the LBW group, when expressed relative to insulin sensitivity (disposition index = insulin secretion × insulin action). We propose that reduced insulin-stimulated glycolysis precedes overt insulin resistance in LBW men. A lower insulin secretion may contribute to impaired glucose tolerance and ultimately lead to diabetes. *Diabetes* 51:1271–1280, 2002

Several studies have linked size at birth, or indices of poor fetal growth, with later development of impaired glucose tolerance (IGT) and type 2 diabetes (1–13). On the basis of their observations in two independent British populations, Barker and Hales (5) proposed that IGT and type 2 diabetes may arise as a result of "programming," a term used to describe persistent changes in organ structure and/or function caused by exposure to adverse environmental influences during critical periods of development (14,15). A number of studies have shown that non- (or pre-) diabetic subjects with low birth weight are insulin resistant and thus predisposed to development of type 2 diabetes (6,8,13,16–24). However, the majority of those previous studies used indirect measures of insulin resistance (i.e., homeostasis model assessment, or glucose and insulin responses during an oral glucose tolerance test [OGTT]). Two recent studies (8,20) used the gold standard technique to measure insulin sensitivity in vivo: subjects aged 70 and older (8), with uncertain information on family history of diabetes, who were insulin resistant during a hyperinsulinemic-euglycemic clamp, and 25-year-old men and women who had birth weights in the lowest third percentile (20). In the latter study, fat mass was significantly higher in the low birth weight (LBW) subjects and may per se have caused insulin resistance (25). None of these studies addressed the cellular mechanisms underlying the insulin resistance.

It was originally thought that the adverse effect of intrauterine growth restriction on glucose homeostasis was mediated through programming of the fetal endocrine pancreas (5). The idea was encouraged by animal experiments showing impaired β-cell function in offspring exposed to a low-protein diet during gestation (26–33). LBW has been associated with reduced 30-min insulin response after glucose ingestion in young nondiabetic men, relative to glucose and adjusted for BMI (34), and correlated negatively with β-cell function in a cohort of normoglycemic or glucose-intolerant first-degree relatives of patients with type 2 diabetes (35). However, a number of studies found no impact of LBW on insulin secretion in humans (6,17,18,36). Insulin secretion is related to insulin sensitivity in an inverse hyperbolic manner (37), illustrating the unique ability of the β-cell to compensate for insulin resistance and maintain euglycemia. Accordingly, correct estimation of in vivo insulin secretion requires adjustment for the corresponding insulin sensitivity, i.e., calculation of a disposition index (D_i). None of the above studies reported insulin secretion indices.

The aim of the present study was to resolve whether a birth weight below the 10th percentile was associated with impaired insulin action and/or insulin secretion in a well-matched Caucasian population of 19-year-old glucose-

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AUC, area under the curve; D_i, disposition index; EGP, endogenous glucose production; EGS, exogenous glucose storage; FFA, free fatty acids; FFM, fat-free mass; GF, glycolytic flux; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; IA, insulin action; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; LBW, low birth weight; NOGM, nonoxidative glucose metabolism; OGTT, oral glucose tolerance test; R_a, appearance rate; R_d, disposal rate; S_i, sensitivity index; TCA, tricarboxylic acid; $V\text{O}_{2max}$, maximal aerobic capacity.

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tolerant men. Peripheral and hepatic insulin action as well as intracellular partitioning of glucose fluxes was determined using two-step hyperinsulinemic-euglycemic clamps in conjunction with indirect calorimetry and [3-^{3}H]glucose infusion. Insulin secretion was assessed in response to both oral and intravenous glucose administration, and D_i's were calculated accordingly.

RESEARCH DESIGN AND METHODS

Particpants. Forty singleton men who were born at term (39–41 weeks) in 1980 in Copenhagen County were identified and recruited from the Danish Medical Birth Registry, according to birth weight. Twenty men had had birth weights below the 10th percentile (<2,800 g [week 39], <2,960 g [week 40], and <3,010 g [week 41]), and 20 men had had birth weights in the upper normal range (50–75th percentile; 3,390–3,700 g [week 39], 3,500–3,800 g [week 40], and 3,660–4,000 g [week 41]). None of the participants had a family history of diabetes (parents, grandparents), hypertension, or ischemic heart disease. In addition, none of them received medication known to affect glucose homeostasis. All participants had normal glucose tolerance after a standard 75-g OGTT, according to the World Health Organization criteria. The participants provided written informed consent before participation. The protocol was approved by the regional ethical committee, and procedures were performed according to the principles of The Helsinki Declaration.

Experimental protocol. The participants were instructed to abstain from strenuous physical activity and alcohol and to consume a diet rich in carbohydrate for a period of at least 48 h before the experiment. Smoking was not permitted during the experimental period. The participants reported to the laboratory at 0800 after a 10-h overnight fast. Standardized meals were served at 15 min (breakfast), 3 h and 15 min (lunch), 9 h (dinner), and 12 h and 30 min (sandwich), and a standardized light exercise on a bicycle was performed at 2 and 5 h. On the next day, after a 10-h fast, a polyethylene catheter was placed in the antecubital vein for test infusions. A second catheter was placed in a dorsal hand vein of the contralateral arm for blood sampling. The hand was placed in a heated Plexiglas box to ensure arterialization of the venous sample. A primed-continuous infusion of [3-^{3}H] tritiated glucose (bolus 10.9 μCi, 0.109 μCi/min) was initiated at 0 h and continued throughout the 2-h basal period, the 30-min intravenous glucose tolerance test (IVGTT), and two consecutive insulin clamps (in total 270 min). A 1-min intravenous glucose bolus (0.3 g/kg body wt) was given immediately after the 2-h basal period. Blood samples for glucose, insulin, and C-peptide were drawn at 0, 2, 4, 6, 8, 10, 15, 20, and 30 min. A primed-continuous insulin infusion (square wave bolus 2 IU, 10m U/m^2 per min) was begun at 150 min, continued for 120 min during the low physiological insulin clamp, and increased to 40 mU/m^2 per min during the 120-min high physiological insulin clamp. Steady state was defined as the last 30 min of the each 2-h basal, low- and high-insulin clamp period, when tracer equilibrium was anticipated. Indirect calorimetry was performed during all three steady-state periods using a computerized flow-through canopy gas analyzer system (Deltatrac; Datex, Helsinki, Finland) as previously described (38). Variable infusion of "cold" glucose (180 g/l) enriched with tritiated glucose (13.75 μCi/500 ml [10 mU/m^2 per min], 55 μCi/500 ml [40 mU/m^2 per min]) was used to maintain euglycemia during insulin infusion. Plasma glucose concentration was monitored every 5–10 min during basal and insulin-stimulated steady-state periods using an automated glucose oxidation method (Glucose Analyzer 2; Beckman Instruments, Fullerton, CA); blood samples for measurements of insulin, C-peptide, glucagon, D-β-hydroxybutyrate, glycerol, free fatty acids (FFA), lactate, and alanine were drawn every 30 min and of tritiated glucose/water every 10 min during steady-state periods and every 30 min for the rest of the study period.

On a separate day, a standard 75-g OGTT was performed after a 10-h overnight fast. Blood samples for plasma glucose were drawn at 0, 30, 60, 90, and 120 min; blood samples for insulin, C-peptide, glucagon-like peptide-1 (GLP-1), and gastric inhibitory polypeptide (GIP) were drawn at 0, 30, and 120 min. Height was determined as the mean of three measures by a stadiometer, a manual scale measured weight, and blood pressure was determined as the mean of two measures after 5 min of rest. Waist-to-hip ratio was measured at the level of umbilicus and greater trochanters. Body composition, i.e., lean body mass and fat mass, was determined by dual-energy X-ray absorptiometry scan (39). A submaximal exercise test with continuous monitoring of heart rate was performed on a bicycle ergometer, using a workload of 400–1,400 kpm/min. The mean heart rate during the last 2 min of work (170 beats/min > heart rate > 120 beats/min) was used for estimation of the maximal aerobic capacity ($V_{O_{2max}}$) as described by Åstrand (40).

Analytical procedures. Plasma glucose was measured bedside by an automated glucose oxidase method (Glucose Analyzer 2). Plasma insulin and C-peptide blood samples were centrifuged immediately at 4°C and stored at −80°C for later analysis. Plasma insulin and C-peptide concentrations were determined by 1235 AutoDELPHIA automatic immunoassay system (Wallac Oy, Turku, Finland). The plasma insulin assay had a detection limit of ~3 pmol/l. Cross-reactivity with intact proinsulin was 0.1%, 0.4% with 32–33 split proinsulin and 66% with 64–65 split proinsulin, intra-assay coefficient of variation 4.5%, and interassay variation 7%. Detection limit of the C-peptide assay was 5 pmol/l. Cross-reactivity with intact proinsulin was 51%, 35% with 32–33 split proinsulin and 92% with 64–64 split proinsulin (no detectable cross-reactivity with insulin), intra-assay variation of 5%, and interassay variation 8%. Tritiated glucose and water was measured as described by Beck-Nielsen and colleagues and Hother-Nielsen et al. (38,41). Plasma FFA were quantified by enzymatic colorimetric method (Wako, Neuss, Germany), and plasma lactate was quantified on an automatic lactate analyzer (YSI 23AM; Yellow Springs International, Yellow Springs, OH). Concentrations of alanine, D-β-hydroxybutyrate, and glycerol were measured by the fluorometric method. Total GIP was measured using the COOH-terminally directed antiserum R65 (42), which reacts fully with intact GIP and the N-terminally truncated metabolite, GIP (3–42), but not with the so-called 8-kDa GIP, whose chemical nature and relation to GIP secretion are uncertain. The assay has a detection limit of <2 pmol/l and an intra-assay variation of ~6%. Human GIP (Pennisula Laboratories Europe, St. Helens, Meyerside, U.K.) was used as standard, and radiolabeled GIP came from Amersham Pharmacia Biotech (Little Chalfont, Buckinghamshire, U.K.). COOH-terminal GLP-1 immunoreactivity was determined by radioimmunoassay, measuring total GLP-1 (sum of intact peptide plus primary metabolite) as described previously (43), using standards of synthetic GLP-1(7-36)amide(=proglucagon 78-107amide) and antiserum no. 89390. The assay cross-reacts <0.01% with COOH-terminally truncated fragments and 83% with GLP-1 (9-36) amide and has a detection limit below 1 pmol/l. Intra-assay and interassay coefficients of variation were 6 and 16% at 40 pmol/l, respectively. The glucagon assay (44) is directed against the COOH-terminus of the glucagon molecule (antibody code no. 4305) and, therefore, measures glucagon of mainly pancreatic origin. Detection limit and intra-assay coefficient of variation of the assay used was 1 pmol/l and <6%, respectively. For glucagon and GLP-1 analysis, plasma was extracted with ethanol (final concentration, 70% vol/vol) before analysis.

Statistical methods. Nonparametric statistical methods (Mann-Whitney *U* test for unpaired data, Wilcoxon's test for paired data, and Spearman's ρ for correlation analysis) were used in the data analysis. Friedman's test (nonparametric repeated measurement ANOVA) was applied when comparing mean values for clamp data during steady state (Instat Statistical Package; GraphPad, San Diego, CA). D_i's were in addition analyzed on the log scale using a mixed linear model (45) with fixed effects of type of index ($D_{i\ 1,OGTT}$, $D_{i\ 2,OGTT}$, and $D_{i\ IVGTT}$) and birth weight group (LBW or control) and a random effect of person. We tested the assumption that the group differences were the same regardless of the D_i used, i.e., if there was in fact a difference between insulin secretion assessed by oral and intravenous test. $P < 0.05$ was considered significant in two-tailed analysis. Data are presented as mean ± SE (descriptive data in Table 1 as mean ± SD).

Calculations

Basal and insulin-stimulated glucose turnover rates. Appearance rates (R_a), disposal rates (R_d), and endogenous glucose production (EGP) rates were calculated at 10-min intervals during predefined steady state, using Steele's non–steady-state equations (46). In the calculations, distribution volume of glucose was 200 ml/kg body wt, and the pool fraction was 0.65. Rates of whole-body glycolysis were estimated from the increment per unit time in tritiated water ([cpm per ml per min] × total body water mass [ml]/[3-^{3}H]glucose specific activity [cpm per mmol]) (47). Plasma water was estimated as 93% of total plasma volume, and total body water mass was assumed to be 65% of the body mass (48). EGP was calculated by subtracting the rate of the exogenous glucose infusion from the rate of appearance of [3-H_3]glucose (R_a), exogenous glucose storage (EGS) as R_d minus glycolytic flux (GF), and nonoxidative glucose metabolism (NOGM) as R_d minus glucose oxidation. Glucose turnover rates are expressed in mg/kg fat-free mass (FFM)/min and presented throughout this article as mean values of the 30-min steady-state periods. The sensitivity index ($S_{i\ Rd}$) was defined as ([$R_{d\ 40\ mU}$ − $R_{d\ basal}$]/[(p-insulin$_{40\ mU}$ − p-insulin$_{basal}$) · p-glucose$_{40\ mU}$] [mg · kg FFM^{-1} · min^{-1} · pmol/l insulin^{-1} · mmol/l glucose^{-1}]).

OGTT. Total and incremental areas under the curve (AUCs) were calculated by trapezoidal method for insulin (pmol · l^{-1} · min) and glucose (mmol · l^{-1} · min), using the 0-, 30-, and 120-min insulin concentrations and 0-, 30-, 60-, 90-, and 120-min glucose concentrations, respectively. The incremental insulin response was defined as ([p-insulin$_{30\ min}$ − p-insulin$_{basal}$]/[p-glucose$_{30\ min}$ − p-glucose$_{basal}$]) (pmol/l insulin per mmol/l glucose) and Phi_2 as (total AUC$_{insulin}$/total AUC$_{glucose}$) (pmol/l insulin per mmol/l glucose).

IVGTT. Total and incremental AUCs for insulin (pmol · l^{-1} · min) and glucose

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TABLE 1
Subject characteristics at examination

	LBW subjects	Control subjects	P
Weight (kg)	73.6 ± 8.5	74.7 ± 13.1	NS
Height (cm)	178.5 ± 4.0	181.7 ± 4.8	0.03
BMI (kg/m^2)	23.1 ± 2.7	22.6 ± 3.6	NS
Waist-to-hip ratio	0.82 ± 0.04	0.81 ± 0.06	NS
Systolic BP (mmHg)	111.3 ± 11.7	114.8 ± 11.5	NS
Diastolic BP (mmHg)	65.5 ± 8.9	67.8 ± 9.8	NS
Mid-arterial BP (mmHg)	80.8 ± 8.7	83.4 ± 8.0	NS
f-p triglycerides (mmol/l)	1.09 ± 0.66	0.92 ± 0.30	NS
f-p cholesterol, total (mmol/l)	4.03 ± 0.70	3.88 ± 0.56	NS
f-p LDL cholesterol (mmol/l)	2.37 ± 0.56	2.16 ± 0.44	NS
f-p HDL cholesterol (mmol/l)	1.16 ± 0.26	1.31 ± 0.21	0.05
Fat mass$_{DXA}$ (kg)	15.6 ± 6.8	15.6 ± 6.9	NS
Lean body mass$_{DXA}$ (kg)	54.9 ± 4.3	56.9 ± 7.3	NS
$V\text{O}_{2\text{max}}$ (l/min)	3.4 ± 0.4	3.5 ± 0.7	NS

Data are means ± SD. BP, blood pressure; f-p, fasting plasma.

(mmol · l^{-1} · min) were calculated using the 0-, 2-, 4-, 6-, 8-, and 10-min concentrations after initiation of the glucose bolus. Phi$_1$ was defined as (total AUC$_{insulin}$/total AUC$_{glucose}$) (pmol/l insulin per mmol/l glucose).

***D_i*.** We calculated two D_i's based on OGTT data and one based on IVGTT data: $D_{i\ 1,OGTT}$ was calculated as (incremental insulin response · $S_{i\ Rd}$) and $D_{i\ 2,OGTT}$ as (Phi$_2$ · $S_{i\ Rd}$) (mg · kg FFM^{-1} · min^{-1} · mmol/l glucose^{-2}). $D_{i\ IVGTT}$, was calculated as (Phi$_1$ · $S_{i\ Rd}$) (mg · kg FFM^{-1} · min^{-1} · mmol/l glucose^{-2}). It should be noted that calculation of the D_i is usually based on simultaneous estimates of insulin secretion and insulin sensitivity during a frequently sampled IVGTT (37). Here we have used a clamp-based estimate of S_i rather than Bergman's minimal model estimate, and less frequent blood sampling for estimation of acute insulin response. The comparability of our measures to that described by Kahn et al. (37) has not been demonstrated.

RESULTS

Subject characteristics (Table 1). Weight at birth differed by ~1 kg (2,702 ± 202 vs. 3,801 ± 99 g [mean ± SD]). In addition, both birth length (48.5 ± 0.6 vs. 52.7 ± 0.4 cm; $P < 0.0001$) and ponderal index (24.0 ± 0.9 vs. 26.2 ± 0.5 kg/m^3; $P = 0.0004$) were lower in the LBW group. Current height was lower in the LBW group. However, the groups were similar with regard to current weight, BMI, waist-to-hip ratio, systolic and diastolic blood pressures, mid-arterial blood pressure, fasting plasma triglycerides, total and LDL cholesterol, body composition (i.e., lean body mass and fat mass), and $V\text{O}_2$ max. HDL cholesterol was lower in the LBW group.

Clamp studies

Basal and insulin-stimulated concentrations of plasma glucose, insulin, C-peptide, glucagon, D-β-hydroxybutyrate, glycerol, FFA, lactate, and alanine (Fig. 1). Plasma glycerol concentrations were significantly lower in the LBW group during basal steady state (51.8 ± 3.7 vs. 67.3 ± 7.8 μmol/l; $P = 0.04$) and borderline significantly lower at low insulin concentrations (21.8 ± 3.0 vs. 29.9 ± 3.2 μmol/l; $P = 0.07$; Fig. 1*G*). Basal and insulin-stimulated plasma glucose, insulin, C-peptide, glucagon, D-β-hydroxybutyrate, FFA, lactate, and alanine (not shown) concentrations were comparable in the two groups; however, the LBW group had a tendency toward higher plasma glucagon during insulin infusion (Fig. 1*D*), lower FFA concentration at basal, and low insulin concentrations (Fig. 1*H*).

Basal and insulin-stimulated glucose turnover rates (Fig. 2) Tracer equilibrium, i.e., constant specific activity of glucose tracer, was reached in both groups during predefined 30-min basal and insulin clamp steady-state periods (Fig. 1*J*). The LBW group had significantly higher plasma glucose specific activity than the control group at basal and high insulin clamp concentrations, despite similar glucose infusion rates (average, 9–11%). With the exception of a slight increase in glucose disposal (Fig. 2*A*), infusion of insulin at the low infusion rate did not produce significant change in glucose flux through any of the major pathways. Plasma concentrations of tritiated water increased linearly with time in both LBW and control subjects ($r^2 = 0.71 \pm 0.06$; $r^2 = 0.75 \pm 0.06$; Fig. 1*K*). However, the calculated glyolytic flux (GF) rates were significantly lower in the LBW group during high insulin clamp concentrations (3.7 ± 0.5 vs. 5.1 ± 0.6 mg · kg FFM^{-1} · min^{-1}; $P = 0.03$; Fig. 2*D*), with no differences between the groups during basal steady state or at low insulin clamp concentrations. EGP was suppressed by 50% in the low insulin clamp, with only minor nonsignificant further suppression from low to high physiological insulin concentrations (Fig. 2*C*). The LBW group had significantly lower EGP at high insulin concentrations (1.1 ± 0.3 vs. 2.0 ± 0.3; $P < 0.05$), with similar basal and low insulin clamp values in the two groups. Basal and insulin-stimulated glucose disposal, glucose oxidation, lipid oxidation (data not shown), glucose storage, and NOGM were similar in the two groups.

Insulin secretion

OGTT (Fig. 3, Table 2). Fasting plasma glucose was slightly but significantly higher in the LBW group (5.6 ± 0.1 vs. 5.4 ± 0.1 mmol/l; $P = 0.05$), whereas a higher 2-h postload plasma glucose reached only borderline significance (5.9 ± 0.2 vs. 5.3 ± 0.2 mmol/l; $P = 0.07$). Thirty-minute postload plasma insulin was lower in the LBW group but was not quite significant (331.2 ± 72.5 vs. 391.9 ± 72.5 pmol/l; $P = 0.07$). The incremental insulin response (Ins$_{incr,\ 0–30\ min}$) was similar in the two groups. The total and incremental AUCs for insulin tended to be lower in the LBW group, whereas total and incremental AUC$_{glucose}$ was similar between the groups. As a result, Phi$_2$ (AUC$_{insulin}$/AUC$_{glucose}$) was lower in the LBW group (33.6 ± 6.5 vs. 37.4 ± 3.7; $P = 0.09$; Fig. 3, Table 2). The 30-min plasma GLP-1 concentration was lower in the LBW group (22.5 ± 1.9 vs. 31.5 ± 4.5 pmol/l; $P = 0.08$). Neither

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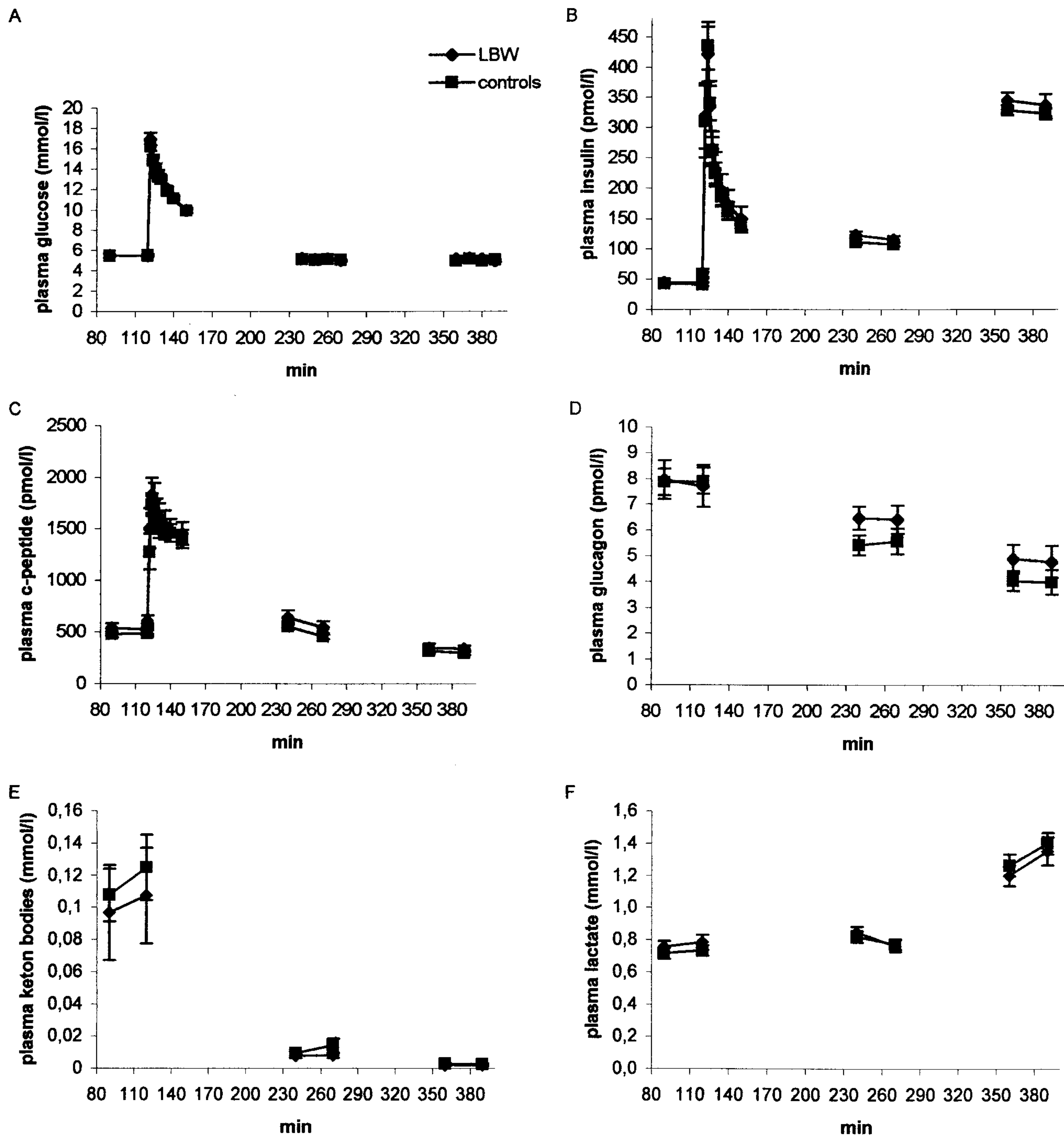

FIG. 1. Clamp and IVGTT data. Plasma glucose (*A*), insulin (*B*), C-peptide (*C*), glucagon (*D*), D-β-hydroxybutyrate (*E*), lactate (*F*), glycerol (*G*), FFA (*H*), counts per minute (cpm) per mg glucose (*I*), glucose specific activity (*J*), and tritiated water specific activity (*K*) in LBW versus control subjects. Data are means ± SE. *$P < 0.05$; **$P < 0.01$.

plasma GIP concentrations nor total and incremental AUCs for GLP-1 and GIP differed between the groups.

***IVGTT (Fig. 1A–C*, Table 2).** There were no significant differences in plasma glucose, insulin, or C-peptide concentrations between the groups at any time point; however, there was a tendency toward higher total $AUC_{glucose}$ in the LBW group. None of the calculated parameters—incremental AUC for glucose, total and incremental AUCs for insulin and C-peptide, or Phi_1—differed significantly between the groups.

***D_i*.** When the corresponding insulin sensitivity ($S_{i\ Rd}$) was accounted for by calculation of a disposition index, $D_{i1,\ OGTT}$ (incremental insulin response × $S_{i\ Rd}$) and $D_{i\ 2,\ OGTT}$ (Phi_2 × $S_{i\ Rd}$) were significantly lower in the LBW group (Table 2). There was a tendency toward lower $D_{i\ IVGTT}$ in the LBW group; however, this difference did not reach statistical significance. Analysis of D_i's on the original scale showed gross violation of variance homogeneity assumptions, which was not the case when data were log-transformed. We found no interaction between type of index ($D_{i\ 1,OGTT}$, $D_{i\ 2,OGTT}$, or $D_{i\ IVGTT}$) and birth weight group (LBW or control; Wald test, $P = 0.493$), i.e., the group difference can

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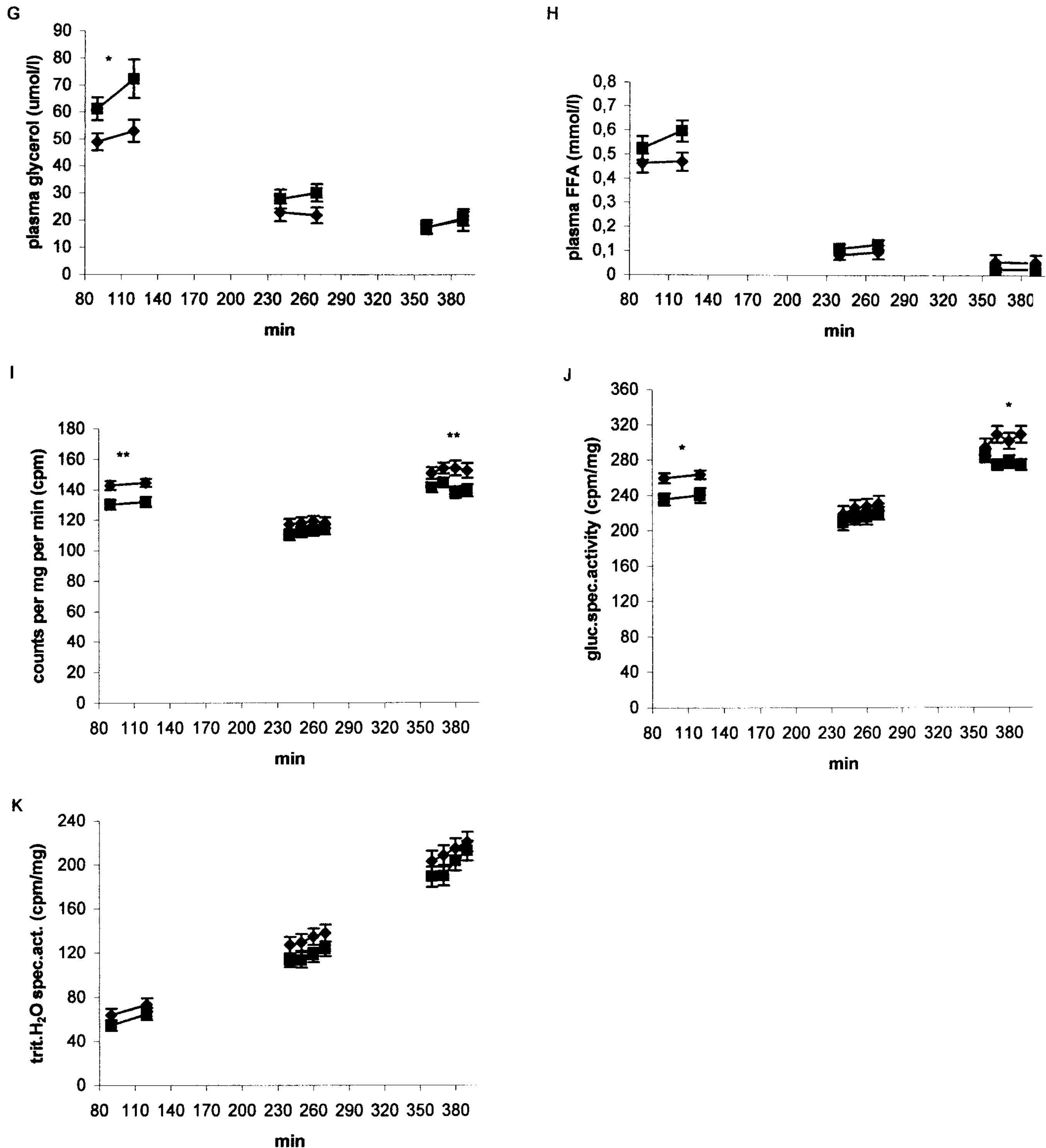

FIG. 1. Continued

be summarized in one number, the ratio between D_i's in the LBW and control groups, which was 0.705 (95% CI, 0.521–0.955; P = 0.0295). That means that insulin secretion in the LBW group was reduced by ~30% in comparison with the control group, regardless of the type of test (oral or intravenous) used.

Correlations. Insulin secretion during OGTT and IVGTT was inversely related to insulin sensitivity during the clamp (LBW: Phi_2 versus $S_{i\ Rd}$, R = −0.73, P = 0.0003; incremental insulin response versus $S_{i\ Rd}$, R = −0.53, P = 0.02; Phi_1 versus $S_{i\ Rd}$, R = −0.39, P = 0.06. Control subjects: Phi_2 versus $S_{i\ Rd}$, R = −0.53, P = 0.02; incremental insulin response versus $S_{i\ Rd}$, R = −0.32, P = 0.06; Phi_1 versus $S_{i\ Rd}$, R = −0.42, P = 0.06). Insulin-stimulated GF (40 mU/m^2 per min) correlated with 2-h postload glucose (LBW: R = −0.55, P = 0.01; control subjects: R = −0.46, P = 0.04; Fig. 4). We found significant correlation between insulin-stimulated GF (40 mU/m^2 per min) and glucose

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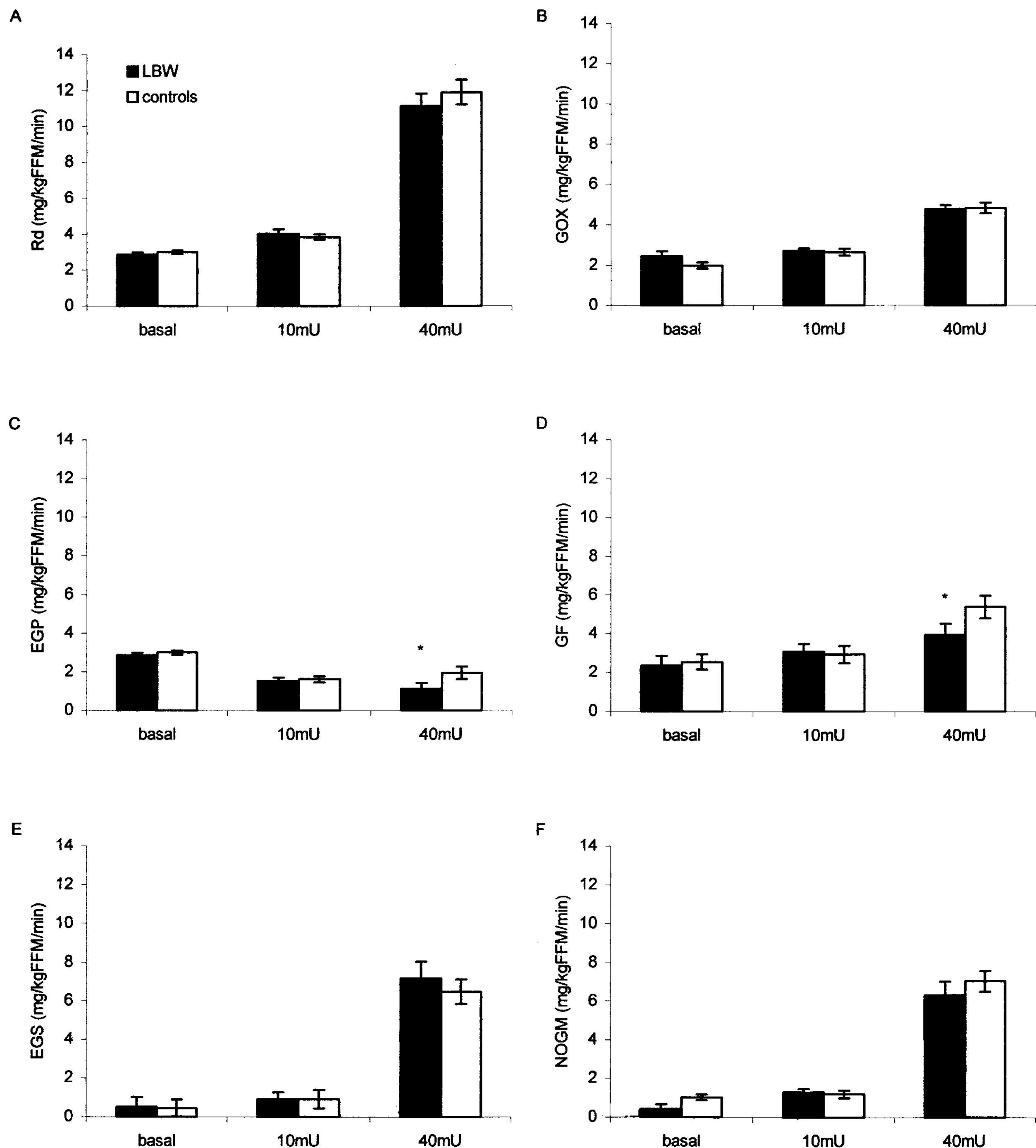

FIG. 2. Glucose fluxes during basal, low (10 mU/m^2 per min), and high (40 mU/m^2 per min) insulin clamp steady state in LBW and control subjects. Glucose disposal (*A*), glucose oxidation (*B*), endogenous glucose production (*C*), glycolytic flux (*D*), exogenous glucose storage (*E*), and nonoxidative glucose metabolism (*F*). Data are means ± SE. *$P < 0.05$ for LBW vs. control subjects.

oxidation (40 mU/m^2 per min) in control subjects ($r = 0.54$, $P = 0.01$) but not in the LBW group ($r = 0.03$, $P = 0.92$).

DISCUSSION

"The fetal origins hypothesis" (5) is now widely recognized, yet the underlying cellular mechanism(s) has not been established. Briefly, our study revealed four novel and potentially important findings: *1*) reduced insulin-stimulated glycolysis in the face of normal whole-body peripheral glucose disposal; *2*) enhanced suppression of hepatic glucose production during high physiological insulin concentrations; *3*) lower fasting steady-state plasma glycerol concentrations, possibly reflecting a lower lipolytic rate; and *4*) reduced glucose-stimulated insulin secretion, when adjusted for insulin sensitivity (assessed during clamp).

We chose to study young, healthy, glucose-tolerant men who had had birth weights in the lower 10th percentile and whose parents and grandparents did not have diabetes. By doing so, we tried to eliminate major confounders such as "diabetes genes" and metabolic defects that could be secondary to aging, obesity, glucose- and lipotoxicity, and

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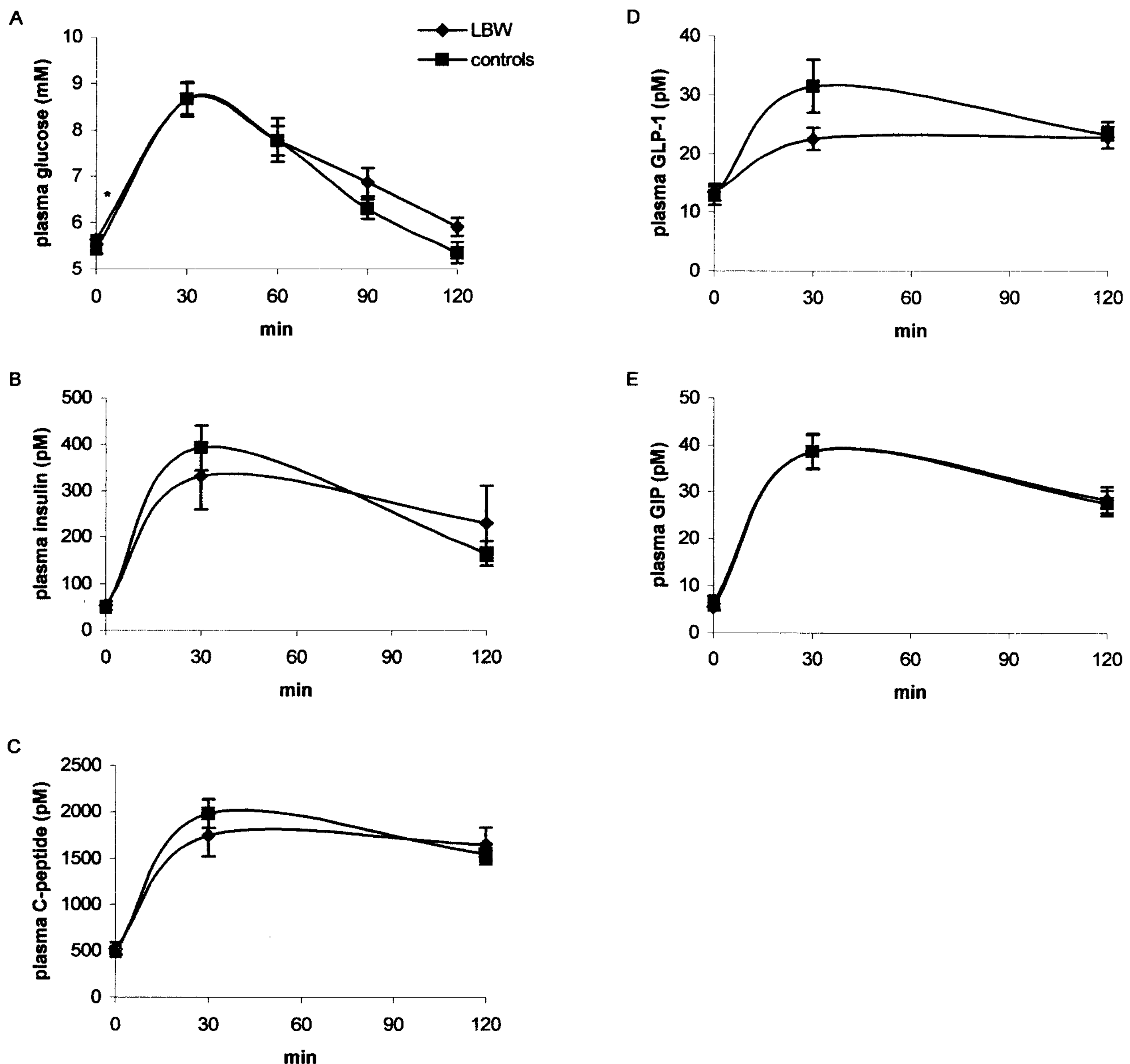

FIG. 3. Seventy-five-gram OGTT in LBW participants and control subjects. Plasma glucose (*A*), insulin (*B*), C-peptide (*C*), GLP-1 (*D*), and GIP (*E*). Data are means ± SE. *$P < 0.05$.

so forth. Even so, this group of 19-year-old lean men had slightly but significantly higher fasting plasma glucose and higher 2-h postload glucose ($P = 0.07$).

Are young LBW subjects insulin resistant? Several studies have shown higher prevalence of insulin resistance in middle-aged or elderly subjects who had been LBW infants (1–4,6–9,11). Data in younger populations are scarce and mostly indirect: highly significant inverse relations between birth weight and insulin concentrations before and after a standard glucose challenge were reported in a study of 30-year-old Mexican-Americans and non-Hispanic whites in San Antonio (13), and in a study of 21-year-olds in Haguenau, France (21). Birth weight was positively associated with the insulin sensitivity index (MINMOD) in a cohort of young healthy Danes (17) and Australian men (18), independent of BMI and fat distribution. The only other study that used the gold standard hyperinsulinemic clamp technique (20) found reduced whole-body glucose uptake in 25-year-old men and women who had had birth weights below the third percentile, but only after adjusting for body fat mass. The LBW subjects in that study, however, had significantly higher total body fat mass than their control subjects. We speculate that this finding may indeed explain the difference between that and our study. We propose that overt insulin resistance associated with LBW occurs later, succeeding normal or perhaps even enhanced insulin sensitivity at a younger age, as seen in rats (49) and monozygotic twins (50).

Reduced insulin-stimulated GF. The use of tritiated glucose and indirect calorimetry during a clamp enabled us to look in more detail at cellular glucose metabolism. This study is the first to report decreased GF during insulin stimulation, in the face of a normal overall glucose disposal. In fact, it may be speculated that decreased insulin-stimulated GF represents a primary defect associated with LBW, preceding and potentially leading to insulin resis-

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TABLE 2
Disposition indices

	LBW subjects	Control subjects	P
Clamp (40 mU/m^2 per min)			
Si_{Rd}	0.00583 ± 0.00055	0.00638 ± 0.00048	NS
OGTT			
Incremental insulin secretion (0–30 min)	94.0 ± 14.8	106.1 ± 10.1	NS
$AUC_{insulin}$ (0–120 min)	31031 ± 7806	31677 ± 3320	NS
$AUC_{glucose}$ (0–120 min)	872.3 ± 33.3	843.2 ± 22.8	NS
Phi_2 ($AUC_{insulin}/AUC_{glucose}$)	33.6 ± 5.6	37.4 ± 3.7	NS
$D_{i1, OGTT}$ (based on incremental insulin secretion)	0.4692 ± 0.0889	0.6319 ± 0.0637	0.03
$D_{i2, OGTT}$ (based on Phi_2)	0.1425 ± 0.0108	0.2187 ± 0.0191	0.006
IVGTT			
$AUC_{insulin}$ (0–10 min)	2,968.5 ± 347.9	2,973.6 ± 262.9	NS
$AUC_{glucose}$ (0–10 min)	138.1 ± 3.1	134.8 ± 2.1	NS
Phi_1 ($AUC_{insulin}/AUC_{glucose}$)	21.08 ± 2.14	22.09 ± 1.90	NS
Di_{IVGTT} (based on Phi_1)	0.1108 ± 0.0123	0.1356 ± 0.0146	NS*

Data are means ± SE. *Mann-Whitney test. In a mixed linear model, there was no difference between the oral and intravenous indices, and the overall effect of low birth weight was a 30% reduction compared with the control group.

tance and diabetes later in life. In support of our data, Taylor et al. (51) reported reduced muscle glycolytic ATP production during exercise (i.e., not insulin stimulation) using ^{31}P magnetic resonance spectroscopy in normoglycemic insulin-resistant women who were thin at birth. It remains to be determined whether this defect is limited to skeletal muscle (51) or represents a more generalized metabolic defect in tissues of importance for glucose homeostasis, including the liver (52) or β-cell. Such defects may contribute to an increase hepatic glucose production (53) and/or a decrease insulin secretion (54) in patients with type 2 diabetes.

We did not find reduced insulin-stimulated glucose oxidation, which may seem somewhat contradictory to the finding of reduced exogenous GF. However, indirect calorimetry measures total flux through the tricarboxylic acid (TCA) cycle, i.e., exogenous glucose molecules deriving from cell membrane glucose transport and endogenous glucose from glycogen stores. Oxidation of glucose from glycogen stores will not appear in the tracer calculations. Moreover, indirect calorimetry does not provide an estimate of the glucose that has been directed to nonoxidative glycolysis (lactate and alanine production). Thus, it is possible that the lower insulin-stimulated GF was counterbalanced by an increased glycogenolysis in the LBW men. Alternatively, the decrease in glycolysis may have resulted in reduction of nonoxidative glycolysis only, which did not affect absolute flux through the TCA cycle.

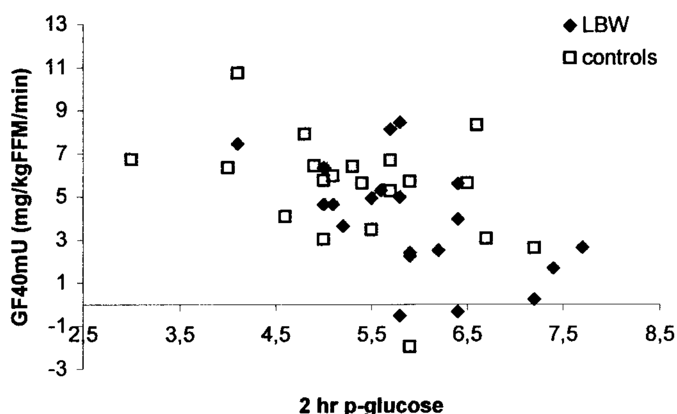

FIG. 4. Correlation between insulin-stimulated GF ($GF_{40\ mU}$) and 2-h postload glucose in LBW and control subjects. $R = -0.55$, $P = 0.01$ (LBW); $R = -0.46$, $P = 0.04$ (control).

Better suppression of glucose production during insulin stimulation. The enhanced insulin suppression of EGP in the LBW men may well represent a compensatory mechanism to ensure normal glucose tolerance. It was shown recently that insulin-resistant 20-year-old men who had LBW had increased glucose effectiveness as assessed by an IVGTT (18), similarly to insulin-resistant first-degree relatives of patients with type 2 diabetes (55). Glucose effectiveness is the ability of glucose per se to promote its own uptake and to suppress EGP. The glucose transporter molecule GLUT1 has been proposed as a candidate to mediate those cellular effects of glucose (56). Recent animal studies showed that intrauterine growth retardation induced by uterine artery ligation is associated with an increased hepatic (57) and skeletal muscle (58) expression of GLUT1 in the neonatal period. Although it is not known whether these changes persist into adulthood, it may be speculated that parallel or similar compensatory changes in expression patterns of glucose transporters may occur as a result of altered organ programming in men who had LBW. Key enzymes in glycolysis and gluconeogenesis (GK, PEPCK) were altered in the gluconeogenic direction in livers from adult male rat reduced-protein offspring (53). It is interesting that in parallel with our findings of lower EGP and (nonsignificantly) higher clamp glucagon concentrations in the LBW group, rat livers from young adult rats had reduced glucose output in response to glucagon (59), i.e., glucagon resistance, possibly as a result of a lower number of glucagon receptors and a threefold increase in the glucose transporter GLUT2 (59).

Lower fasting plasma glycerol concentrations. Some (60,61) but not all (18) studies have indicated that an adverse intrauterine environment predisposes to adult obesity. It is unknown to what extent an increased body fat mass or altered body fat distribution contributes to insulin resistance associated with LBW. Although theoretically the result of increased hepatic glycerol clearance,

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the lower fasting plasma glycerol concentrations in the LBW subjects in our study, may reflect a lower lipolytic rate, which in the setting of a high-caloric intake or low physical activity could lead to fat accumulation and insulin resistance later in life (13).

Reduced insulin secretion after adjustment for insulin sensitivity. Previous studies did not take insulin sensitivity into account when evaluating insulin secretion and for this reason may have underestimated the effect of birth weight. We measured insulin secretion in response to both an oral and intravenous glucose stimulus. There were no significant differences between the groups in the absolute responses, but when the corresponding insulin sensitivity (assessed during clamp, not by minimal model analysis of frequently sampled IVGTT data as in the study by Kahn et al. [37]) was accounted for, insulin secretion D_i (oral and intravenous) were significantly reduced by ~30% in the LBW group. In support of our data, Robinson et al. (34) found that LBW was associated with lower 30-min plasma insulin and proinsulin concentrations during OGTT in young men with a mean age of 21 years (when adjusted for corresponding plasma glucose levels and BMI). Jaquet et al. (20) found hyperinsulinemia in response to a standard oral glucose load in their study of 25-year-old men and women. However, these LBW subjects were insulin resistant as assessed by hyperinsulinemic-euglycemic clamp. Offspring of women who were exposed to severe restrictions in food intake during the famine in the Netherlands in 1944–1945 (aged ~50 years) had higher 120-min plasma glucose concentrations but comparable fasting and 120-min plasma insulin/proinsulin concentrations and relative insulin increment after oral glucose ingestion (11). These data are suggestive of a relative insulin secretion defect. Young (17,18) and middle-aged (6,36) populations had normal absolute insulin secretion in response to intravenous glucose; there was no information on insulin sensitivity. We speculate that with time, accumulation of body fat, and increasing insulin resistance, the relative insulin secretion defect observed in these young men who had had LBW will contribute to impair glucose tolerance and perhaps in time lead to overt diabetes. Lower incremental GLP-1 responses may further impair glucose-stimulated insulin secretion during a meal. Defective GLP-1 secretion has been demonstrated in patients with type 2 diabetes (62), and studies in monozygotic twins discordant for type 2 diabetes have indicated that defects in GLP-1 secretion may be of nongenetic origin (62,63).

Implications. Our data add to the mounting evidence of key metabolic processes being programmed during fetal life. Although we do not find large or highly significant differences, we think that our being able to detect various metabolic differences in an otherwise well-matched population of young adult men merits continued interest in this field of research. With our knowledge of type 2 diabetes as a progressive disease that is precipitated by obesity (and age), it is likely that it is the combination of a number of defects programmed during fetal life, a Westernized diet, and a sedentary lifestyle that eventually result in overt diabetes.

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