Metabolic Factors Contributing to Increased Resting Metabolic Rate and Decreased Insulin-Induced Thermogenesis During the Development of Type 2 Diabetes

Christian Weyer, Clifton Bogardus, and Richard E. Pratley

Previous studies have indicated that individuals with type 2 diabetes have an increased resting metabolic rate (RMR) but decreased insulin-induced thermogenesis (IIT) compared with those with normal glucose tolerance (NGT). When and by which mechanisms these abnormalities occur during the development of diabetes remain unknown. In 560 Pima Indians, sleeping metabolic rate (respiratory chamber) was higher not only in subjects with diabetes (+4.9%, $P < 0.001$) but also in those with impaired glucose tolerance (IGT) (+2.7%, $P < 0.01$) compared with subjects with NGT. Longitudinally, RMR (ventilated hood) increased progressively in 17 subjects in whom glucose tolerance deteriorated from NGT to IGT (+4.2%) to diabetes (+2.6%) over 5.1 ± 1.4 years ($P < 0.05$ for trend). In parallel, IIT (% increase in metabolic rate during an insulin/glucose infusion) decreased during the transition from NGT (11.7%) to IGT (7.3%) to diabetes (6.5%) ($P < 0.05$ for trend). In 151 subjects, basal endogenous glucose output (3-^{3}H-glucose), fasting insulin and free fatty acid concentrations, and glucose disposal (hyperinsulinemic clamp) were significant determinants of RMR, independent of body composition, age, and sex. Nonoxidative and oxidative glucose disposal, RMR, and fasting insulin and glucose concentrations were determinants of IIT. Differences in RMR and IIT between glucose tolerance groups decreased after adjusting for these factors. These findings indicate that increases in RMR and decreases in IIT occur early in the development of type 2 diabetes, and that both changes are related to the progressive metabolic abnormalities that occur during the development of the disease. *Diabetes* 48:1607–1614, 1999

From the Clinical Diabetes and Nutrition Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona.

Address correspondence to Christian Weyer, MD, Clinical Diabetes and Nutrition Section, National Institutes of Health, 4212 N. 16th St., Room 5-41, Phoenix, AZ 85016. E-mail:cweyer@phx.niddk.nih.gov.

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EGO, endogenous glucose output; EMBS, estimated metabolic body size; FFA, free fatty acid; FFM, fat-free mass; FM, fat mass; IGT, impaired glucose tolerance; IIT, insulin-induced thermogenesis; INS, fasting plasma insulin; NGT, normal glucose tolerance; NOGD, nonoxidative glucose disposal; OGD, oxidative glucose disposal; PA-EE, physical-activity–related energy expenditure; RMR, resting metabolic rate; SMR, sleeping metabolic rate; SNS, sympathetic nervous system; SPA, spontaneous physical activity; 24-EE, 24-h energy expenditure; UCP, uncoupling protein.

Several cross-sectional studies have indicated that individuals with type 2 diabetes have a higher mean resting metabolic rate (RMR) (1–3) but a decreased thermic response to food intake (4,5) and/or insulin/glucose infusions (insulin-induced thermogenesis [IIT]) (6–10) than do individuals with normal glucose tolerance (NGT). It is unknown when and by which mechanisms these abnormalities occur during the development of type 2 diabetes.

The increased RMR in individuals with type 2 diabetes could be a late abnormality, secondary to their metabolic deterioration, as suggested by the observation that patients with poorly controlled type 1 diabetes also have an increased RMR (11). However, Nair et al. (3) reported that measured RMR was higher in individuals with impaired glucose tolerance (IGT) than was predicted on the basis of their age, sex, and body composition, suggesting that an increased RMR may precede the development of overt hyperglycemia. A reduced thermic response to food and insulin/glucose infusions has been reported in several clinical conditions often associated with IGT, such as obesity (12–14), pregnancy (15), and previous gestational diabetes (4). Moreover, insulin resistance, a common prediabetic abnormality, is associated with an impaired thermic effect of food independent of obesity (5). However, Golay et al. (6,16) found no differences in glucose-induced thermogenesis between individuals with IGT and NGT. To date, there have been no direct comparisons of sedentary energy expenditure in subjects with NGT, IGT, and diabetes and no longitudinal studies in which RMR and IIT were measured sequentially throughout the progression from NGT to IGT to diabetes.

The physiological mechanisms responsible for the increased RMR and decreased IIT in individuals with type 2 diabetes are also poorly understood. Several mechanisms have been proposed to explain the increased RMR in type 2 diabetes, including increases in protein turnover (17), futile substrate cycling (18), gluconeogenesis (19), plasma glucagon (20), and sympathetic nervous system (SNS) activity (1). Similarly, various explanations have been proposed to explain the reduced IIT in type 2 diabetes, including defects in both "obligatory" (energy cost of glucose storage) and "facultative" (SNS activity, protein synthesis, etc.) thermogenesis (21–24).

To determine the time course of the changes in RMR and IIT during the development of diabetes and possible under-

TABLE 1
Physical characteristics of 560 Pima Indians with NGT, IGT, or diabetes, in whom energy expenditure was measured in a respiratory chamber

	NGT	IGT	Diabetes
n	365	127	68
Males/females	249/116	56/71	31/37
Age (years)	28 ± 8*	31 ± 9†	35 ± 12†
Body weight (kg)	92.2 ± 24.5*	100.1 ± 25.3†	107.3 ± 27.0‡
Body fat (%)	31 ± 9*	36 ± 8†	39 ± 7‡
FM (kg)	34.1 ± 16.8*	42.8 ± 17.3†	49.3 ± 18.6‡
FFM (kg)	58.1 ± 12.1*	57.3 ± 12.4†	58.0 ± 12.2‡
Waist-to-thigh ratio	1.63 ± 0.16*	1.69 ± 0.16†	1.82 ± 0.21‡

Data are *n* or means ± SD. Group comparisons are adjusted for age and sex. Values not sharing a common symbol (*,†,‡) are significantly different from each other ($P < 0.05$).

lying mechanisms, we analyzed data from cross-sectional and longitudinal studies in Pima Indians, a population with a high prevalence of IGT and type 2 diabetes (25,26). First, in a cross-sectional analysis, we compared sleeping metabolic rate (SMR), measured over several hours in a respiratory chamber, in 560 subjects with NGT, IGT, or diabetes, to test the notion that sedentary energy expenditure may be increased not only in individuals with diabetes but also in those with IGT. Second, we analyzed data from an ongoing longitudinal study (25,26) to determine when and to what extent changes in RMR and IIT occur during the deterioration of glucose tolerance from NGT to IGT to diabetes. Finally, we sought to identify physiological mechanisms underlying changes in RMR and IIT in a group of 151 individuals in whom RMR; IIT; fasting glucose, insulin, and free fatty acid (FFA) concentrations; endogenous glucose output (EGO); and insulin action (including glucose oxidation and storage) had been measured.

RESEARCH DESIGN AND METHODS

Subjects. Subjects in all three studies were Native Americans from the Pima tribe or the closely related Tohono O'Odham (Papago) tribe. They were healthy (apart from having diabetes, obesity, or both) according to the results of a physical examination and routine laboratory tests and not taking any medication. Before testing in all studies, subjects stayed at least 3 days on the metabolic ward of the Clinical Diabetes and Nutrition Section of the National Institutes of Health in Phoenix, AZ, where they abstained from strenuous exercise and were fed a weight-maintaining diet (50% carbohydrate, 30% fat, and 20% protein). After a 12-h overnight fast, glucose tolerance was assessed by a 75-g oral glucose tolerance test (27). For the respiratory chamber analysis, all Native American subjects between the ages of 18 and 70 years who had been studied in our respiratory chamber since 1984 were included. Subjects for the other two analyses ranged in age from 20 to 50 years and were selected from among the participants in an ongoing longitudinal study of the metabolic determinants of type 2 diabetes initiated in 1982 (25,26). The protocols were approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases and by the Tribal Council of the Gila River Indian Community, and all subjects provided written informed consent before participation.

Body composition was estimated by underwater weighing with simultaneous determination of residual lung volume by helium dilution (28) or by total-body dual energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) (29). Percent body fat, fat mass (FM), and fat-free mass (FFM) were calculated as described (30). A previously derived conversion equation (31) was used to make measurements comparable between the two methods. Waist and thigh circumferences were measured at the umbilicus and the gluteal fold in the supine and standing positions, respectively, and the waist-to-thigh ratio was calculated as an index of body fat distribution (32). All statistical analyses were performed using software of the SAS Institute (Cary, NC).

Methods

Respiratory chamber study. SMR, 24-h energy expenditure (24-EE), and physical-activity–related energy expenditure (PA-EE) were measured in 560 subjects (Table 1) in a human respiratory chamber as described (33). In brief, volunteers entered the chamber at 7:45 A.M. after an overnight fast and remained there until 7:00 A.M. the following morning. Subjects were fed an isocaloric diet according to previously determined equations (34), with meals provided at 8:00 A.M., 11:30 A.M., and 5:00 P.M. and a snack at 8:00 P.M. The rate of energy expenditure was measured continuously, calculated for each 15-min interval and then extrapolated to 24 h (24-EE) (33). Spontaneous physical activity (SPA) was detected by radar sensors and expressed as percent time over the 24-h period in which activity was detected. SMR was defined as the average energy expenditure of all 15-min periods between 11:30 P.M. and 5 A.M. during which SPA was <1.5% (33). PA-EE was calculated by multiplication of SPA values by the slope of the regression line of energy expenditure versus SPA (33). SMR, 24-EE, and PA-EE were compared among the NGT, IGT, and diabetes groups by general linear regression analyses with simultaneous adjustment for FFM, FM, age, and sex.

Longitudinal study. In the second study, RMR and IIT were longitudinally assessed in 17 subjects (Table 2) in whom glucose tolerance deteriorated from NGT to IGT to diabetes over a mean interval of 5.1 ± 1.4 years (25). Individuals

TABLE 2
Anthropometric changes in 17 Pima Indians in whom glucose tolerance deteriorated from NGT to IGT to diabetes over 5.1 ± 1.4 years

	NGT	IGT	Diabetes	*P*
Time intervals (years)		1.8 ± 0.8	3.3 ± 1.4	
Body weight (kg)	93.7 ± 17.0	99.3 ± 17.9*	106.9 ± 20.4*	<0.0001
Body fat (%)	38 ± 6	39 ± 6	41 ± 6†	<0.01
FM (kg)	36.3 ± 9.8	38.6 ± 10.0†	43.6 ± 11.1*	<0.0001
FFM (kg)	57.4 ± 10.9	60.7 ± 11.4*	63.3 ± 12.9†	<0.0001
Waist-to-thigh ratio	1.67 ± 0.16	1.73 ± 0.15†	1.76 ± 0.17	<0.01

Data are means ± SD. Symbols indicate significant changes between NGT and IGT and between IGT and diabetes (*$P < 0.001$; †$P < 0.05$). *P* values in the right column refer to the overall time effect.

TABLE 3
Physical characteristics of 151 Pima Indians with NGT, IGT, or diabetes, in whom determinants of RMR and IIT were assessed

	NGT	IGT	Diabetes
n	89	24	38
Males/females	56/33	9/15	11/27
Age (years)	24 ± 5*	28 ± 5†	28 ± 6†
Body weight (kg)	94.6 ± 24.6*	104.9 ± 27.4†	99.0 ± 22.3*
Body fat (%)	32 ± 9*	37 ± 8*	38 ± 6*
FM (kg)	31.5 ± 14.8*	39.8 ± 15.9†	37.9 ± 12.5*
FFM (kg)	63.1 ± 13.3*	65.1 ± 14.0†	61.1 ± 12.6*
Waist-to-thigh ratio	1.63 ± 0.16*	1.74 ± 0.15†	1.73 ± 0.13†

Data are *n* or means ± SD. Group comparisons are adjusted for age and sex. Values not sharing a common symbol (*,†) are significantly different from each other ($P < 0.05$).

who initially had NGT were recruited for this study and then restudied at approximately annual intervals (25,26). Of the 404 subjects enrolled since 1982, all subjects were included who had become diabetic and who had been studied at each stage during the progression from NGT to IGT, and to diabetes ($n = 17$) (25). Metabolic rate (MR) was measured at each visit by indirect calorimetry using a ventilated hood system, before (RMR) and throughout a two-step hyperinsulinemic glucose clamp (25,26). In brief, after an overnight fast, a primed continuous intravenous insulin infusion was administered for 100 min at a constant rate of 40 mU · m^{-2} body surface area · min^{-1} (low dose), followed by a second 100-min infusion at a rate of 400 mU · m^{-2} · min^{-1} (high dose). Total insulin-stimulated glucose disposal (M) was calculated for the last 40 min of the high-dose insulin infusion as described (25,26). Plasma glucose concentrations were maintained at basal levels with a variable infusion of 20% glucose. Indirect calorimetry was used to calculate rates of insulin-stimulated oxidative glucose disposal (OGD) and nonoxidative glucose disposal (NOGD) (25,26). RMR was measured for 40 min at baseline. During the clamp, MR was unchanged at the end of the low-dose insulin infusion, but was significantly ($P < 0.0001$) increased at the end of the high-dose insulin infusion. Therefore, IIT (% increase in MR above baseline) was calculated only for the high-dose insulin infusion. In 2 of the 17 subjects, IIT was not assessed at each of the three glucose tolerance stages because of technical problems. The obligatory component of IIT was estimated from the theoretical energy cost of storing glucose as glycogen (35). Accordingly, the rate of glucose storage (NOGD [g/min]) was multiplied by the energy content of glucose (3.75 kcal/g) and the molar ratio of ATP hydrolysis during glycogen synthesis (2/38 = 5.3%) (35). The facultative component of IIT was calculated as the difference between total and obligatory IIT. RMR and IIT were adjusted for FFM and FM. Changes in RMR and IIT over time were analyzed by repeated-measures analysis of variance.

Study of the determinants of RMR and IIT. From the same study (25,26), we selected all subjects with either NGT, IGT, or diabetes (Table 3) in whom measurements of insulin action, EGO, and fasting plasma concentrations of glucose, insulin, and FFA were available, in addition to measurements of RMR and IIT ($n = 151$). EGO was determined before the clamp using a primed (30 μCi), continuous (0.3 μCi/min) 3-^{3}H-glucose infusion (25,26). Values for M, OGD, NOGD, and EGO given in the text and tables are normalized to estimated metabolic body size (EMBS = FFM + 17.7 kg) (36), while absolute rates (mg/min) were used in the regression analyses (see below). Plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations were measured by radioimmunoassay, using either the Herbert modification (37) of the method of Yalow and Berson (38) or an automated analyzer (Concept 4; ICN, Costa Mesa, CA). Fasting plasma FFA concentrations were determined using a modification of the method of Soloni and Sardina (39).

Initially, RMR and IIT were compared among the three glucose tolerance groups after adjustment for age, sex, FFM, and FM only. Then, stepwise and multiple linear regression analyses were performed to identify further physiological determinants of RMR and IIT. The coefficients of determination (R^2) derived from the stepwise model represent the percentage of variance in RMR and IIT explained by each factor and by all factors together. All significant determinants of RMR and IIT were then entered into multiple linear regression models to compare the three groups after additional adjustment for the physiological covariates. These models were also used to obtain the residual variances in RMR and IIT that remained after adjustment for the covariates (root of the mean square error). The adjusted values of SMR, RMR, and IIT in the three studies are given as least-square means. Linear regression analysis was used to assess the relationship among RMR, IIT, and their determinants.

RESULTS

Respiratory chamber study. The physical characteristics of the 560 subjects are summarized in Table 1. Body weight, percent body fat, FM, FFM, and waist-to-thigh ratio (age- and sex-adjusted) were highest in the individuals with diabetes. After adjustment for age, sex, FFM, and FM, SMR was 4.9% higher in subjects with diabetes (+81 ± 22 kcal/d, $P < 0.001$) and 2.7% higher in subjects with IGT (+44 ± 16 kcal/d, $P < 0.01$) compared with those with NGT (Fig. 1). Adjusted 24-EE was 2.3% higher in subjects with diabetes (+53 ± 22 kcal/d, $P < 0.05$), but was not significantly higher in subjects with IGT (+18 ± 17 kcal/d), compared with those with NGT (2,309 kcal/d). Adjusted PA-EE was not different among subjects with NGT, IGT, and diabetes (375, 359, and 362 kcal/d, respectively).

Longitudinal study. In the 17 subjects (15 women, 2 men; age 26 ± 6 years) in whom glucose tolerance deteriorated from NGT to IGT to diabetes over 5.1 ± 1.4 years, body weight increased by 13.2 ± 7.3 kg (Table 2). The weight gain was due to an increase in both FM and FFM and was accompanied by significant increases in percent body fat and waist-to-thigh ratio (Table 2).

After adjustment for FFM and FM, RMR increased by 4.2% (74 ± 53 kcal/d) during the transition from NGT to IGT ($P < 0.06$) and by another 2.6% (47 ± 42 kcal/d) with further progression to diabetes ($P < 0.06$) ($P < 0.05$ for overall time effect) (Fig. 2).

IIT decreased during the development of diabetes ($P < 0.01$ for overall time effect). The major decrease in IIT

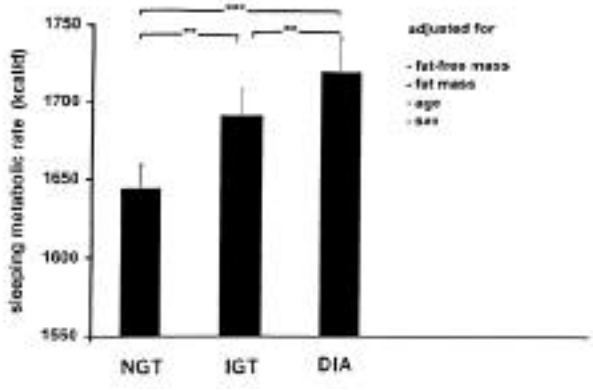

FIG. 1. SMR in 560 Pima Indian subjects with NGT, IGT, or diabetes (DIA). SMR is adjusted for age, sex, FFM, and FM (least-square mean ± SE). Asterisks indicate significant differences among the three groups ($P < 0.01$, ***$P < 0.001$).**

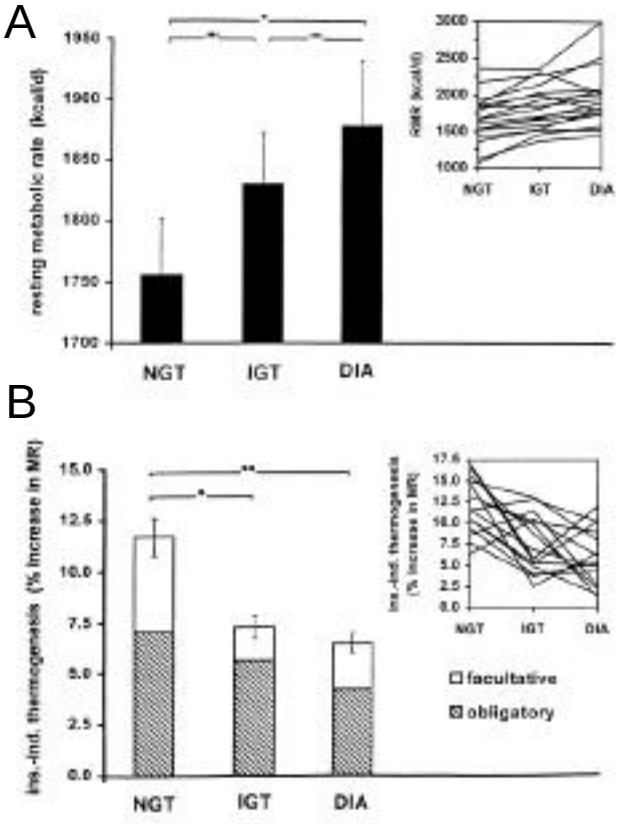

FIG. 2. Longitudinal changes in RMR (*A*) (*n* = 17) and IIT (*B*) (*n* = 15) in Pima Indians, in whom glucose tolerance deteriorated from NGT to IGT to diabetes (DIA) over 5.1 ± 1.4 years (25). RMR and IIT are adjusted for FFM and FM (least-square mean ± SE). The subcolumns of IIT represent changes in estimated obligatory and facultative thermogenesis, as calculated using the estimations of Flatt (35). Asterisks indicate significant changes among the three stages ((*)*P* = 0.06, **P* < 0.05, ***P* < 0.01). Individual results are given in the insets in the upper right corners. MR, metabolic rate.

occurred during the transition from NGT (11.7%) to IGT (7.3%) ($P < 0.05$), whereas the decrease in IIT with further progression from IGT to diabetes (6.5%) was not significant (Fig. 2). Insulin-stimulated NOGD decreased with development of diabetes (from 5.4 ± 1.0 [NGT] to 4.4 ± 1.5 [IGT] to 3.2 ± 1.2 [diabetes] mg · kg^{-1} EMBS · min^{-1}, $P < 0.0001$ for overall time effect). In parallel, the estimated increase in energy expenditure attributable to glucose storage (obligatory thermogenesis) decreased, from 7.1% (NGT) to 5.6% (IGT) to 4.2% (diabetes) (Fig. 2). The remaining proportion of IIT not explained by glucose storage (facultative thermogenesis) also decreased, from 4.6% (NGT) to 1.7% (IGT) to 2.3% (diabetes) (Fig. 2).

Determinants of RMR and IIT. The physical characteristics of the 151 subjects in this analysis are summarized in Table 3. After adjustment for age and sex, individuals with IGT had higher body weight, FM, and FFM than individuals with NGT and diabetes, while percent body fat was not significantly different among the three groups (Table 3).

RMR. After adjustment for age, sex, FFM, and FM, RMR was higher in subjects with diabetes than in those with NGT (+60 ± 31 kcal/d, $P < 0.05$) (Fig. 3). RMR in subjects with IGT tended to be higher than in subjects with NGT (+27 ± 26 kcal/d) and lower than in subjects with diabetes (–33 ± 33 kcal/d), but these differences did not reach statistical significance (Fig. 3). FFM, FM, age, and sex explained 79% of the variability (R^2) in RMR, and adjusting for these factors reduced the variance from 289 to 132 kcal/d. EGO, fasting plasma insulin (INS) and FFA concentrations, and insulin-stimulated glucose disposal (M) (Table 4) were all independent determinants ($P < 0.05$) of RMR, in addition to FFM, FM, and sex (age not significant): RMR = 595 +10.2 FFM (kg) + 2.8 FM (kg) + 168 (males) + 3.3 INS (μU/l)+1.2 EGO (mg/min) + 0.2 M (mg/min) + 0.2 FFA (μmol/l). The additional four factors explained another 3% of the variability in RMR (total R^2 = 82%), reducing the variance from 132 to 119 kcal/d. After adjusting for the four factors in addition to FFM, FM, and sex, there were no significant differences in RMR among the three groups (Fig. 3).

IIT. After adjusting for age, sex, FFM, and FM, IIT was higher in subjects with NGT compared with subjects with IGT and diabetes (Fig. 3). These factors explained only 8% of the variability (R^2) in IIT, and their inclusion in the model reduced the variance from 6.7 to 6.5%. Both NOGD and OGD as well as RMR and fasting insulin (INS) and fasting glucose (GLU) concentrations (Table 4) were independent determinants ($P < 0.05$) of IIT, in addition to FFM, FM, age, and sex: IIT = 29 – 0.2 age (years) + 3.3 (males) + 0.2 FFM (kg) + 0.1 FM (kg) + 0.02 NOGD (mg/min) + 0.05 OGD (mg/min) – 0.03 RMR (kcal/d) + 0.06 INS (μU/l) + 0.04 GLU (mg/dl).

The additional five factors explained another 53% of the variability in IIT (total R^2 = 61%), reducing the variance from 6.5 to 4.2%. As with RMR, there were no significant differences in IIT among the three groups after adjustment for the additional physiological factors (Fig. 3).

DISCUSSION

Previous studies have indicated an increased sedentary energy expenditure (1–3) but a decreased thermic effect of food (4,5) and/or glucose/insulin infusions (6–10) in individuals with type 2 diabetes compared with nondiabetic individuals. It has been unclear when and by which mechanisms these abnormalities occur during the development of type 2 diabetes. The results of the present series of cross-sectional and longitudinal studies reveal that increases in RMR and decreases in IIT occur early during the development of diabetes and appear to be related to the progressive metabolic abnormalities, independent of changes in body composition.

Sedentary energy expenditure. The results of our first study demonstrate that SMR, measured in a respiratory chamber, is increased not only in individuals with type 2 diabetes, but also in those with IGT, compared with subjects with NGT. The finding that sedentary energy expenditure is increased in otherwise healthy individuals with IGT has not, to our knowledge, been previously reported. Nair et al. (3) found that RMR in individuals with IGT was higher than predicted on the basis of their age and body composition. In their study, however, the small number of subjects precluded a direct comparison of individuals with IGT and NGT. Moreover, RMR was measured for only 45 min in the morning using a ventilated hood system. The respiratory chamber method used in the present study allows accurate measurement of sedentary energy expenditure over several hours during sleep (SMR), which is less affected by environmental influences than RMR. Using data collected systematically over several years, we were able to examine the effect of glucose tolerance on SMR in a large number of subjects covering a wide range of age and body size and to demonstrate significant

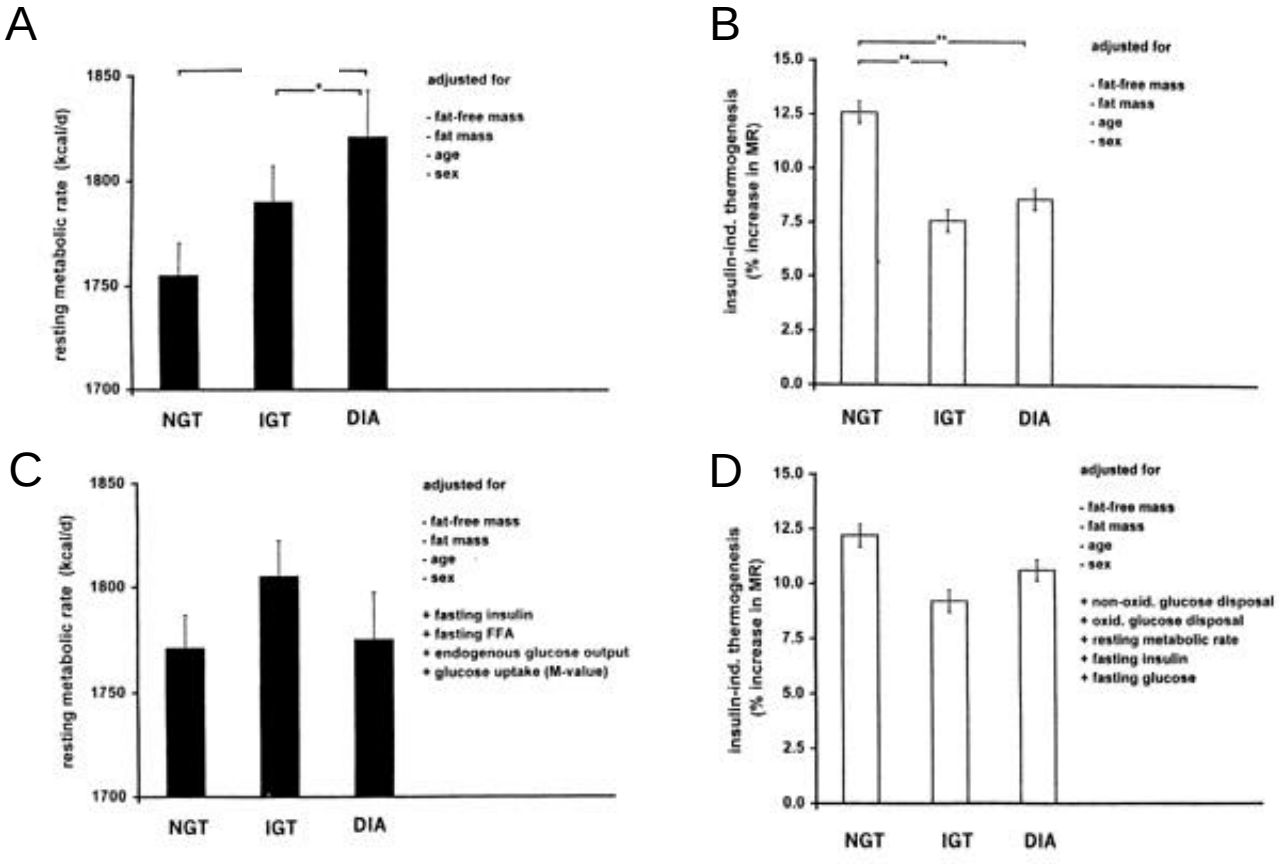

FIG. 3. RMR (*A* and *C*) and IIT (*B* and *D*) in 151 Pima Indian subjects with NGT, IGT, or diabetes (DIA). RMR and IIT (least square means ± SE) differed significantly (P* < 0.05, ***P* < 0.01) among the groups when adjusted for age, sex, FFM, and FM (*A* and *B*). These differences diminished (*C* and *D*) when RMR and IIT were additionally adjusted for the physiological determinants identified in multiple regression analyses (Table 4). MR, metabolic rate.**

($P < 0.01$), albeit small, differences in adjusted SMR between individuals with NGT versus IGT (+44 kcal/d) and with IGT versus diabetes (+37 kcal/d).

Our finding of increased SMR in individuals with IGT suggests that an increased sedentary energy expenditure may represent an early abnormality in the development of type 2 diabetes rather than a late consequence of poor metabolic control. It is evident, however, that the time course of the increase in RMR can be determined only in a longitudinal study in which subjects are followed throughout the development of diabetes. We therefore analyzed RMR measurements performed sequentially in 17 subjects in whom glucose tolerance deteriorated from NGT to IGT to diabetes over an average of 5 years (25). RMR increased by 4.2% during the transition from NGT to IGT, independent of simultaneous changes in body composition. Progression to diabetes was accompanied by a further 2.6% increase in RMR. These data confirm that sedentary energy expenditure increases early during the development of diabetes and indicate that this increase must be mediated by physiological factors other than changes in body composition.

An increased RMR has been demonstrated in several clinical syndromes commonly associated with IGT, such as cancer (40,41) and thermal injury (42). Much of the excess energy expenditure in these conditions can be attributed to disease-specific mechanisms such as tumor wasting and heat loss, respectively. Nevertheless, patients with these syndromes show many of the metabolic abnormalities typically found in otherwise healthy individuals with IGT and type 2 diabetes including hyperinsulinemia, increased EGO, and elevated fasting plasma FFA concentrations (40–42). We therefore examined whether these factors were related to RMR in healthy individuals. In multiple regression analyses, EGO, fasting insulin, and FFA (but not glucose) concentrations and insulin-stimulated glucose disposal were each significant determinants of RMR, independently of established determinants such as FFM, FM, and sex.

Of these four additional factors, EGO was quantitatively the most significant. Gluconeogenesis, the major source of the increased EGO in type 2 diabetes (43), is known to be an energy-consuming metabolic pathway (44). In an earlier study (1), SMR, measured in a respiratory chamber, was positively correlated with EGO in Pima Indians. Consoli et al. (43) calculated that in patients with type 2 diabetes, the energy cost of increased gluconeogenesis could account for more than half of the observed increase in metabolic rate. A higher EGO is a relatively late finding in the development of type 2 diabetes and is typically not evident until the transition from IGT to diabetes (25). The extent to which the energy cost of EGO contributes to increased RMR is, therefore, probably less in individuals with IGT than in those with diabetes. Other substrate cycles, such as the Cori cycle (45) and the glucose-alanine cycle (46), are also increased in type 2 diabetes and, thus,

TABLE 4
Determinants of RMR and IIT in 151 Pima Indians with NGT, IGT, or diabetes

	NGT	IGT	Diabetes	*r* (Pearson) RMR	*r* (Pearson) IIT
EGO (mg · kg^{-1} EMBS · min^{-1})	1.9 ± 0.2*	1.8 ± 0.2*	2.2 ± 0.5†	0.61§	—
Glucose disposal (mg · kg^{-1} EMBS · min^{-1})					
Total (M)	9.4 ± 2.0*	7.6 ± 2.0†	6.7 ± 1.4‡	0.23‖	—
NOGD	5.9 ± 1.6*	4.5 ± 1.5†	3.7 ± 1.3‡	—	0.49§
OGD	3.5 ± 0.7*	3.1 ± 0.6†	3.0 ± 0.5†	—	0.42§
RMR (kcal/d)	1,755 ± 277*	1,789 ± 319†	1,821 ± 302‡	—	–0.29§
Fasting glucose (mmol/l)	5.1 ± 0.4*	5.8 ± 0.3†	7.4 ± 2.3‡	—	–0.17¶
Fasting insulin (pmol/l)	204 ± 102*	300 ± 120†	378 ± 126‡	0.32§	–0.37§
Fasting FFA (μmol/l)	332 ± 96*	354 ± 131*	388 ± 106*	0.09	—

Data are means ± SD or *r*. Group comparisons are adjusted for age and sex. Values not sharing a common symbol (*,†,‡) are significantly different from each other ($P < 0.05$). The correlation coefficients (*r*) for the relationships between RMR, ITT, and their determinants are given in the two right columns (§$P < 0.001$; ‖$P < 0.01$; ¶$P < 0.05$). Note that the absolute rates (mg/min) of EGO, M, NOGD, and OGD were used in the correlation and regression analyses.

could contribute to the increase in RMR in diabetic individuals, but these pathways were not assessed in this study.

The association between RMR and fasting insulin concentration could be due, in part, to the postulated interaction between insulin and the activity of the SNS (1,47,48). Infusion of insulin stimulates both RMR (6–10, 23) and SNS activity (49), and patients with type 2 diabetes are commonly hyperinsulinemic. This led Daly and Landsberg (48) to hypothesize that increased insulin-mediated SNS activity may play an important role in the pathogenesis of hypertension in individuals with IGT and type 2 diabetes. It is possible that the same mechanism contributes to the increased RMR in these individuals. There is limited evidence that SNS activity is increased in individuals with early type 2 diabetes (50,51) and, in fact, the opposite is found in people with diabetes of long duration, particularly when complicated by diabetic neuropathy (52). In the present study, only patients with mild diabetes of short duration and no clinically apparent neuropathy were studied. Fasting insulin concentrations are commonly regarded as a surrogate marker of insulin resistance. In our multiple regression analysis, however, insulin action (M) was positively, not negatively, correlated with RMR, and this relationship was independent of fasting insulin concentrations.

FFAs are an important substrate for gluconeogenesis and could thereby contribute to increased RMR. The relation of FFA and RMR was independent of EGO in our multiple regression analysis, however, suggesting that other mechanisms are involved. In burn patients, futile FFA cycling is thought to be an important mechanism for increased energy expenditure (40). Thus, energy-consuming substrate cycles other than gluconeogenesis may mediate the relation between FFA and RMR. Alternatively, FFA may contribute to increased RMR via stimulation of mitochondrial uncoupling proteins (UCPs) (53,54). As early as 1976, Himms-Hagen (53) suggested that FFAs stimulate UCP-1. More recently, elevated FFA concentrations were reported to stimulate UCP-3 expression in rats (54).

IIT. In our cross-sectional analysis, IIT was markedly reduced in individuals with IGT and diabetes compared with those with NGT, suggesting that impaired IIT also represents an early abnormality in the natural history of diabetes. This was confirmed by our longitudinal data indicating that almost all of the decrease in IIT occurred during the transition from NGT to IGT, whereas only a marginal, nonsignificant decrease was evident during further progression from IGT to diabetes. This finding agrees with previous cross-sectional studies demonstrating reduced IIT and thermic effect of food in conditions frequently associated with IGT, such as obesity (12–14), previous gestational diabetes (4), or acromegaly (55). In the only other longitudinal study, Golay et al. (16) reported a decrease in glucose-induced thermogenesis over 6 years in obese individuals who initially had NGT or IGT. In their study, however, glucose tolerance remained unchanged in the majority of subjects, and none progressed from NGT to diabetes (16).

By assessing the rate of glucose storage simultaneously with the measurement of IIT, we were able to estimate the relative proportions of obligatory and facultative thermogenesis contributing to the observed decrease in IIT. The results indicate that the observed decrease in IIT during the development of IGT and type 2 diabetes is a consequence of declines in both obligatory and facultative thermogenesis. The decrease in obligatory thermogenesis could be anticipated since glucose storage is known to decrease with the development of diabetes (26). However, the decline in IIT cannot be explained solely by the decrease in obligatory thermogenesis, because the estimated proportion of facultative thermogenesis also decreased, particularly during transition from NGT to IGT.

In linear regression analyses, NOGD and OGD, RMR, and fasting glucose and insulin concentrations were independent determinants of IIT, in addition to FFM, FM, age, and sex. While NOGD (obligatory thermogenesis) alone explained 24% of the variance in IIT, another 29% was explained by the remaining four metabolic factors not attributable to obligatory thermogenesis and only 8% by FFM, FM, age, and sex.

RMR and OGD were quantitatively the most important determinants of IIT not attributable to obligatory thermogenesis. The negative correlation between RMR and IIT may simply be due to the fact that a greater relative (%) increase in MR can be expected when the baseline MR is low. On the other hand, the net increase in MR during the clamp (IIT) is a consequence of both the stimulatory effects of insulin on glucose storage and SNS activity and the inhibitory effects

of insulin on EGO and FFA. Among individuals with a higher RMR at baseline (in whom EGO and FFA are higher), the effects of insulin to suppress EGO and FFA will be relatively greater; hence, a smaller increase in MR (IIT) might occur. The positive correlation between glucose oxidation and IIT is explained by the biochemical nature of the process, since the measurement of MR by indirect calorimetry is based on the measurement of oxygen consumption and carbon dioxide production. Previous studies demonstrated that much of the thermic response to glucose/insulin infusion can be inhibited by β-adrenergic blockade, suggesting that facultative thermogenesis is, in part, mediated by SNS activity (56,57). This notion is supported by the fact that glucose-induced thermogenesis is positively correlated with heart-rate variability, a surrogate measure of SNS activity (58), and that the impaired thermic effect of food in obese individuals is associated with a reduced increase in urinary norepinephrine excretion after meals (13). In a previous study using the microneurography technique (59), we have shown that SNS activity increases after oral glucose ingestion in Pima Indians in proportion to the increase in insulin levels. Thus, SNS activity may contribute to the observed relation between IIT and both glucose oxidation and insulin concentrations.

The differences in RMR and IIT among the different glucose tolerance groups that were present after adjusting for age, sex, and body composition alone largely diminished after additional adjustment for the newly identified determinants. While this clearly demonstrates the physiological importance of these factors, further experimental studies are warranted to uncover the exact biochemical mechanisms underlying the abnormalities in RMR and IIT in individuals with IGT and diabetes.

A noteworthy finding was that the longitudinal changes in RMR and IIT were not uniform and that, for instance, IIT increased in 4 of the 15 subjects during the transition from NGT to IGT. Thus, as with other metabolic abnormalities, there appears to be significant interindividual variability in the changes in RMR and IIT during the development of diabetes. As changes in RMR and IIT during the development of diabetes may also vary among different ethnic groups, it is unknown whether our findings in Pima Indians are generalizable to other populations. However, the Pima Indians are prototypic with respect to most other metabolic abnormalities commonly associated with type 2 diabetes in other populations (25,26).

The clinical significance of increased RMR and decreased IIT in type 2 diabetes is a matter of debate. Some authors have proposed that reduced glucose-induced thermogenesis contributes to the development of obesity (60) and the propensity for relapse after weight loss (12), while others argue that in individuals with diabetes, this defect may be compensated for by the simultaneous increase in RMR (3). In support of the latter argument, 24-EE was only slightly increased in people with diabetes and not increased in those with IGT. Our findings of increasing RMR during the development of diabetes may thus be primarily of pathophysiological interest. However, we have previously shown that even a small difference in RMR (70 kcal/d) can have a significant impact on long-term body-weight regulation (61), and it is possible that even a slight increase in RMR contributes to a limitation of further weight gain after the onset of IGT and diabetes. Two intervention studies in individuals with type 2 diabetes have shown that increased RMR and a decreased thermogenic effect of food can be normalized by antidiabetic treatment with sulfonylureas or insulin. Our present results suggest that this may not be attributable to the lowering of glucose per se, since fasting glucose concentration was not a determinant of RMR and only a weak determinant of IIT. Instead, it must be assumed that part of this normalization of RMR and IIT was accounted for by a normalization of increased EGO, FFA, and insulin concentrations as well as improvements in insulin action and glucose storage that typically occur with improvement of metabolic control.

In conclusion, we have shown that the development of type 2 diabetes is accompanied by an increase in RMR and a decrease in IIT and that both abnormalities occur early, with the transition from NGT to IGT. These changes are not attributable solely to changes in body size and composition, but appear to be related to the progressive metabolic abnormalities that occur during the development of type 2 diabetes.

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