Low Sex-Hormone–Binding Globulin Concentration as Independent Risk Factor for Development of NIDDM

12-Yr Follow-Up of Population Study of Women in Gothenburg, Sweden

GÖRAN LINDSTEDT, PER-ARNE LUNDBERG, LEIF LAPIDUS, HANS LUNDGREN, CALLE BENGTSSON, AND PER BJÖRNTORP

Serum sex-hormone–binding globulin (SHBG) and corticosteroid-binding globulin (CBG) concentrations were evaluated as risk factors for the development of non-insulin-dependent diabetes mellitus (NIDDM), myocardial infarction, stroke, and premature death in a prospective study of 1462 randomly selected women, aged 38–60 yr, over 12 yr of observation. In multivariate analysis, taking only age into consideration as a confounding factor, low initial concentration of SHBG was significantly correlated to the incidence of NIDDM and stroke, and high initial concentration of CBG was correlated to the incidence of NIDDM. There were also significant correlations between SHBG and CBG concentrations on one hand and possible risk factors for the end points studied, such as serum triglycerides, serum cholesterol, fasting blood glucose, body mass, body mass index, waist/hip ratio, smoking habits, and systolic blood pressure, on the other. When these possible confounders, in addition to age, were taken into consideration in multivariate analyses, only the inverse significant correlation between SHBG and NIDDM remained. The increased incidence of diabetes was confined to the lowest quintile of SHBG values, where it was 5-fold higher than in the remaining group. This incidence was further increased to 8- and 11-fold in the lowest 10 and 5% of the values, respectively. We conclude that SHBG is a uniquely strong independent risk factor for the development of NIDDM in women. *Diabetes* 40:123–28, 1991

Abdominal obesity (i.e., the male type of obesity) is a risk factor for the development of cardiovascular disease (1,2) and diabetes mellitus (3,4) and for overall mortality (1,2) in men and women. In women, abdominal obesity is followed by elevated free testosterone and low sex-hormone–binding globulin (SHBG) concentrations (5). Previously, these biochemical correlates to hyperandrogenicity in women had never been examined as predictors of disease in prospective studies. In this study, SHBG and corticosteroid-binding globulin (CBG) were determined in a population sample of women aged 38, 46, 50, 54, and 60 yr participating in a health survey in 1968–1969, and the results were related to the 12-yr incidence of diabetes mellitus, cardiovascular disease, stroke, and overall mortality to established risk factors for these end points (6). Low SHBG concentration was found to be a uniquely strong independent risk factor for diabetes mellitus.

RESEARCH DESIGN AND METHODS

In 1968–1969, 1462 women in Gothenburg, Sweden, aged 38, 46, 50, 54, or 60 yr were selected at random. The systematic sampling method, based on date of birth, and a high participation rate (90.1%) ensured that the participants were a representative cross section of women from the community of the ages studied. Table 1 shows the numbers of participants in the initial examination. The population sample was followed up in 1974–1975 and 1980–1981. Most of the nonparticipants were interviewed by telephone or letter concerning their history of myocardial infarction, stroke, and diabetes mellitus. In this way, information was obtained from a total of 1351 women (97.4% of those participating in 1968–1969 were still alive in 1980–1981).

In the initial 1968–1969 study, blood samples were taken after overnight fasting, and blood glucose (7), serum triglycerides (8), and serum cholesterol (9) were analyzed. Serum SHBG and CBG were determined in 1986 by radioimmunoassay of the samples collected in 1968–1969. The details of the SHBG and CBG immunoassays, including validation for use in the frozen serum specimens, have been described (10). Body weight was measured to the nearest

From the Departments of Clinical Chemistry, Medicine I, and Medicine II, Sahlgren's Hospital, and the Department of Primary Health Care, University of Gothenburg, Gothenburg, Sweden.

Address correspondence and reprint requests to Leif Lapidus, MD, PhD, Department of Medicine II, Sahlgren's Hospital, S-413 45 Gothenburg, Sweden.

Received for publication 12 September 1989 and accepted in revised form 5 September 1990.

TABLE 1
Numbers of subjects in each age group participating in population study of women in Gothenburg, Sweden

Year of birth	Age during follow-up (yr)	Women participating in 1968–1969 (*n*)
1930	38→50	372 (91.4)
1922	46→58	431 (90.0)
1918	50→62	398 (91.3)
1914	54→66	180 (88.7)
1908	60→72	81 (83.5)
Total	38–60*, 50–72†	1462 (90.1)

Values in parentheses are participation rates (%).
*Age range in 1968–1969 study.
†Age range at end of follow-up.

0.1 kg with a balance scale. The women wore only briefs when being weighed. Body height without shoes was measured to the nearest 0.5 cm, and body mass index was calculated (kg/m^2). Waist circumfererence was measured to the nearest 1 mm at the level midway between the lower rib margin and the iliac crest with a steel tape measure, and hip circumference was measured to the nearest 1 mm at the widest point between the hips and buttocks. Blood pressure was recorded with a mercury manometer after ~5 min rest with the subject in the seated position. Information about smoking habits was obtained via a standardized interview. Information about menopausal state was also obtained in an interview. The women were subdivided into premenopausal and postmenopausal groups according to their menstrual state at the time of the initial study in 1968–1969. Serum insulin was determined at the initial 1968–1969 examination in all women aged 50 yr by a double-antibody method (Amersham International, Aylesbury, UK).

For nonfatal myocardial infarction, each participant was asked if she had any history of chest pain, as were most of the nonparticipants in the follow-up studies. Records of all women who were admitted to the hospital due to chest pain were studied, and the diagnosis of acute myocardial infarction was defined as the presence of at least two of the following criteria: central chest pain, transient rise of serum aminotransferase activities, and typical electrocardiographic changes of recent onset. These criteria have been described in detail elsewhere (11). A diagnosis of fatal myocardial infarction was accepted if it was stated on the death certificate.

Stroke was defined as previously described (12). If nonfatal, diagnosis at the hospital was required. Stroke was also registered for subjects who showed signs consistent with a recent cerebrovascular accident at postmortem examination and for whom stroke was recorded as the major cause of death on their death certificate.

Each participant was asked whether she had diabetes, as were most of the nonparticipants in the follow-up studies. If a physician stated that a patient had diabetes, we accepted the diagnosis (n = 32). Women with a fasting serum glucose concentration ≥6 mM who were not known to have diabetes were referred to a special diabetes unit for further examination. Women who then had two fasting capillary whole-blood samples with glucose concentrations ≥7 mM were defined as diabetic (n = 11) according to the World Health Organization's former recommendations (13).

Confirmation of whether the women were alive or dead at the end of the 12 yr was obtained for 1458 (99.7%) of the initial 1462 participants; death certificates were obtained for all participants who died. Necropsies were performed in 5 (63%) of 8 patients whose deaths were attributed to myocardial infarction and in 27 (40%) of 67 women for whom myocardial infarction was not stated on the death certificate.

Patients positive for myocardial infarction at the initial examination were excluded when the risk for myocardial infarction as an end point was assessed. Similarly, patients already positive for other end points at the initial examination were excluded in the final calculations for corresponding end points.

Values were calculated by standard methods and are expressed as means ± SD. Student's t test was used to test the hypothesis of no difference in mean values between two groups. Associations between graded or continuous variables were tested by Pitman's nonparametric permutation test (14). In adjusting for confounding variables, an extension of Mantel-Haenszel's procedure for permutation testing was used (15). Two-tailed tests were used, and $P < 0.05$ was considered significant.

RESULTS

Of the 1424 women initially free from signs of myocardial infarction from whom we obtained valid follow-up data, 23 (1.6%) developed myocardial infarction during the follow-up period (8 fatal, 15 nonfatal). New signs of stroke were recorded in 13 women (0.9%). Forty-three women (3%) developed diabetes mellitus. A total of 75 women (5.1%) died during the follow-up period.

Table 2 shows the P values for nonparametric correlations

TABLE 2
Nonparametric correlations (age-adjusted P values) between initial sex-hormone–binding globulin (SHBG) and corticosteroid-binding globulin (CBG) concentrations and 12-yr incidences of myocardial infarction, stroke, diabetes mellitus, and overall mortality

	n	Myocardial infarction	Stroke	Diabetes mellitus	Mortality
SHBG					
Premenopausal	840	NS	NS	0.002*	NS
Postmenopausal	446	NS	NS	<0.001*	0.007*
All		NS	0.02*	<0.001*	NS
CBG					
Premenopausal	840	0.02	NS	NS	NS
Postmenopausal	446	0.04	NS	0.02	NS
All		0.03	NS	0.02	NS

*Negative correlation.

between initial serum concentrations of SHBG and CGB and the 12-yr incidences of myocardial infarction, stroke, diabetes mellitus, and overall mortality. When all women were examined, age was considered as a potential background factor. A low concentration of SHBG was found to be a strong risk factor the development of diabetes mellitus ($P < 0.001$). This was also the case when premenopausal and postmenopausal women were analyzed separately. Low values of SHBG also predicted stroke in all women ($P = 0.02$) but mortality only among postmenopausal women ($P = 0.007$). SHBG was not a risk factor for the incidence of myocardial infarction, but there was a tendency toward an inverse relationship.

CBG was a risk factor for myocardial infarction ($P = 0.03$) and for diabetes ($P = 0.02$). CBG was not related to the incidence of stroke or to total mortality.

Figure 1 shows the distribution of the SHBG values in the total group of women and in those who developed diabetes, demonstrating the generally low concentrations in those who developed diabetes. The 43 women who became diabetic had markedly lower values of SHBG at the start of the observation period (55 ± 31 nM) than those who remained free from diabetes (88 ± 55 nM; $P < 0.001$). Despite the small numbers of women in the different age groups, the differences were significant in each of the age strata except in the youngest and oldest groups, where only 4 and 3 women, respectively, became diabetic.

Figure 2 shows the incidence of diabetes during the follow-up period in the quintiles of SHBG standardized for age. The first quintile of SHBG showed a >5-fold incidence of diabetes compared with the other four quintiles, separately or together. This incidence increased to 8- and 11-fold, respectively, when the first 10 and 5% of the distribution of SHBG were compared with the rest of the group. There was no gradient in the incidence of diabetes among the last four quintiles.

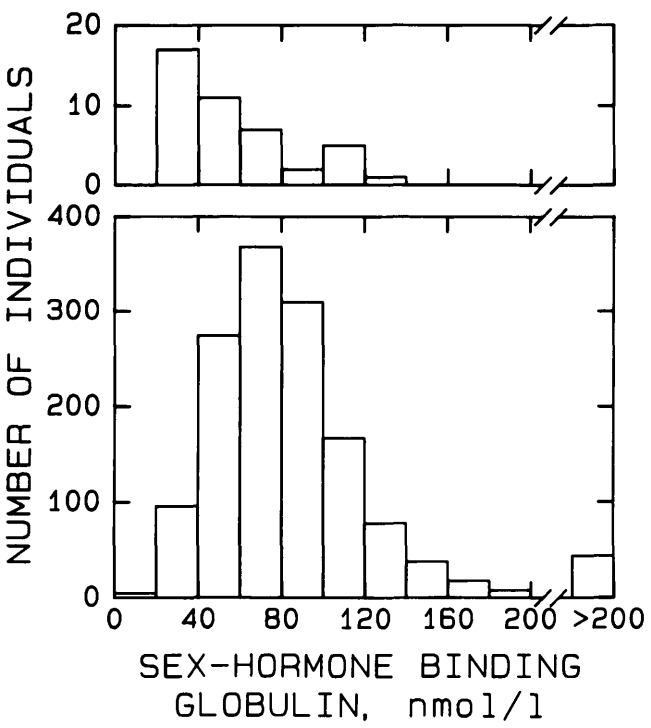

FIG. 1. Distribution of values for serum sex-hormone–binding globulin concentration by radioimmunoassay in 1986 of specimens collected in 1968–1969 health survey of women (see METHODS). *Top*, women who developed diabetes mellitus during ensuing 12 yr (from left to right *n* = 17, 11, 7, 2, 5, 1). *Bottom*, total population.

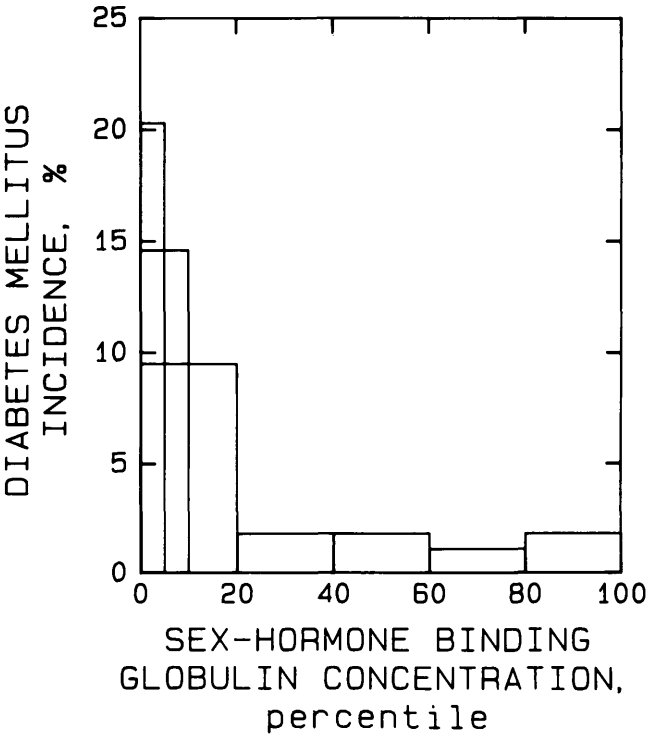

FIG. 2. Incidence of diabetes mellitus during 12-yr versus initial concentration of sex-hormone–binding globulin.

Table 3 gives the results from statistical analyses of relationships between SHBG and CBG on one hand and age, serum lipid concentrations, fasting blood glucose, measurements of general and regional obesity, smoking, and blood pressure on the other. Only serum cholesterol did not correlate with SHBG concentration. The correlations were usually negative with the exception of age, smoking, and triglycerides in premenopausal women. CBG was positively correlated to age, serum cholesterol, and smoking and was negatively correlated to serum triglycerides, body mass, and body mass index.

The interrelationships between SHBG and waist/hip ratio or body mass index and incidence of diabetes are illustrated in Fig. 3. There seems to be an interaction between these three variables, with a clustering of diabetes incidence with higher waist/hip ratio or body mass index in combination with low SHBG values.

Because of the findings mentioned above, the background variables serum triglycerides, serum cholesterol and blood glucose, body mass, body mass index, waist/hip ratio, smoking habits, and systolic blood pressure were added in multivariate analyses, and the remaining significant predictors for the end points myocardial infarction, stroke, diabetes mellitus, and mortality were examined. We found that the significant correlations between SHBG and stroke did not remain in the multivariate analyses. Furthermore, the significant age-specific correlations between CBG, myocardial infarction, and diabetes did not remain in the extended mul-

TABLE 3
Age-adjusted nonparametric correlations (*P* values) of concentrations of sex-hormone–binding globulin (SHBG) and corticosteroid-binding globulin (CBG) versus selected variables

	Age	Serum triglycerides	Serum cholesterol	Blood glucose	Body mass	Body mass index	Waist/hip ratio	Smoking	Systolic blood pressure
SHBG									
Premenopausal		0.006	NS	0.01*	<0.001*	<0.001*	<0.001*	0.02	0.006*
Postmenopausal		<0.001*	NS	0.03*	<0.001*	<0.001*	<0.001*	NS	NS
All	<0.001	NS	NS	0.001*	<0.001*	<0.001*	<0.001*	0.01	<0.001*
CBG									
Premenopausal		<0.001*	NS	NS	<0.002*	0.003*	NS	0.02	NS
Postmenopausal		NS	0.001	NS	<0.001*	<0.001*	NS	NS	NS
All	0.008	<0.001*	0.01	NS	<0.001*	<0.001*	NS	0.003	

*Negative correlation.

tivariate analyses. In contrast, the association between SHBG and the incidence of diabetes remained as a significant independent inverse correlation in the total group of women. This was also the case for the subgroups of premenopausal and postmenopausal women when analyzed separately. Furthermore, low SHBG concentration remained as an independent significant risk factor for mortality among postmenopausal women. Serum insulin was determined only in women aged 50 yr; therefore, serum insulin could be included as a potential confounding variable only for that age stratum. Serum insulin is a strong risk factor for diabetes in these women (16). When serum insulin was included in the multivariate analyses, we observed that SHBG was significantly correlated to diabetes incidence independent of serum insulin. However, when SHBG was included as a background variable, the significant relationship between serum insulin and incidence of diabetes also persisted.

DISCUSSION

This prospective study of women shows the strong power of low SHBG values, a sign of hyperandrogenicity, to predict the development of diabetes. This is independent of other previously established risk factors for diabetes, such as age and high blood glucose or plasma lipids, hypertension, and generalized or regional obesity. Hyperandrogenicity thus seems to be a strong predictor of development of diabetes in women, which was previously not sufficiently observed. The increased risk was found entirely with the lowest quintile of SHGB and was markedly increased with lower values within this quintile. Thus, in the lowest 5% of the distribution, the incidence of diabetes was one in five women. Our results suggest therefore that determination of SHBG might be useful for screening women at risk of developing diabetes because of its high power of discrimination and its relative ease for deciding the borderline of the population at risk.

CBG, another steroid-hormone–binding globulin, was also associated with the incidence of diabetes and myocardial infarction; however, it was not an independent risk factor. The strong correlation between SHBG and measurements of generalized body mass increase or abdominal obesity (waist/hip ratio) found herein is in good agreement with findings in case-control studies that have shown that women with abdominal obesity have increased free testosterone and low SHBG concentrations in parallel with insulin resistance (5,17).

Generalized and abdominal obesity have previously been found to be strong risk factors for the development of diabetes in both women (3) and men (4). Despite the remaining predictive power of a low SHBG level after the obesity factors have been taken into account, generalized and abdominal obesity cannot be ruled out as contributing factors to the development of diabetes, as also suggested by analyses in which all of these factors were considered (Fig. 3).

Several clinical syndromes in women are characterized by a combination of hyperandrogenicity, hyperinsulinemia, insulin resistance, and non-insulin-dependent diabetes mellitus (NIDDM) with or without obesity, and measurements of hyperandrogenicity usually correlate with plasma insulin concentrations (18–20). Of particular interest is the polycystic ovary syndrome (18–23), in which hyperinsulinemia has been found to be associated with hyperandrogenicity in the absence of obesity (21–23). Hyperinsulinemia is a strong risk factor for the development of diabetes (16,24).

In the subgroup of the women in this study, fasting plasma insulin showed a strong negative correlation with SHBG ($r = -0.243$, $P < 0.001$), as reported previously (25). In multivariate analyses in this subgroup, SHBG remained a significant predictor for diabetes when insulin was included as a background factor and vice versa. These observations suggest a close statistical relationship between SHBG and insulin and the incidence of diabetes.

Several studies have shown that women with abdominal obesity and hyperandrogenicity have muscle tissue characterized by low insulin sensitivity (26). Such muscles have few insulin-sensitive slow-twitch muscle fibers and few capillaries, which are also male characteristics (26,27; E. Skarfors, H. Lithell, J. Selinus, B. Sultin, unpublished observations). Signs of generalized insulin resistance correlate strongly with these muscle characteristics (26,27). Other studies have shown that administration of exogenous androgens to children (28), women (29), and in excess to male weight lifters (30) is followed by a marked increase in plasma insulin concentrations. Studies have shown that female rats exposed to testosterone, resulting in blood concentrations similar to those found in hyperandrogenic women, develop marked insulin resistance. This is localized to muscle tissue, which shows remarkable trend toward fewer red insulin-sensitive fibers (31,32). Thus, it is possible that hyperandrogenicity in women, indicated by a low SHBG concentration, actually induces insulin resistance through changes in mus-

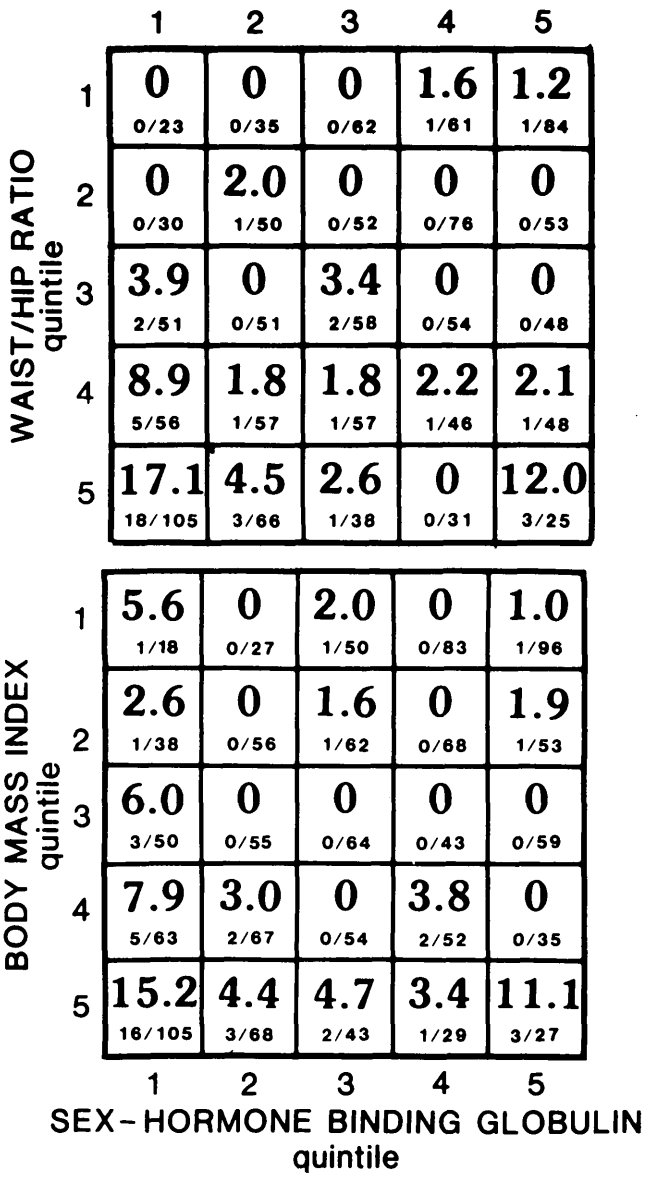

FIG. 3. Incidence of diabetes in relation to age-specific quintiles of waist/hip ratio and sex-hormone-binding globulin (SHBG) and to body mass index and SHBG. In each *square*, large numerals denote percent incidence during 12-yr period, and small numerals show absolute numbers from which incidence was calculated.

cle, increasing the risk to develop diabetes mellitus. Abdominally localized obesity is another male trait that might be an additional consequence of female hyperandrogenicity. Although low SHBG is associated with a risk for development of NIDDM independent of obesity factors, the presence of obesity probably amplifies this risk.

However, other observations suggest that insulin may increase androgen production, resulting in hyperandrogenicity (33–35). It is thus not yet possible to resolve the question of which is the primary factor—increased insulin concentration or hyperandrogenicity. Our observations and experimental evidence demonstrate the importance of further studies of the role of hyperandrogenicity in the development of diabetes in women.

ACKNOWLEDGMENTS

This study was supported by Grants 27X-4578 and B91-19X-251-29C from the Swedish Medical Research Council and Göteborgs Läkaresällskap.

REFERENCES

1. Lapidus L, Bengtsson C, Larsson B, Pennert K, Rybo E, Sjöström L: Distribution of adipose tissue and risk of cardiovascular disease and death: 12 year follow-up participants in the population study of women in Gothenburg, Sweden. *Br Med J* 289:1261–63, 1984
2. Larsson B, Svärdsudd K, Welin L, Wilhelmsen L, Björntorp P: Abdominal adipose tissue distribution, obesity and risk of cardiovascular disease and death: 13 year follow-up of participants in the study of men born 1913. *Br Med J* 288:1401–404, 1984
3. Lundgren H, Bengtsson C, Blohmé G, Lapidus L: Adiposity and adipose tissue distribution in relation to incidence of diabetes in women: results from a prospective population study in Gothenburg, Sweden. *Int J Obes* 13:413–24, 1989
4. Ohlsson L-O, Larsson B, Svärdsudd K, Welin L, Eriksson H, Wilhelmsen L, Björntorp P, Tibblin G: The influence of body fat distribution on the incidence of diabetes mellitus: 13.5 years of follow-up of the participants in the study of men born 1913. *Diabetes* 34:1055–58, 1985
5. Evans PJ, Hoffman RG, Kalkhoff RK, Kissebah AH: Relationship of androgenic activity to body fat topography, fat cell morphology and metabolic aberrations in menopausal women. *J Clin Endocrinol Metab* 57:304–10, 1983
6. Bengtsson C: Ischemic heart disease in women: a study based on randomized population sample of women and women with myocardial infarction in Göteborg, Sweden. *Acta Med Scand Suppl* 549:1–128, 1973
7. Hoffman WS: A rapid photoelectric method for the determination of glucose in blood and urine. *J Biol Chem* 120:51–55, 1937
8. Lofland HB Jr: A semiautomated procedure for the determination of triglycerides in serum. *Anal Biochem* 9:393–400, 1964
9. Levine JB, Zak B: Automated determination of serum total cholesterol. *Clin Chim Acta* 10:381–84, 1964
10. Lapidus L, Lindstedt G, Lundberg PA, Bengtsson C, Gredmark T: Concentrations of sex-hormone binding globulin and corticosteroid binding globulin in serum in relation to cardiovascular risk factors and 12-year incidence of cardiovascular disease and overall mortality in postmenopausal women. *Clin Chem* 82:146–52, 1986
11. Elmfeldt D, Wilhelmsen L, Tibblin G, Vedin A, Wilhelmsson C, Bengtsson C: Registration of myocardial infarction in the city of Göteborg, Sweden: a community study. *J Chronic Dis* 28:173–86, 1975
12. Harmsen P, Tibblin G: A stroke register in Göteborg, Sweden. *Acta Med Scand* 191:463–70, 1972
13. World Health Organization: *WHO Expert Committee on Diabetes Mellitus*. Geneva, World Health Org., 1980, p. 1–80 (Tech. Rep. Ser., no. 646)
14. Bradley JV: *Distribution-Free Statistical Tests*. Englewood Cliffs, NJ, Prentice-Hall, 1968 p. 68–86
15. Mantel N: Chi-square tests with one degree of freedom, extension of the Mantel-Haenszel procedure. *J Am Stat Assoc* 58:690–700, 1963
16. Lundgren H, Bengtsson C, Blohmé G, Lapidus L, Waldenström J: Fasting serum insulin and early insulin response as risk determinants for developing diabetes: results from a 12-year follow up of women aged 50 in Gothenburg, Sweden. *Diabetic Med.* 7:407–13, 1990
17. Peiris AN, Mueller RA, Struve MF, Smith HA, Kissebah AH: Relationships of androgenic activity to body fat topography, fat cell morphology and metabolic aberrations in menopausal women. *J Clin Endocrinol Metab* 64:162–69, 1987
18. Achard C, Thiers J: Le virilisme pilaire et son association a l'insuffisance glycolitique (diabete des femmes à barbe). *Bull Acad Med* 86:51–66, 1921
19. Goldzieher JW: Polycystic ovary syndrome. *Fertil Steril* 35:371–402, 1981
20. Burgen GA, Givens JR, Kitabchi AE: Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. *J Clin Endocrinol Metab* 50:113–20, 1980
21. Dunaif A, Graf M, Mandelli J, Laumas V, Dobrjansky A: Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance, and/or hyperinsulinemia. *J Clin Endocrinol Metab* 65:499–507, 1987
22. Rebuffé-Scrive M, Cullberg G, Lundberg PA, Lindstedt G, Björntorp P: Anthropometric variables and metabolism in polycystic ovarian disease. *Horm Metab Res* 21:391–97, 1989
23. Dunaif A, Segal KR, Futterweit W, Dobrjanski A: Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes* 38:1165–74, 1989
24. Sicree RA, Zimmet PZ, King HOM, Coventry JS: Plasma insulin response among Nauruans: prediction of deterioration in glucose tolerance over 6 yr. *Diabetes* 36:179–86, 1987
25. Haffner SM, Katz MS, Stern MP, Dunn JF: The relationship of sex-hor-

mones to hyperinsulinemia and hyperglycemia. *Metabolism* 37:683–88, 1988
26. Krotiewski M, Björntorp P: Muscle tissue in obesity with different distribution of adipose tissue, effects of physical training. *Int J Obesity* 10:331–41, 1986
27. Lillioja S, Young AA, Culter CL, Ivy JL, Abbott WGH, Zawadzki JK, Yki-Järvinen H, Christin L, Secomb TW, Bogardus C: Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. *J Clin Invest* 80:415–24, 1987
28. Woodard TJ, Burghen GA, Kitabchi AE, Wilimas JA: Glucose intolerance and insulin resistance in aplastic anemia treated with oxymetholone. *J Clin Endocrinol Metab* 53:905–908, 1981
29. Landon JC, Wynn V, Samols E: The effect of anabolic steroids on blood sugar and plasma insulin levels in man. *Metabolism* 12:924–30, 1963
30. Cohen JC, Hickman R: Insulin resistance and diminished glucose tolerance in power lifters ingesting anabolic steroids. *J Clin Endocrinol Metab* 64:960–64, 1987
31. Holmäng A, Björntorp P: Effects of testosterone on insulin sensitivity and muscle morphology of female rats (Abstract). *Int J Obesity* 13 (Suppl. 1):65, 1989
32. Holmäng A, Svedberg J, Jennische E, Björntorp P: Effects of testosterone on muscle insulin sensitivity and morphology in female rats. *Am J Physiol* 259:E555–60, 1990
33. Plymate SR, Matej LA, Jones RE, Friedl KE: Inhibition of sex-hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. *J Clin Endocrinol Metab* 67:460–64, 1988
34. Nestler JE, Barlascini CO, Matt DW, Steingold KA, Plymate SR, Clore JN, Blackard WG: Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic ovarian syndrome. *J Clin Endocrinol Metab* 68:1027–32, 1989
35. Poretsky L, Karlin MF: The gonadotrophic function of insulin. *Endocr Rev* 8:132–41, 1987