0021-972X/97/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 82, No. 2
Printed in U.S.A.

Glucose Metabolism in Identical Twins Discordant for Obesity. The Critical Role of Visceral Fat*

TAPANI RÖNNEMAA, MARKKU KOSKENVUO, JUKKA MARNIEMI, TERHI KOIVUNEN, ANTTI SAJANTILA, AILA RISSANEN, MERJA KAITSAARI, CLAUDE BOUCHARD, AND JAAKKO KAPRIO

Departments of Medicine (T.R.), Public Health (Ma.K.), and Diagnostic Radiology (T.K.), University of Turku, FIN-20520 Turku, Finland; Research and Development Centre (J.M., Me.K.), Social Insurance Institution, FIN-20720, Turku, Finland; Department of Human Molecular Genetics (A.J.), National Public Health Institute and Department of Forensic Medicine, University of Helsinki, FIN-00300 Helsinki, Finland; Department of Psychiatry, Helsinki University Central Hospital (A.R.), FIN-00180 Helsinki, Finland; Physical Activity Sciences Laboratory (C.B.), Laval University, Quebec G1K 7PH, Canada; and Department of Public Health (J.K.), University of Helsinki, FIN-00014 Helsinki, Finland

ABSTRACT

Obesity, especially intraabdominally deposited fatness, is associated with reduced insulin sensitivity. However, it is not well established whether this association is confounded by genetic factors. We studied 23 monozygous twin pairs (14 female, 9 male), 33–59 yr old, who had, on the average, 18 kg intrapair difference in body weight. A 75-g oral glucose tolerance test with glucose and insulin measurements at 30-min intervals was performed, and fat distribution was determined with magnetic resonance imaging. The pairs were divided into two groups by the gender-specific median of the abdominal visceral fat area (AVF) in the obese co-twins. In the high-AVF pairs, the mean area under curve (AUC) for glucose (mmol × min/L) was 758 *vs.* 968 ($P = 0.001$), AUC for insulin (mU × min/L) was 4320 *vs.* 8741 ($P = 0.001$), and insulin sensitivity index (mg × L × L/mmol × mU × min) was 71.5 *vs.* 45.9 ($P < 0.001$) in the lean and obese co-twins, respectively. In the low AVF pairs, the mean AUC for glucose was 669 *vs.* 706 (not significant), AUC for insulin was 3323 *vs.* 4241 (not significant), and the sensitivity index was 85.2 *vs.* 73.7 ($P = 0.04$) in the lean and obese co-twins, respectively. In subjects who are genetically identical but who are discordant for body mass, only those who differ most in visceral fat level are characterized by major alterations in insulin sensitivity and glucose tolerance. (*J Clin Endocrinol Metab* **82:** 383–387, 1997)

IT IS WELL established that obesity is an independent risk factor for noninsulin-dependent diabetes mellitus (1). Several studies in the 1980's suggested that distribution of body fat was an important determinant of the disturbing effect of obesity on glucose metabolism (2–4). It was shown that accumulation of intraabdominal fat was associated with reduced insulin sensitivity. However, some recent studies have proposed that sc fat accumulation (5), especially truncal sc fat (6), may be as harmful as, or even more disadvantageous for glucose metabolism, than intraabdominal fat deposition.

When comparing the glucose metabolism in unrelated obese and nonobese subjects, as has been the case in previous studies, differences in the genetic background of the subjects were not taken into account. However, genetic factors may be of importance, as approximately 50% of the variation in body fat distribution is genetically determined (7). One strategy to assess the independent impact of obesity on glucose metabolism without the confounding effects of genetic factors is to examine identical twins who are discordant for obesity. Therefore, we compared glucose metabolism in 23 identical nondiabetic twin pairs who had, on the average, a 7-kg/m^2 difference in body mass index (BMI). Special attention was paid to the distribution of body fat, as determined by magnetic resonance imaging.

Received June 20, 1996. Revision received October 25, 1996. Accepted October 31, 1996.

Address all correspondence and requests for reprints to: Dr. Tapani Rönnemaa, Department of Medicine, University of Turku, FIN-20520 Turku, Finland.

* This study was supported in part by a grant from the Academy of Finland.

Subjects and Methods

Subjects

The Finnish Twin Cohort includes all pairs (4307 monozygous, 9581 like-sexed dizygous pairs) of adult Finnish twins born before 1958 and alive in 1975 (8). Based on a postal questionnaire sent to the twins in 1990, identical twin pairs born between 1932 and 1957 and discordant for obesity were identified. Discordance for obesity was defined as a BMI difference of at least 4 kg/m^2 and, in addition, the BMI of the obese co-twin had to be more than 27 kg/m^2, and the BMI of the lean co-twin had to be less than 25 kg/m^2. Subjects with a history of thyroid disorders, psychiatric diseases, diabetes, major musculoskeletal problems, and other diseases, or taking medications (*e.g.* diuretics or β-blockers) possibly affecting glucose metabolism were excluded. All eligible twin pairs, based on a response letter, were invited to take part in the present study in 1992, provided that they still fulfilled the criteria. A total of 28 twin pairs were examined.

The physical examination revealed that two of the pairs had a BMI difference less than 3 kg/m^2, and they were excluded. Three pairs had a BMI difference between 3 and 4 kg/m^2, and they were included in the final study population. The obese co-twin of one pair was found to have previously undiagnosed overt diabetes mellitus, and this pair was excluded.

Zygosity of the twin pairs was originally based on a validated self-administered questionnaire (9). The monozygosity of the pairs of the present study was confirmed by dermatomatoglyphic analysis of fingertip prints (10, 11) by a highly experienced expert. All except six pairs were confirmed to be monozygotic. DNA samples of the six pairs with

uncertain zygosity were typed for markers at six different polymorphic gene loci (DIS80, APOB, D17S30, COL2A1, VWA, and HUMTH). Four of the pairs were found to be monozygotic, and the two other pairs were dizygotic; these two pairs were excluded.

Thus, the final study sample consisted of 23 nondiabetic identical twin pairs (14 female, 9 male) with more than 3 kg/m^2 difference in BMI and having no diseases or medications possibly affecting the results.

Methods

Glucose metabolism was assessed in a 2-h glucose (75 g) tolerance test with glucose and insulin measurements at 0, 30, 60, 90, and 120 min. Serum glucose was measured by the glucose dehydrogenase method (Merck Diagnostica, Darmstadt, Germany). Plasma insulin was measured by RIA (Pharmacia Diagnostics, Uppsala, Sweden). The antiserum of this kit is specific for insulin and does not cross-react with proinsulin or C-peptide (cross-reactivities $< 0.1\%$). An insulin sensitivity index was calculated according to the formula of Cederholm and Wibell (12). Serum free fatty acids (FFAs) were measured enzymatically after an overnight fast using acyl-CoA synthetase, acyl CoA oxidase, and peroxidase (Wako Chemicals, Neuss, Germany). Smoking was defined as regular smoking at the time of examination.

Percentage of body fat was determined using the so-called four-component method. The method is based on the division of body mass into four components with different densities: fat tissue 0.9007 g/cm^3), water (0.994 g/cm^3), minerals (3.042 g/cm^3), and proteins (1.34 g/cm^3). Water mass was estimated by bioelectric impedance method (BIA-101A/S, RJL System Inc., Clemens, MI). Mineral mass was estimated by dual-energy x-ray absorptiometry (Norland XR26, Norland Corporation, Fort Atkinson, WI). The density of the whole body was estimated by underwater weighing corrected for information on body water and mineral mass. The proportion of fat tissue was calculated from the density of the whole body according to the formula of Siri (13).

The distribution of body fat was measured by magnetic resonance imaging (MRI) (14). Imaging was performed at 0.1 tesla (Mega4^R, Instrumentarium Co., Imaging Division, Helsinki, Finland). Axial and sagittal localizers were used to obtain a transaxial T1-weighted image (relaxation time/echo time = 155/20, slice thickness 10 mm) at the level of the fourth lumbar vertebra. Visceral and sc fat areas were measured. MRI was not performed in three pairs, either because of claustrophobia or because of temporary malfunctioning of the MRI equipment. These three pairs were excluded from analyses concerning body fat distribution.

A paired *t* test was used to compare means and the McNemar's test to compare the proportions of obese and lean co-twins. Pearson correlation coefficients were calculated to quantify the association between intrapair differences in adiposity or its distribution and intrapair differences in variables of glucose metabolism. Multiple regression analyses were performed to analyze the independent contributions of visceral fat and body fat percentage to glucose metabolism. All statistical analyses were performed using Statistical Analysis System (SAS) statistical programs.

Results

The average difference in weight between the obese and lean co-twins was 16 kg among males and 19 kg among females (Table 1). In both genders, the abdominal visceral fat (AVF) area was twice as large in the obese co-twins as in the lean co-twins. The difference in sc fat area between obese and lean co-twins was greater among female than male pairs. Fasting serum glucose, area under curve (AUC) for glucose, fasting insulin, and AUC for insulin were significantly greater in obese co-twins compared with lean co-twins. Accordingly, the insulin sensitivity index was lower in the obese co-twins. Intrapair differences in indices of glucose metabolism were somewhat greater among males compared with females. Serum FFA concentrations were similar in obese and lean co-twins.

To characterize the specific impact of fat distribution on glucose metabolism, the twin pairs were divided according to the median value of AVF area of the obese co-twins (Fig. 1). Gender-specific median values were used for males and females, and results from both genders were combined. In both subgroups of AVF, the abdominal sc fat area was almost twice as large in the obese co-twins as in the nonobese co-twins. The intrapair differences in fasting glucose and insulin, AUC for glucose, and AUC for insulin, as well as the insulin sensitivity index between the obese and nonobese co-twins, were statistically highly significant among the high-AVF area pairs. In contrast, the corresponding intrapair differences were small or nonsignificant among the pairs with low AVF fat area. The proportion of smokers and serum FFA concentrations did not differ between obese and lean co-twins in either subgroup of AVF. When a division of twin pairs was made by the median value of waist to hip ratio of the obese co-twins, the results were essentially similar (data not shown) compared with results obtained using division of the pairs by AVF.

When the twin pairs were divided according to the gender-

TABLE 1. Characteristics and glucose metabolism of the co-twins (mean ± SE)

	Men (9 pairs)			Women (14 pairs)		
	Obese	P^a	Nonobese	Obese	P^a	Nonobese
Age (yr)	44 ± 2		44 ± 2	46 ± 2		46 ± 2
Height (cm)	176 ± 3	NS	175 ± 3	163 ± 2	NS	163 ± 2
Weight (kg)	88.9 ± 2.9	<0.001	72.9 ± 3.2	79.1 ± 1.7	<0.001	59.8 ± 1.6
BMI (kg/m^2)	28.8 ± 0.5	<0.001	23.7 ± 0.3	30.0 ± 0.9	<0.001	22.4 ± 0.4
No. of smokers	4/9	NS	7/9	3/14	NS	5/14
% Body fat	25.2 ± 1.1	0.02	20.8 ± 1.3	39.7 ± 1.4	<0.001	27.4 ± 1.5
AVF area (cm^2)	128 ± 24	0.003	57 ± 9	69 ± 10	<0.001	30 ± 4
ASF area (cm^2)	238 ± 22	<0.001	172 ± 19	303 ± 13	<0.001	156 ± 12
Fasting serum glucose (mmol/L)	5.20 ± 0.16	0.018	4.95 ± 0.15	5.03 ± 0.14	0.021	4.77 ± 0.08
AUC glucose (mmol × min/L)	915 ± 87	0.008	724 ± 60	803 ± 48	0.007	699 ± 30
Fasting plasma insulin (mU/L)	11.0 ± 1.5	0.002	6.1 ± 0.8	9.9 ± 1.4	0.025	6.4 ± 0.9
AUC insulin (mU × min/L)	6846 ± 1128	0.004	3302 ± 325	6226 ± 864	0.015	4156 ± 364
Insulin SI (mg × L × L/mmol × mU × min)	54.7 ± 7.9	<0.001	81.8 ± 7.6	61.0 ± 5.4	0.001	75.5 ± 4.5
FFA (mmol/L)	0.69 ± 0.07	NS	0.61 ± 0.11	0.88 ± 0.07	NS	0.84 ± 0.10

AUC, area under curve during an oral glucose tolerance test; SI, sensitivity index; AVF, abdominal visceral fat; ASF, abdominal subcutaneous fat.

[a] Significance of mean intra-pair difference (paired t-test, except for smoking McNemar's test).

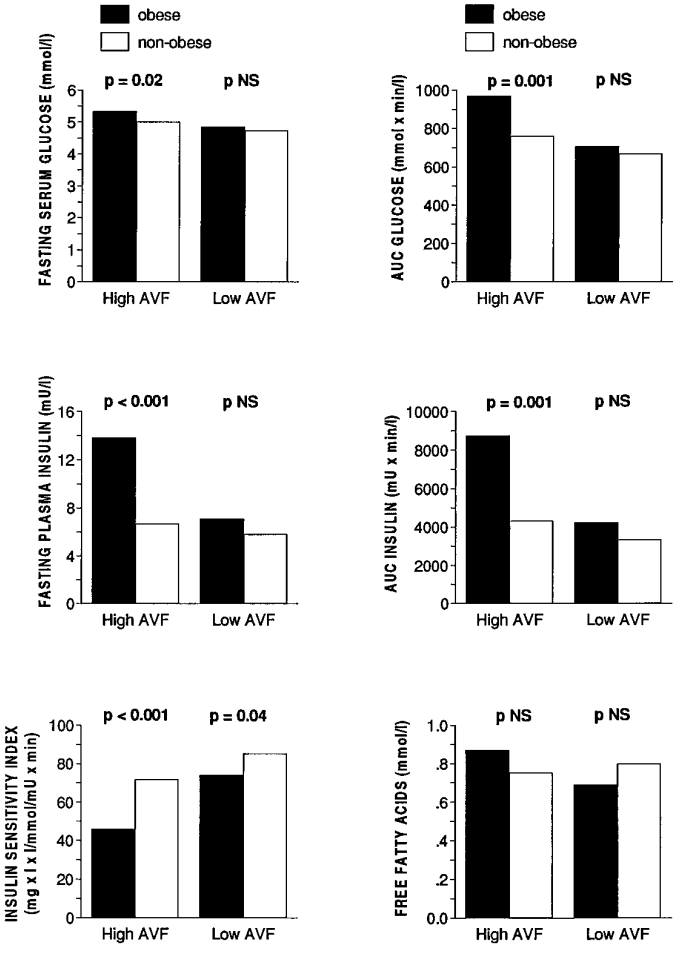

FIG. 1. Glucose metabolism in twins stratified by gender-specific median value of abdominal visceral fat (AVF) area in the obese co-twin. *Columns* represent mean values, genders are combined. Pair *t* test was used except for smoking McNemar's test. High AVF, above median; low AVF, below median value. In the high-AVF pairs, mean BMI was 30.2 and 22.5 kg/m^2 ($P < 0.001$), mean abdominal subcutaneous fat (ASF) area was 276 and 141 cm^2 ($P < 0.001$), mean AVF area was 127 and 49 cm^2 ($P < 0.001$), and number of smokers was 2/10 and 5/10 (P not significant) in the obese and lean co-twins, respectively. In the low AVF pairs, mean BMI was 27.9 and 23.2 kg/m^2 ($P < 0.001$), mean ASF area was 280 and 184 cm^2 ($P < 0.001$), mean AVF area was 58 and 32 cm^2 ($P = 0.001$), and number of smokers was 4/10 and 5/10 (P not significant) in the obese and lean co-twins, respectively.

specific median value of abdominal sc fat (ASF) area in the obese co-twins, the intrapair differences in glucose metabolism were similar among high- and low-ASF subgroups. For example, AUC of insulin was 6369 ± 1136 and 4022 ± 519 mU × min/L ($P = 0.019$) in obese and lean co-twins of the high-ASF subgroup, and 6592 ± 1108 and 3620 ± 340 mU × min/L ($P = 0.022$) in obese and lean co-twins of the low ASF subgroup, respectively.

To quantify the relationship between adiposity or its distribution with glucose metabolism, we correlated intrapair differences in adiposity variables to indicators of insulin sensitivity (Table 2, Fig. 2). Intrapair differences in BMI and percentage of body fat did not show any significant correlation to the metabolic variables. Intrapair differences in AVF area were those most significantly correlated to intrapair

TABLE 2. Pearson correlation coefficients between intrapair differences in glucose metabolism and intrapair differences in indices of obesity and its distribution. Genders are combined

	BMI	% body fat	AVF area	ASF area
Fasting serum glucose	0.23	0.08	0.37	−0.07
AUC glucose	0.36	0.10	0.72[a]	−0.15
Fasting plasma insulin	0.18	0.42	0.51[b]	0.19
AUC insulin	0.34	0.31	0.73[a]	0.05
Insulin sensitivity index	−0.23	−0.07	−0.57[c]	0.12
Free fatty acids	0.19	0.32	0.23	0.04

AVF, abdominal visceral fat; ASF, abdominal subcutaneous fat.
[a] $p < 0.001$.
[b] $p < 0.05$.
[c] $p < 0.01$.

differences in glucose and insulin AUCs and to intrapair difference in insulin sensitivity index. In contrast, intrapair differences in sc fat area showed no correlation to intrapair differences in indices of glucose metabolism. In multiple regression analyses, the association between intrapair differences in AVF area and differences in glucose metabolism remained significant when adjustment was made for differences in body fat percentage (Table 3). The whole model explained 31–50% of the variation in intrapair differences in glucose metabolism.

Discussion

Our results show that in identical twins discordant for obesity, the obese co-twins exhibit disturbed glucose metabolism compared with the leaner co-twins. However, despite a similar difference in BMI in subgroups of identical twins stratified according to indicators of body fat distribution, a clear-cut difference in insulin sensitivity between the obese and lean co-twins was observed only in the subgroup with a large amount of AVF. Accordingly, the intrapair difference in insulin sensitivity was inversely correlated to visceral fat but not with sc fat or overall adiposity. Our results are thus in accordance with earlier findings in study cohorts of subjects not related by nascency, showing the importance of visceral fat deposition in disturbed glucose metabolism (2, 4, 15, 16) but are in contrast to the results of a recent study in which the amount of truncal sc fat showed the strongest inverse correlation to insulin sensitivity (6). In the latter study, the population was racially heterogeneous. In our study, all subjects were Caucasians, and because obese and lean individuals were genetically identical, the association between visceral fat and insulin resistance is entirely a consequence of adipose mass differences, *i.e.* the degree of visceral obesity, and is not confounded by genetic influences.

Discordance for obesity in identical twins is a rare phenomenon. We found only 50 out of approximately 1500 twin pairs fulfilling our discordance criterion. Therefore, an important question is whether all twin pairs studied were really monozygous. We paid special attention to this by performing fingertip print analyses in all pairs and an analysis of alleles at six marker loci in those pairs in whom the identity could not be fully confirmed by fingertip print characteristics.

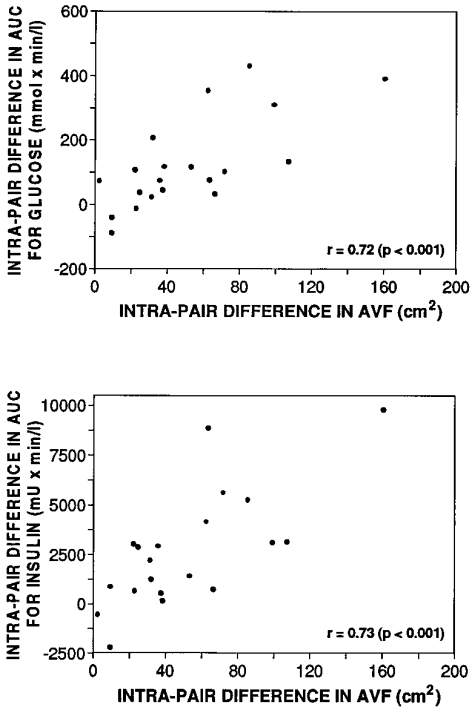

FIG. 2. Correlation between intrapair differences (obese *vs.* nonobese) in abdominal visceral fat (AVF) area and intrapair differences in AUC for glucose and insulin during an oral glucose tolerance test. Genders are combined.

Therefore, it is highly unlikely that any of the pairs studied would have been dizygous. Of course, we cannot exclude the possibility that postconception tissue-selective mutation potentially affecting adipose tissue could have occurred in some of the pairs.

To study glucose metabolism, we performed an oral glucose tolerance test with samples collected every 30 min for glucose and insulin measurements. As indicators of insulin sensitivity, we used fasting insulin, insulin area, and a sensitivity index calculated according to Cederholm & Wibell (12). Although we did not directly measure insulin sensitivity by the hyperinsulinemic-euglycemic clamp technique (17), our indicators, even fasting insulin, are well correlated with clamp results in nondiabetic subjects (18). Moreover, all indicators of insulin sensitivity in the present study exhibit consistent relationships with body fat and its distribution. We used MRI at the level of the fourth lumbar vertebra to assess body fat distribution. Results obtained with MRI correlate strongly with those obtained with computed tomography, and the reproducibility of the MRI method is good (19).

Although visceral fat accumulation itself seems to be responsible for the low insulin sensitivity in subjects with this type of obesity, other environmental factors could act as confounders. For example, smoking is related to insulin resistance (20), and it may also favor upper body fat accumulation (21). In our study, the proportion of smokers did not differ significantly between obese and nonobese co-twins when analyzed as whole groups or as subgroups with either visceral or sc fat accumulation. In fact, the number of smokers tended to be somewhat higher among lean co-twins, suggesting that the results were not confounded by smoking.

TABLE 3. Multiple regression analyses on the association between intrapair differences in glucose metabolism and intrapair differences in AVF area and percentage of body fat

Variable of glucose metabolism	Regression coefficient	*P*-value	R^2
AUC glucose			0.50
AVF area	2.57	0.002	
Percentage of body fat	−1.83	0.65	
Fasting insulin			0.47
AVF area	0.06	0.022	
Percentage of body fat	0.31	0.035	
AUC insulin			0.50
AVF area	54.4	<0.001	
Percentage of body fat	124.1	0.11	
Insulin sensitivity index			0.31
AVF area	−0.20	0.020	
Percentage of body fat	0.13	0.79	

AUC, area under curve during an oral glucose tolerance test; AVF, abdominal visceral fat; R^2, squared multiple correlation coefficient of the whole model including intra-pair differences in AVF area and in percentage of body fat. In corresponding multiple regression analyses where intra-pair differences in abdominal sc fat area were included instead of differences in visceral fat area, the regression coefficients for the differences in sc fat area were not significant for any variable of glucose metabolism (*P*-values ranged from 0.16 to 0.47).

Regarding the mechanism by which visceral fat may result in reduced insulin sensitivity, it has been proposed that increased flux of FFAs from intraabdominal fat stores to the liver and systemic circulation leads to a higher preponderance of tissues to fat use instead of glucose as fuel (22, 23). We did not measure FFA kinetics, but similar FFA levels in obese and lean co-twins, independent of fat distribution, suggest that if the flux of FFAs from visceral fat is increased, it is likely compensated by their increased use (24).

We conclude that when genetic factors are controlled for, subjects prone to AVF accumulation are more susceptible to experiencing reduced insulin sensitivity, whereas subjects with a tendency to ASF deposition exhibit only modest or no disturbances in glucose metabolism.

Acknowledgment

We thank Professor Terry E. Reed, Indiana University School of Medicine, for analyzing the fingertip prints.

References

1. **Knowler WC, Pettit DJ, Savage PJ, Bennett PH.** 1981 Diabetes incidence in Pima Indians: contributions of obesity and parental diabetes. Am J Epidemiol. 113:144–156.
2. **Kissebah A, Vydelingum N, Murray R, et al.** 1982 Relation of body fat distribution to metabolic complications of obesity. J Clin Endocrinol Metab. 54:254–260.
3. **Evans DJ, Hoffmann RG, Kalkhoff RK, Kissebah AH.** 1984 Relationship of body fat topography to insulin sensitivity and metabolic profiles in premenopausal women. Metabolism. 33:68–75.
4. **Björntorp P.** 1990 "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. Arteriosclerosis. 10:493–496.
5. **Young TK, Gelskey DE.** 1995 Is noncentral obesity metabolically benign? Implications for prevention from a population survey. JAMA 274:1939–1941.
6. **Abate N, Garg A, Peshock RM, Stray-Gundersen J, Grundy SM.** 1995 Relationships of generalized and regional adiposity to insulin sensitivity in men. J Clin Invest. 96:88–98.
7. **Bouchard C.** 1995 Genetics and the metabolic syndrome. Int J Obes. [Suppl 1]19:S52–S59.

8. **Kaprio J, Sarna S, Koskenvuo M, Rantasalo I.** 1978 The Finnish twin registry: formation and compilation, questionnaire study, zygosity determination procedures and research program. Prog Clin Biol Res. 24B:179–184.
9. **Sarna S, Kaprio J, Sistonen P, Koskenvuo M.** 1978 Diagnosis of twin zygosity by mailed questionnaire. Hum Hered. 28:241–254.
10. **Smith SM, Penrose LS.** 1955 Monozygotic and dizygotic twin analysis. Ann Hum Genet. 19:273–289.
11. **Reed T.** 1986 Blind assessment of zygosity using dermatoglyphics from the NHLBI twin study. American Dermatoglyphics Association Newsletter. 5:6–11.
12. **Cederholm J, Wibell L.** 1990 Insulin release and peripheral sensitivity at the oral glucose tolerance test. Diabetes Res Clin Pract. 10:167–175.
13. **Siri WE.** 1956 The gross composition of the body. In: Lawrence JH, Tobias CA, eds. Advances in biological and medical physics, Vol 4. New York: Academic; 239–280.
14. **Staten MA, Totty WG, Kohrt WM.** 1989 Measurement of fat distribution by magnetic resonance imaging. Invest Radiol. 24:345–349.
15. **Despres J-P, Moorjani S, Lupien PJ, Tremblay A, Nadeau A, Bouchard C.** 1990 Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. Arteriosclerosis. 10:497–511.
16. **Sparrow D, Borkan GA, Gerzof SG, Wisniewski C, Silbert CK.** 1986 Relationship of fat distribution to glucose tolerance. Results of computed tomography in male participants of the Normative Aging Study. Diabetes. 35:411–415.
17. **DeFronzo RA, Tobin JD, Andres R.** 1979 Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. 237:E214–E223.
18. **Laakso M.** How good a marker is insulin level for insulin resistance? 1993 Am J Epidemiol. 137:959–965.
19. **Terry JG, Hinson WH, Evans GW, Schreiner PJ, Hagaman AP, Crouse JR.** 1995 Evaluation of magnetic resonance imaging for quantification of intra-abdominal fat in human beings by spin-echo and inversion-recovery protocols. Am J Clin Nutr. 62:297–301.
20. **Facchini FS, Hollenbeck CB, Jeppesen J, Chen Y-DI, Reaven GM.** 1992 Insulin resistance and cigarette smoking. Lancet. 339:1128–1130.
21. **Shimokata H, Muller DC, Andres R.** 1989 Studies in the distribution of body fat. III. Effects of cigarette smoking. JAMA 261:1169–1173.
22. **Randle P, Garland P, Hales C, Newsholme E.** 1963 The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet. 1:785–789.
23. **Kissebah AH, Krakower GR.** 1994 Regional obesity and morbidity. Physiol Rev. 74:761–811.
24. **Kissebah AH, Peiris AN.** 1989 Biology of regional body fat distribution: relationship to non-insulin-dependent diabetes mellitus. Diabetes Metab Rev. 5:83–109.