Endogenous Glucose Production, Insulin Sensitivity, and Insulin Secretion in Normal Glucose-Tolerant Pima Indians With Low Birth Weight

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Individuals with low birth weight (LBW) are at increased risk of developing type 2 diabetes in later life. Whether impairments in endogenous glucose production (EGP), insulin action, insulin secretion, or a combination thereof account for this association is unclear. We, therefore, examined these parameters in Pima Indians with normal glucose tolerance. Body composition, glucose and insulin responses during a 75-g oral glucose tolerance test (OGTT), EGP, insulin-stimulated glucose disposal during low- and high-dose insulin infusion (M-low and M-high, hyperinsulinemic glucose clamp), and acute insulin response (AIR) to a 25-g intravenous glucose challenge were measured in 230 Pima Indians (147 men and 83 women, aged 25 ± 0.4 years [mean ± SE; range, 18 to 44]) with normal glucose tolerance. A subgroup of 63 subjects additionally underwent biopsies of subcutaneous adipose tissue for determination of adipocyte cell size and lipolysis. Subjects in the lowest quartile of birth weight (birth weight: 2,891 ± 33 g, LBW, n = 58) were compared to those whose birth weight was in the upper 3 quartiles (birth weight: 3,657 ± 28 g, NBW, n = 172). Age- and sex-adjusted body mass index (BMI), percent body fat, and waist-to-thigh ratio (WTR) were similar in LBW and NBW subjects. Suppression of EGP during the clamp was less in LBW than in NBW subjects before ($P = .002$) and after adjustment for age, sex, percent body fat, and M-low ($P = .02$). M-low and M-high were less in LBW than in NBW subjects before ($P = .05$ and $P = .01$) and after adjustment for age, sex, percent body fat, and WTR ($P = .04$ and $P = .05$). AIR was not different in LBW compared to NBW subjects before adjustments ($P = .06$), but it was lower in LBW than in NBW subjects after adjustment for age, sex, percent body fat, and M-low ($P = .02$), suggesting that AIR did not increase appropriately for the decrease in insulin-stimulated glucose disposal (M). In addition, average adipocyte cell size ($P = .08$) and basal lipolysis ($P = .02$) were higher in the LBW than in the NBW group. These results show that Pima Indians with LBW manifest a variety of impairments in metabolism in adulthood. Among these, a lesser insulin-stimulated suppression of EGP and a lesser insulin secretory capacity are the predominant ones. We conclude that interaction of multiple defects may contribute to increased susceptibility to type 2 diabetes among individuals with LBW.

A LARGE NUMBER of studies indicate that low birth weight (LBW) is associated with an increased risk of developing type 2 diabetes later in life.[1-10] This association, originally described in 1991,[1] has been replicated in studies in the United Kingdom,[2] Sweden,[3,4] and elsewhere[5,6] during the last decade. In the United States, two large longitudinal studies of health professionals, which together provide information on more than 90,000 men and women, confirmed that LBW is associated with an increased risk of developing diabetes.[5,6] Subjects in these studies were mostly Caucasians, but an association of LBW and an increased risk of diabetes has been found in other ethnic groups as well.[7-9] Among the Pima Indians of Arizona, a population with an extraordinarily high prevalence of type 2 diabetes,[11] higher risks for diabetes are apparent in both low– and high–birth weight groups.[7] However, after accounting for the effects of maternal diabetes during pregnancy, an inverse relation between birth weight and subsequent incidence of diabetes is observed, similar to that reported in other populations.

Although the evidence linking birth weight and diabetes is remarkably consistent, there is less agreement about the mechanisms underlying this association. Originally, it was hypothesized that fetal malnutrition during critical developmental periods caused a permanent adaptation or "programming" of the pancreas that impaired the ability to secrete insulin.[1] This is supported by animal studies demonstrating that food restriction during late gestation can impair pancreatic β-cell development and insulin secretion.[12,13] Observations that LBW is associated with abnormalities in pancreatic β-cell development in humans are not consistent.[14,15] A few studies have shown that LBW is associated with impaired insulin secretion,[1,16,17] but several others have not.[18-23] In contrast, a number of reports have suggested that LBW is associated with insulin resistance.[3,4,8,10,22-27]

Most studies to date have examined the effect of birth weight on indirect measures of obesity, insulin secretion and insulin action. Insulin secretion is best evaluated relative to the degree of insulin resistance. However, insulin secretion and action have rarely been measured in the same study. In many cases, individuals with impaired glucose tolerance and, occasionally, diabetes have been included. Since even mild degrees of glucose intolerance are associated with impairments in insulin secretion and action,[28] it is not possible to ascertain from these studies whether the observed metabolic defects are an effect of LBW per se, or are secondary to hyperglycemia. More recently, it was shown that LBW is associated with low insulin secretory capacity in 19-year-old male Caucasian subjects.[29] Although, in that study subjects with LBW were shown to have significantly lower glycolytic flux, total glucose disposal was also lower but not statistically significant. Thus, whether the increased risk of diabetes in LBW individuals is attributable only

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to primary defects in insulin secretion or additionally decreased whole-body insulin sensitivity and increased endogenous glucose production (EGP) remains unclear. We, therefore, examined the relationship between birth weight and metabolic risk factors for diabetes in Pima Indians with normal glucose tolerance who had undergone detailed measures of body composition, EGP, insulin action, and insulin secretion. In a limited number of individuals who underwent biopsies of subcutaneous abdominal adipose tissue, in vitro data on adipocyte cell size and lipolysis were available.

MATERIALS AND METHODS

Subjects

Birth weight records were available on 230 Pima Indians with normal glucose tolerance according to the 1999 World Health Organization (WHO) diagnostic criteria[30] who had participated in a longitudinal study of the metabolic predictors of type 2 diabetes.[31] Where subjects had been seen on repeated occasions, data were analyzed from their first visit to the National Institutes of Health Clinical Research Unit in Phoenix. The age of the subjects was 25 ± 0.4 years (mean ± SE; range, 18 to 44) and the time period when the test were performed ranged from 1983 through 2001. Data on the age of diagnosis of diabetes in the parents of these subjects was obtained from epidemiologic studies of type 2 diabetes conducted since 1965 among the members of the Gila River Indian Community.[11] Status of parental diabetes was obtained from the date the study was performed in the offspring. As the median age in the offspring was 25 years (range, 18 to 44 years), most of the parents were in their mid 40s. Subjects known to be the offspring of a diabetic pregnancy (2-hour oral glucose tolerance test [OGTT]) were excluded from all analyses. All subjects provided written informed consent prior to participation in this study, which was approved by the National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board and the Tribal Council of the Gila River Indian Community. All subjects were in good general health as judged by a medical history, physical examination, and routine laboratory tests, and none took medications known to influence plasma glucose or insulin concentrations. Subjects were admitted to the Clinical Research Unit at Phoenix Indian Medical Center for 10 to 14 days and were fed a weight-maintaining diet for at least 3 days prior to metabolic testing.

Methods

Anthropometric measurements. Body mass index (BMI) was calculated as weight divided by height-squared (kg/m^2). Body density was measured by underwater weighing with simultaneous determination of residual lung volume by helium dilution, and percent body fat, fat mass, and fat-free mass were calculated as described[32] until January 1996. Thereafter, body composition was measured by total-body dual-energy x-ray absorptiometry (DEXA)[33] (DPX-L; Lunar Radiation Corp, Madison, WI). Measurements of body composition using the 2 different methods were made comparable using a conversion equation previously derived in our laboratory.[34] Waist circumference was measured at the umbilicus, while supine and thigh circumference was measured at the gluteal fold while standing. The waist-to-thigh ratio (WTR) was calculated as an index of body fat distribution.[31]

Oral glucose tolerance test. After a 12-hour overnight fast, subjects underwent a 3-hour 75-g OGTT. Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations prior to 1987 were determined by the Herbert modification[35] of the manual radioimmunoassay of Yalow and Berson,[35,36] prior to 1998 using an automated radioimmunoassay analyzer (Concept 4, Inc, Horsham, PA), and currently by an automated chemiluminescent assay (Access; Beckman Coulter, Fullerton, CA). The mean interassay coefficients of variation for plasma insulin concentrations were 7%, 12%, and 4% for the 3 methods. Insulin concentrations measured using the Concept 4 and Access methods were made comparable to the manual method by an algorithm established on the basis of 542 samples (Concept 4 *v* manual) and 250 samples (Access *v* Concept 4).

Intravenous glucose tolerance test. Insulin secretory responses to glucose were measured in response to a 25-g intravenous glucose bolus. The acute insulin response (AIR) was calculated as the mean increment in plasma insulin concentrations above basal in samples obtained 3, 4, and 5 minutes after the injection of glucose and was adjusted for the mean plasma glucose concentrations calculated from 3, 4, and 5 minutes.

Two-step hyperinsulinemic-euglycemic glucose clamp. Insulin action was assessed at physiologic and supraphysiologic insulin concentrations using a 2-step hyperinsulinemic-euglycemic glucose clamp.[31] After a 12-hour overnight fast, basal EGP was determined using a primed (30 μCi), continuous (0.3 μCi/min) 3-[^{3}H]-glucose infusion. A primed, continuous intravenous insulin infusion was then administered for 100 minutes at a final rate of 240 pmol/L per m^2 body surface area per minute (low dose), followed by a second 100-minute primed, continuous infusion at a rate of 2,400 pmol/L per m^2 per minute (high dose). These infusions achieved steady-state plasma insulin concentrations of 874 ± 18 pmol/L and 15,716 ± 428 pmol/L, respectively (mean ± SE). Plasma glucose concentrations were maintained at 5.5 mmol/L with a variable infusion of a 20% glucose solution. The rate of total insulin-stimulated glucose disposal (M) was calculated for the last 40 minutes of the low-dose (M-low) and high-dose (M-high) insulin infusion and adjusted to a steady-state glucose concentration of 5.5 mmol/L.[31] M-low was also adjusted for EGP measured during the low-dose insulin infusion and steady-state insulin concentrations as previously described.[31] EGP was assumed to be 0 during the high-dose insulin in infusion. All measurements derived from the glucose clamp were expressed per kilogram estimated metabolic body size (EMBS = fat-free mass + 17.7 kg).[37]

Fat biopsy and in vitro characterization of adipocytes. In a subgroup of 63 subjects (8 male/7 female LBW and 25 male/23 female normal birth weight [NBW]) data on average cell size and basal lipolysis of adipocytes obtained from subcutaneous abdominal adipose tissue biopsies were available. The procedures for fat biopsies and assessment of adipocyte size have been previously described in detail.[37-40] In brief, subcutaneous abominal adipose tissue was removed from the periumbilical region by either a surgical procedure or percutaneous needle biopsy. Immediately following the biopsy, fat specimens were fixed in 2% osmoic acid for 48 hours and then used to prepare a suspension of adipocytes in normal saline. Adipocyte size was measured electronically using a Coulter channelizer (model 2B; Coulter Electronics, Hialeah, FL) with a 400-μm aperture equipped with a logarithmic scale expander. Basal lipolysis of the isolated adipocytes was determined as previously described.[38-41]

Statistical Analyses

All data were analyzed using the procedures implemented in SAS (SAS Institute, Cary, NC). Fasting and 2-hour insulin concentrations, AIR, and M-low were $\log_{10}$-transformed to normalize their distribution prior to parametric analyses. In Pima Indians both low and high birth weight are associated with an increased risk of developing type 2 diabetes. As high birth weight is associated with maternal diabetes during pregnancy, the relationship of high birth weight with type 2 diabetes was not seen after adjustment for maternal diabetes during pregnancy and the relationship between birth weight and the risk of type 2 diabetes was linear.[7] In the present analyses we wanted to exclude a potentially confounding effect of the in utero environment on

Table 1. Physical Characteristics of LBW and NBW Groups

	LBW	NBW	P*
N (M/F)	58 (37/21)	172 (110/62)	.99
Birth weight (g)	2891 ± 33	3657 ± 28	
Age (yr)	27 ± 1	25 ± 0.4	.05
Height (cm)	166 ± 1	168 ± 1	.08
Weight (kg)	93 ± 3	92 ± 2	.89
Fat mass (kg)	31 ± 2	30 ± 1	.71
Fat-free mass (kg)	62 ± 2	63 ± 1	.45
BMI (kg/m^2)	34 ± 1	33 ± 1	.68
Body fat (%)	32 ± 1	31 ± 1	.86
WTR	1.66 ± 0.02	1.61 ± 0.01	.34

NOTE. Values are mean ± SE. Gender differences between the groups were determined in a chi-square test.

*After adjustments. Anthropometrical variables were adjusted for age and sex in multiple regression analyses.

the relationship between birth weight and metabolic characteristics. Therefore we excluded offspring of diabetic pregnancies. We show the analyses between birth weight and metabolic characteristics as a linear correlation, as well as after dividing birth weight into quartiles by comparing the lowest (LBW group) versus the upper 3 quartiles (NBW group). In generalized estimating equation regression models (PROC GENMOD) of the SAS procedure that account for nuclear family membership and thus allow analyses with all individuals in a sibship,[42] data were adjusted for age, sex, and degree of Pima Indian heritage and expressed as means (±SE). Metabolic variables were additionally adjusted for age, sex, percent body fat (glucose and insulin concentrations during the OGTT); and M-values were adjusted for age, sex, percent body fat, and WTR, and EGP additionally for M-low. In some individuals, EGP during the low-dose insulin clamp was zero (100% suppression of EGP). This creates a non-normal distribution of the data. We present these data, therefore, as a dichotomous trait, ie, individuals either suppressed EGP by 100% or not, as well as a quantitative variable. The AIR was adjusted for age, sex, percent body fat, and M-low. A P value less than .05 was considered significant.

RESULTS

Physical Characteristics

The physical characteristics of the LBW and NBW groups are summarized in Table 1. The cut-off birth weight between the lowest quartile and the upper three quartiles was 3,220 g for males and 3,068 g for females. Subjects in the LBW group were older and tended to be somewhat shorter than in the NBW group. Mean birth weight was 21% lower in the LBW group than in the NBW group. There were no significant differences in BMI, percent body fat, fat mass, or fat-free mass between the groups. The pattern of body fat distribution, indexed as the WTR, also did not differ between groups.

Parental Diabetes

As parental diabetes was shown to be a associated with onset of type 2 diabetes in the offspring, LBW, and low insulin secretory function in Pima Indians,[43,44] information on prevalence of parental diabetes in the 2 groups was included. Data on prevalence of parental diabetes were available from 201 subjects (51 LBW group and 150 NBW group). There was no difference in the prevalence of diabetes among the mothers (54 *v* 64%, LBW *v* NBW, $P = .21$, chi-square test). Among the fathers, prevalence of diabetes was higher in the LBW than the NBW group (62 *v* 40%, LBW *v* NBW, $P = .008$, chi-square test). The mean age of onset in mothers and fathers was similar in LBW and NBW groups (40 ± 1 *v* 40 ± 1 years, $P = .67$ in mothers and 44 ± 2 *v* 41 ± 1 years, $P = .54$ in fathers).

Glucose Tolerance

Fasting glucose and area under the curve for glucose during the OGTT, both adjusted for age, sex, and percent body fat, tended to be negatively correlated with birth weight ($r = -0.12$, $P = .07$ for fasting glucose and $r = -0.11$, $P = .09$ for area under the curve). Although all subjects had normal glucose tolerance by selection, the mean age and sex adjusted fasting plasma glucose concentration was higher (5.0 ± 0.06 *v* 4.8 ± 0.04 mmol/L, $P = .04$) in the LBW group than in the NBW group. This difference remained significant after adjustment for age, sex, and percent body fat ($P = .04$, Table 2). The area under the curve for plasma glucose, calculated from 0 to 180 minutes, was significantly higher in the LBW group than the NBW group (1,202 ± 25 *v* 1,143 ± 12 mmol/L, $P = .04$). This remained different after adjustment for age, sex, and percent body fat ($P = .07$, Fig 1A). There were no differences in 2-hour glucose concentrations and fasting or 2-hour insulin concentra-

Table 2. Metabolic Characteristics of LBW and NBW Groups

	LBW		NBW			
	Unadjusted	Adjusted	Unadjusted	Adjusted	P*	P†
Fasting glucose (mm)	5.0 ± 0.07	5.09 ± 0.06	4.81 ± 0.04	4.79 ± 0.03	.06	.04
2-h glucose (mmol/L)	6.0 ± 0.15	5.86 ± 0.14	6.10 ± 0.08	6.16 ± 0.08	.26	.42
Log_{10} fasting insulin (pmol/L)	1.56 ± 0.03	2.37 ± 0.02	1.52 ± 0.02	2.29 ± 0.01	.31	.39
Log_{10} 2-h insulin (pmol/L)	2.14 ± 0.05	2.94 ± 0.04	2.08 ± 0.02	2.85 ± 0.02	.27	.52
EGP_{basal} (mg/kg · $EMBS^{-1}$ · min^{-1})	1.92 ± 0.02	1.94 ± 0.02	1.89 ± 0.02	1.88 ± 0.02	.36	.19
$EGP_{insulin}$ (mg/kg · $EMBS^{-1}$ · min^{-1})	0.42 ± 0.05	0.50 ± 0.04	0.27 ± 0.02	0.24 ± 0.02	.003	.02
Log_{10}M-low (mg/kg · $EMBS^{-1}$ · min^{-1})	0.39 ± 0.02	0.37 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	.05	.04
M-high (mg/kg · $EMBS^{-1}$ · min^{-1})	8.67 ± 0.22	8.29 ± 0.22	9.28 ± 0.16	9.40 ± 0.15	.01	.05
Log_{10}AIR (pmol/L)	3.12 ± 0.04	3.08 ± 0.04	3.15 ± 0.02	3.16 ± 0.02	.06	.02

NOTE. Values are mean ± SE.

*Before adjustments; †after adjustments in multiple regression analyses. Glucose and insulin concentrations were adjusted for age, sex and % body fat. M-values were adjusted for age, sex, percent body fat, and WTR. EGP and AIR were adjusted for age, sex, percentage body fat, and M-low. (EMBS = fat-free mass + 17.7 kg).

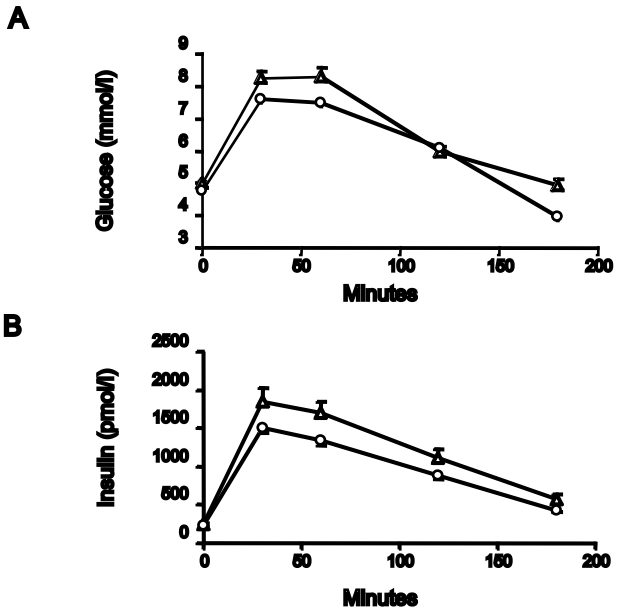

Fig 1. (A) Plasma glucose and (B) plasma insulin concentrations during an OGTT in the whole study population (▲, LBW group; ○, NBW group).

tions during the OGTT or in the area under the curve for plasma insulin concentrations (all $P > .51$, Fig 1B).

Insulin Action

Insulin action at physiological insulin concentrations adjusted for age, sex, percent body fat, and WTR was not ($r = 0.06$, $P = .38$) but insulin action at supraphysiological insulin concentrations was positively correlated with birth weight ($r = 0.12$, $P = .06$). Insulin action at both physiological and supraphysiological insulin concentrations during the 2-step hyperinsulinemic-euglycemic glucose clamp was lower (2.59 ± 0.13 and 8.67 ± 0.22 mg/kg · EMBS^{-1} · min^{-1} and 2.90 ± 0.1 and 9.28 ± 0.16 mg/kg · EMBS^{-1} · min^{-1} [$P = .05$, M-low, and $P = .01$, M-high]) in the LBW than in the NBW group. Insulin action was also lower in the LBW compared to the NBW group after adjustment for age, sex, percent body fat, and WTR ($P = .04$, M-low and $P = .05$, M-high, Table 2 and Fig 2).

Endogenous Glucose Production

EGP in the basal state adjusted for age, sex, percent body fat, and M-low was not ($r = 0.001$, $P = .98$) but EGP during the clamp was negatively correlated with birth weight ($r = -0.18$, $P = .006$). Basal EGP was not different between the groups either before (1.92 ± 0.02 *v* 1.90 ± 0.02 mg/kg · EMBS^{-1} · min^{-1}, $P = .36$, LBW *v* NBW) or after adjustments for age, sex, percent body fat, and M-low ($P = .19$). EGP during the clamp was higher in the LBW than in the NBW group (0.42 ± 0.05 *v* 0.28 ± 0.02 mg/kg · EMBS^{-1} · min^{-1}, $P = .003$). This difference remained significant after adjustment for age, sex, percent body fat, and M-low ($P = .02$, Table 2 and Fig 3). Because of the skewed distribution of EGP during the clamp, we categorized subjects into suppressors and nonsuppressors according to whether EGP was completely suppressed or not. There were fewer suppressors in the LBW than in the NBW group (24% *v* 38%, $P = .06$, chi-square test); however, this difference was not statistically significant.

Acute Insulin Secretory Response

AIR adjusted for age, sex, percent body fat, and M-low was not associated with birth weight ($r = 0.1$, $P = .13$). AIR was

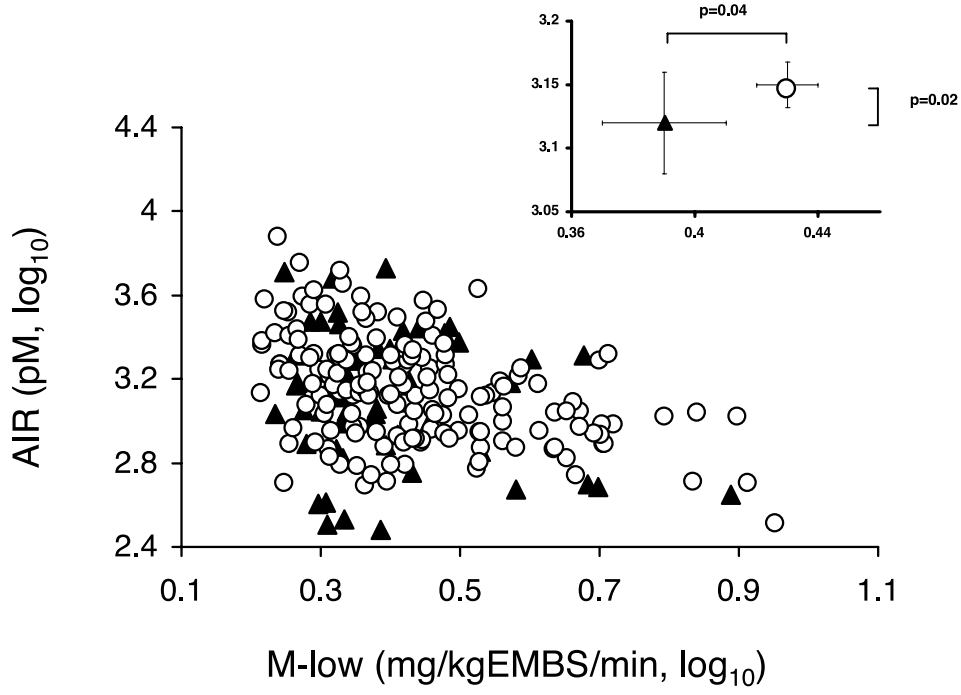

Fig 2. Relationship between M-low and AIR for subjects with LBW (▲) and normal birth weight (○). (Insert) Means ± SE of the 2 groups on a magnified scale. *P* values indicate statistical differences after adjusting for age, sex, % body, and WTR (M-low) and age, sex, % body fat and M-low (AIR). EMBS = [estimated metabolic body size] = fat-free mass + 17.7 kg.

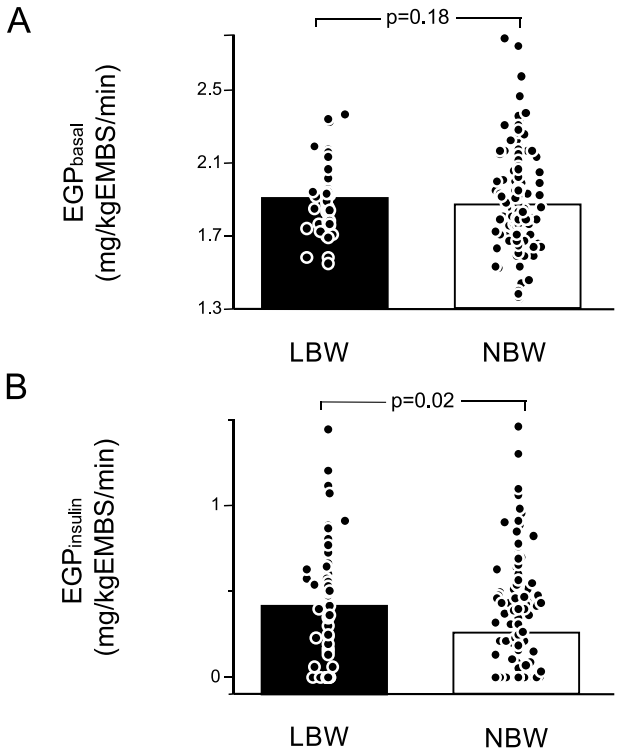

Fig 3. EGP in the basal state (EGP_{basal}, A) and during insulin infusion ($EGP_{insulin}$, B) for subjects with LBW and NBW. *P* values indicate statistical differences after adjusting for age, sex, % body fat, and M-low. EMBS = [estimated metabolic body size] = fat-free mass + 17.7 kg.

not different in the LBW group (1,653 ± 149 pmol/L) compared to the NBW group (1,655 ± 81 pmol/L, $P = .06$). However, AIR was significantly lower in the LBW group than in the NBW group after adjustment for age, sex, percent body fat, and M-low ($P = .02$, Table 2 and Fig 2), suggesting that AIR did not increase appropriately for the decrease in M in the LBW group.

Analyses by Gender

After analyzing males and females separately (147 males and 83 females), the results were similar to the data presented. Insulin action at supraphysiological insulin concentrations ($P = .02$ for males and $P = .05$ for females) and acute insulin secretory response ($P = .02$ for males and $P < .0001$ for females) were significantly lower in the LBW than in the NBW groups for both genders.

Results for Selected Metabolic Characteristics for All Quartiles of Birth Weight

Whole-body insulin sensitivity, EGP, and AIR for subjects divided in quartiles by birth weight are presented in Table 3.

Average Cell Size and Basal Lipolysis of Subcutaneous Abdominal Adipocytes

Among the 63 subjects who underwent fat biopsies, after adjustment for age, sex, and percent body fat, average size of subcutaneous adipocytes was not associated ($r = -0.10$, $P = .42$) and basal lipolysis was negatively associated with birth weight ($r = -0.21$, $P = .1$). Average cell size was higher in the LBW group than in the NBW group (0.89 ± 0.05 *v* 0.78 ± 0.03 $\mu g \cdot cell^{-1}$, $P = .08$). Basal lipolysis was higher in the LBW group than in the NBW group (0.16 ± 0.03 *v* 0.12 ± 0.01 $fmol \cdot cell^{-1} \cdot min^{-1}$, $P = .02$). In this subgroup, as shown for the large group, M-low was lower in the LBW group than in the NBW group (2.18 ± 0.06 *v* 2.68 ± 0.11 $mg/kg \cdot EMBS^{-1} \cdot min^{-1}$, $P = .002$) after adjustment for age, sex, percent body fat, and WTR.

DISCUSSION

An association between LBW and increased risk of type 2 diabetes has been observed in a large number of populations worldwide,[1-10] including the Pima Indians of Arizona.[7] However, the metabolic abnormalities predisposing LBW individuals to diabetes have remained unclear, in part, due to a paucity of comprehensive studies. In the present study, body composition, insulin action, insulin secretion, and EGP were directly measured in a large number of subjects with normal glucose tolerance. In this group, fasting plasma glucose concentrations and EGP during insulin infusion were higher in the LBW group than in the NBW group. Insulin action, directly measured at physiological and supraphysiological levels of hyperinsulinemia during a 2-step euglycemic clamp, was lower in the LBW group than in the NBW group. Additionally, the acute insulin secretory response to intravenous glucose, relative to insulin

Table 3. Whole-Body Insulin Sensitivity, EGP, and AIR for Quartiles of Birth Weight

	1st Quartile	2nd Quartile	3rd Quartile	4th Quartile	ANOVA
EGP_{basal} ($mg/kg \cdot EMBS^{-1} \cdot min^{-1}$)	1.94 ± 0.02	1.90 ± 0.03	1.88 ± 0.03	1.88 ± 0.03	.4
$EGP_{insulin}$ ($mg/kg \cdot EMBS^{-1} \cdot min^{-1}$)	0.50 ± 0.04	0.36 ± 0.04	0.25 ± 0.03	0.12 ± 0.03	<.0001*
Log_{10} M-low ($mg/kg \cdot EMBS^{-1} \cdot min^{-1}$)	0.37 ± 0.01	0.43 ± 0.02	0.49 ± 0.02	0.42 ± 0.02	<.0001*
M-high ($mg/kg \cdot EMBS^{-1} \cdot min^{-1}$)	8.29 ± 0.22	8.93 ± 0.24	9.80 ± 0.28	9.54 ± 0.26	<.0001†
Log_{10} AIR (pmol/L)	3.08 ± 0.04	3.15 ± 0.03	3.19 ± 0.03	3.13 ± 0.03	.08‡

NOTE. Values are mean ± SE after adjustment in multiple regression analyses. M-values were adjusted for age, sex, percentage body fat, and WTR. EGP and AIR were adjusted for age, sex, percentage body fat, and M-low. (EMBS [estimated metabolic body size] = fat-free mass + 17.7 kg). *P* values determined by Duncan's post-hoc test.

Abbreviation: ANOVA, analysis of variance.

*$P < .05$, 1st quartile *v.* 2nd through 4th quartiles.

†$P < .05$, 1st quartile *v.* 3rd and 4th quartiles.

‡$P < .05$, 1st quartile *v.* 3rd quartile. (Duncan's post-hoc test).

resistance, was lower in LBW individuals than in those with NBW. These results suggest that an impairment in insulin secretion, together with an impairment in whole-body and hepatic insulin action, predisposes Pima Indians with LBW to the development of type 2 diabetes.

Several reports have suggested an association between LBW and insulin resistance. A number of studies, including previous investigations in Pima Indians, have reported a negative correlation between fasting insulin concentrations, a widely used surrogate marker of insulin resistance, and birth weight.[3,23,26,27] A limited number of studies employing more direct measures of insulin action such as the insulin tolerance test,[24] the intravenous glucose tolerance with minimal modeling,[23,24,45] and the hyperinsulinemic glucose clamp,[4,25] have also demonstrated that LBW is associated with insulin resistance. Potential sites responsible for this observation may include muscle, liver, and fat. We cannot provide information on in vitro data for mechanisms in muscle and liver. Nevertheless, we were able to analyze data from fat biopsies collected in previous studies, which may not necessarily be representative for the entire Pima population but may contribute to understand the mechanistic link between LBW and insulin resistance. Individuals in the low quartile of birth weight tended to have larger cell size and had significantly higher basal lipolysis and lower insulin action at physiological insulin concentrations than individuals in the higher birth weight quartiles. It remains open, of course, whether this simply represents another manifestation of insulin resistance or is of any primary significance. Nevertheless, at this point, adipose tissue cannot be discounted as a site of metabolic abnormalities associated with LBW.

The mechanism underlying the association between LBW and impaired early insulin secretion remains to be determined. Animal models suggest that fetal malnutrition during critical periods of development may lead to a defect in insulin secretory function in later life.[12] Experimental protein restriction during the last week of pregnancy in rats is associated with abnormalities in β-cell mass and insulin content in the offspring, causing defects in insulin secretion and impaired glucose tolerance.[13] In humans, the hypothesis of LBW being associated with abnormalities in pancreatic β-cell development has been investigated. Intrauterine growth retardation has been associated with reduction of fetal endocrine pancreatic tissue and of the insulin-producing β cells.[15] More recently, however, applying computer-assisted quantitative morphometry of anti-insulin antibody–stained pancreatic tissue, no difference in β-cell mass or insulin content was found.[14] Histological quantification of β-cell mass, however, may not necessarily provide complete information on insulin secretory capacity. Recently, an alternative explanation for the association of LBW and diabetes has been proposed. The "fetal insulin hypothesis" suggests that a low insulin effect during fetal development (as a consequence of a genetic abnormality in insulin secretion and/or insulin action) retards in utero development, leading to LBW.[46] This same genetic defect also increases risk for the subsequent development of diabetes. Indeed, in animal models and in humans, mutations in the glucokinase gene that cause impaired insulin secretion and the development of maturity onset diabetes of the young are associated with LBW.[47,48] Similarly, the *INS* VNTR locus, which is known to regulate insulin gene transcription, is associated with birth weight.[49] In Pima Indians, a polymorphism in tight linkage disequilibrium with *INS-VNTR* was shown to be associated with LBW, but not with type 2 diabetes in family-based analyses.[50] Although mutations in the glucokinase gene are also not associated with diabetes in the Pimas,[51] there is ample evidence that both insulin secretion and insulin action are familial traits in this population.[52] Thus, the association of LBW and low acute insulin secretion may reflect a common genetic basis for these traits in the Pimas. Parenthetically, in a larger group of Pima Indians paternal diabetes was associated with LBW.[43] The same association was observed in this subset. This supports the hypothesis that genetic factors contribute to LBW and the concomitantly increased risk of type 2 diabetes, possibly via paternal transmission. Clearly, nongenetic factors operating through the intrauterine environment such as intrauterine nutrition[53,54] in parallel affecting birth weight and diabetes-relevant traits cannot be excluded.

In addition to manifesting an impairment in early insulin secretion, Pima Indians with LBW had higher plasma glucose concentrations in the fasting state, during the OGTT. Moreover, they had a decreased suppression of EGP rates during insulin infusion than those with NBW. Differences in EGP and, therefore, fasting glucose concentrations, between LBW and NBW individuals could result from differences in the metabolic programming of the liver during critical periods of intrauterine development. Indeed, small-for-gestational-age infants manifest a number of abnormalities in hepatic metabolism[55] and persistent changes in hepatic enzymes associated with glucose metabolism can be demonstrated in the offspring of protein-restricted rats.[56]

Others have reported higher fasting glucose concentrations in subjects with LBW.[57] Recently, in Caucasian males lower EGP during insulin infusion, but not in the basal state was observed in subjects with LBW compared to controls.[29] This was interpreted as compensatory enhanced effectiveness of glucose to suppress EGP, thus ensuring glucose tolerance. In Pima Indians we found the opposite. This may be partially explained by the higher degree of obesity in our study population and, therefore, increased hepatic insulin resistance that may override this protective mechanism operative in lean individuals. This hypothesis is supported by the original findings by Hales et al of a higher risk of diabetes in the LBW/obese versus the LBW/lean adult group.[1] It is also of note that part of the differences between the 2 studies in calculation of EGP may be explained by the different methods that were used. In the study mentioned above the so-called "hot GINF" method was used in contrast to the present report. With this method, a tracer (eg, 3-[^{3}H]-glucose) is coinfused with the glucose during the clamp. This results in a better mixing of endogenous and exogenous glucose and is considered to reduce the possibility of an underestimation of EGP.

It is undisputed that one of the main determinants of whole-body insulin resistance, EGP, and insulin secretory capacity in Pima Indians is obesity. Nevertheless, for a given adiposity there is a broad range of variability in these parameters that can be explained by the individual's size at birth. Therefore, we consider the differences presented here important and indicative of a link between LBW and type 2 diabetes.

It is of note that subjects in the LBW group were 2 years older on average compared to those in the NBW group. Because insulin sensitivity decreases with increasing age,[58] this might explain some of the differences seen between the groups. However, it was shown that the age-related decrease in insulin-stimulated glucose disposal[59,60] and EGP[60] is a function of body composition. For insulin secretory function, age clearly plays an important role.[61] Although we adjusted for age in our analyses, we cannot completely rule out that some of the differences in the metabolic characteristics between adult Pima Indians with LBW and NBW in our study are a function of differences in age.

In summary, in Pima Indians LBW is associated with less suppression of EGP by insulin, impairment in whole-body insulin action, and decreased insulin secretion relative to insulin resistance. Since these defects are evident in individuals with normal glucose tolerance, all of these defects may contribute to increased susceptibility to type 2 diabetes mellitus among individuals with LBW. Whether these abnormalities are genetic or acquired remains to be determined.

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