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Interrelation between plasma testosterone and plasma insulin in healthy adult men: the Telecom Study

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Summary. Plasma insulin is a risk factor for diabetes mellitus and cardiovascular disease in men. We investigated the association between plasma testosterone and plasma insulin in an occupational sample of 1292 healthy adult men. Total plasma testosterone decreased with each decade of age and insulin increased with each decade of age. In these cross-sectional data, this significant graded inverse association between testosterone and insulin was independent of age. The association was reduced but not explained by the addition of obesity and subscapular skinfold to the model. Adjustment for alcohol consumption, cigarette smoking and plasma glucose did not materially alter the association. These results are the reverse of the positive association of androgens with insulin in women and suggest alternative possible explanations for the effect of hyperinsulinaemia on cardiovascular disease risk. Prospective studies will be necessary to determine the direction and causal nature of this association.

Key words: Insulin, insulin resistance, testosterone, androgens, healthy men, cardiovascular risk factors.

We have previously reported a steady stepwise age-related decrease in plasma total and bioavailable testosterone beginning in young adult life in healthy men [1, 2]. Lower testosterone levels were associated with increasing obesity and central adiposity, but were not explained by these characteristics, cigarette smoking or alcohol use [1]. Plasma total testosterone levels have been shown to be negatively correlated with many cardiovascular risk factors in men [3–19]. Insulin resistance increases with age [20–24] and hyperinsulinaemia has been demonstrated to be a risk factor for coronary disease and mortality from three prospective surveys in men [25–27]. In this context, it is surprising that the relationship between plasma testosterone and plasma insulin in men has not been more closely investigated. One large population-based study described insulin as a confounding factor for the association between testosterone and prevalent ischaemic disease [10]. We therefore investigated the relationship between plasma testosterone and plasma insulin levels in a large sample of healthy men recruited from an occupational cohort.

Subjects and methods

From April 1985 to July 1987, 1297 consecutive, healthy Caucasian men, aged 20–60 years, without diabetes mellitus or other known chronic diseases, who voluntarily attended a centre for preventive medicine, were examined in a cross-sectional study. Subjects were "France Telecom" employees working in the Paris area, who are offered a free medical check-up every 5 years for those under 40 years and every 2 years for those over 40 years. Most were sedentary office workers.

A self-administered questionnaire, filled out at home and reviewed by a secretary with the subject during the consultation, inquired about tobacco and alcohol consumption. A physical examination recorded height, weight and subscapular skinfold thickness (with a Holtain skinfold caliper). The body mass index (BMI) [weight(kg)/height(m)^2] was used as an estimate of obesity and subscapular skinfold thickness as an estimate of upper body fat distribution. Blood from fasting subjects was obtained between 08.00 and 09.00 hours. After centrifugation, plasma was separated and frozen at −20 °C until hormones were assayed an average 32 days later. Plasma total testosterone was measured by radioimmunoassay after column chromatography on celite, with 5.1 % intra-assay and 9.2 % inter-assay coefficients of variation [28, 29], and a sensitivity equal to 0.05 ng/ml, less than the range observed in this male population (0.7 to 18.8 ng/ml). Fasting plasma insulin was measured by radioimmunoassay with INSI-PR method (CIS Bio-Industrie; Gif-sur-Yvette, France), providing 13.7 and 5.8 % intra-assay and 8.0 and 8.6 % inter-assay coefficients of variation at insulin levels of 72 and 118 pmol/l respectively, and a sensitivity equal to 30 pmol/l. Plasma glucose measurements were performed the day of venipuncture, fasting and 2-h after a 75-g glucose load, using the glucose-oxidase method [30].

Of the 1297 consecutive, healthy Caucasian men, aged 20–60 years, five were excluded because they did not have both plasma testosterone and insulin measured, for technical reasons. Therefore, 1292 subjects remained for analysis.

Table 1. Description of the 1292 healthy Caucasian men studied according to age

	Age (years)				
	20–29	30–39	40–49	50–59	*p* value
n	420	411	217	244	
Family history of diabetes (%)	9.2	10.5	12.5	9.5	0.62
Smokers (%)	45.4	38.9	34.4	27.0	< 0.0001
Body mass index (kg/m^2)	22.8 ± 2.3	23.5 ± 2.5	25.3 ± 3.0	25.6 ± 2.8	< 0.0001
Subscapular skinfold (mm)	12.7 ± 4.9	15.0 ± 6.1	18.1 ± 6.4	18.7 ± 6.7	< 0.0001
Systolic blood pressure (mm Hg)	127.6 ± 12.2	128.1 ± 11.8	132.2 ± 14.1	137.6 ± 18.1	< 0.0001
Diastolic blood pressure (mm Hg)	73.9 ± 9.0	75.5 ± 8.9	79.2 ± 10.9	82.3 ± 12.2	< 0.0001
Alcohol (g/day)	11.8 ± 24.7	19.2 ± 26.8	26.5 ± 27.8	27.9 ± 27.4	< 0.0001
Cholesterol (mmol/l)	5.40 ± 0.94	5.89 ± 1.06	6.30 ± 1.05	6.42 ± 0.96	< 0.0001
Triglycerides (mmol/l)	0.95 ± 0.57	1.10 ± 0.60	1.23 ± 0.66	1.30 ± 0.84	< 0.0001
Testosterone (ng/ml)	6.69 ± 1.86	6.15 ± 1.89	5.74 ± 1.76	5.53 ± 1.76	< 0.0001
Fasting plasma glucose (mmol/l)	4.99 ± 0.38	5.15 ± 0.39	5.32 ± 0.47	5.41 ± 0.51	< 0.0001
2-h plasma glucose (mmol/l)	5.05 ± 1.15	5.22 ± 1.22	5.71 ± 1.28	6.00 ± 1.60	< 0.0001
Fasting plasma insulin (pmol/l)	44.3 ± 15.2	44.6 ± 14.7	49.2 ± 18.2	48.6 ± 17.1	< 0.0001

For quantitative parameters: Mean ± SD

Table 2. Correlation matrix for age, testosterone, fasting plasma glucose (Go), 2-h plasma glucose (G2), fasting plasma insulin (Io), BMI and subscapular skinfold

	Testos-terone	Go	G2	Io	BMI	Ss. skinfold
Age	−0.24	0.35	0.28	0.11	0.40	0.37
Testosterone		−0.19	−0.21	−0.19	−0.28	−0.27
Go			0.40	0.31	0.32	0.29
G2				0.20	0.23	0.26
Io					0.39	0.37
BMI						0.71

All correlations are significant with $p < 0.0001$.

Statistical analysis

Statistical analysis was conducted with the help of the SAS software on VAX/VMS [31] to perform mean comparisons by variance analysis, to calculate Pearson correlation coefficients (untransformed values shown as the results were not different when log values were used). Mean age (and other covariates)-adjusted plasma testosterone values were also calculated by categorical 15 pmol/l increments of fasting plasma insulin, overall in a first step, then for each quartile of BMI, subscapular skinfold and fasting plasma glucose successively, using analysis of covariance. Correlation between plasma testosterone and fasting plasma insulin (taken as a continuous variable) was also computed after adjustment for age, BMI, skinfold thickness, tobacco and alcohol consumption, fasting and 2-h plasma glucose. A two-tailed p value of ≤ 0.05 was used to indicate statistical significance.

Results

The characteristics of the 1292 Caucasian men aged 20 to 60 years (mean ± SD: 37.2 ± 10.7 years) are shown in Table 1. Plasma testosterone showed a significant stepwise decrease ($p < 0.001$) with each decade of age; in contrast, fasting plasma glucose, 2-h plasma glucose and fasting plasma insulin significantly increased with age (each significant at $p < 0.001$).

Table 2 gives the univariate correlation matrix between these parameters and also BMI and subscapular skinfold. As expected from Table 1, age was significantly negatively correlated with plasma testosterone and positively correlated with fasting plasma glucose, 2-h plasma glucose and fasting plasma insulin. In addition, BMI and subscapular skinfold were each positively correlated with age (Pearson correlation coefficients equal to 0.40 and 0.37 respectively). Plasma testosterone was significantly negatively correlated not only with age, but also with fasting plasma glucose, 2-h plasma glucose, fasting plasma insulin, BMI and subscapular skinfold. Fasting plasma insulin, in addition to its significant positive relation with age ($r = 0.11$) and negative relation with testosterone ($r = -0.19$), was also positively correlated with fasting plasma glucose, 2-h plasma glucose, BMI and subscapular skinfold.

In order to better describe the link between plasma testosterone and fasting plasma insulin, the crude and age-adjusted mean values of plasma testosterone were determined by fasting plasma insulin values (Table 3). There was a significant stepwise decrease in plasma testosterone with increasing levels of insulin, even after adjusting for age ($p < 0.0001$). This decrease was reduced but remained graded and significant after adjusting at the same time for age, BMI, subscapular skinfold, tobacco and alcohol consumption. The mean plasma testosterone values by categorical 15 pmol/l increments of fasting plasma insulin were 6.28, 6.21, 6.14 and 5.79 ng/ml respectively ($p = 0.05$).

Moreover, the relationship between plasma testosterone and fasting plasma insulin remained highly significant after adjustment for fasting and 2-h plasma glucose ($p < 0.0001$). The addition of these two parameters in the model with the above-listed covariates reduced but did not remove the significant correlation between plasma testosterone and fasting plasma insulin ($p < 0.03$).

Table 3. Mean plasma testosterone by level of fasting blood insulin before and after adjustment for age, obesity and tobacco and alcohol consumption

	Fasting blood insulin (pmol/l)				
	≤30	30–45	45–60	>60	*p* value
n	229	533	339	191	
Plasma testosterone (ng/ml)					
Unadjusted mean (SD)	6.49 (1.97)	6.32 (1.89)	6.06 (1.85)	5.38 (1.60)	<0.0001
Age adjusted	6.43	6.29	6.08	5.49	<0.0001
Age, BMI and skinfold adjusted	6.29	6.21	6.13	5.79	0.04
Age, BMI, skinfold, tobacco and alcohol adjusted	6.28	6.21	6.14	5.79	0.05

Tests were performed by analysis of variance; adjustments were performed by analysis of covariance using age, BMI, skinfold, tobacco and alcohol as continuous variables

Figure 1 shows the age-adjusted variations of plasma testosterone in each respective quartile of BMI, subscapular skinfold and fasting plasma glucose between four categories of fasting plasma insulin values. The decrease in plasma testosterone with increasing fasting plasma insulin level was consistent in most quartiles of BMI, subscapular skinfold and fasting plasma glucose despite the instability of relatively small numbers within subgroups, indicating the inverse relationship between plasma testosterone and fasting plasma insulin occurred at all levels of obesity, body fat distribution and plasma glucose. The same trend was observed within each quartile of 2-h plasma glucose (data not shown).

Discussion

In this population of healthy men, testosterone and insulin levels were inversely related, independent of age, obesity, body fat distribution, plasma glucose, tobacco and alcohol consumption. It seems unlikely that this is a chance association, since it is biologically plausible and consistent with another published report [10]. The use of only one single morning blood sample to measure plasma testosterone in men has been proved to be convenient for epidemiological purposes [32]. Furthermore, if a single sample increases the intraindividual variation, then the associations would be reduced, not enhanced. Plasma testosterone is mainly bound to the sex-hormone-binding globulin, which was not measured in this study. Recent studies suggest that a fall in total plasma testosterone is accompanied by a parallel fall in free testosterone [17]. Therefore, measuring total testosterone should not modify the relationship observed here with insulin. The fact that the association is independent of age and obesity does not exclude a role for these attributes as explanatory variables, as some of the association is lost by the addition of obesity and fat distribution to the model. It is possible that a better measure of central adiposity than subscapular skinfold would have better explained the association and provided additional insights into its mechanism. On the other hand, cigarette smoking, alcohol use and plasma glucose levels appear to contribute little to the association, since addition of these attributes to the model did not change the association materially.

Because recruitment was occupation-based and consisted of volunteers, the sample of the study cannot be considered as being representative of the total French adult male population, even if it is probably more representative of healthy people than most samples in other sex hormones studies, often based on patient populations consulting for diseases or sexual dysfunction. Indeed, since the testosterone-insulin association was also noted in a population-based study of older men (aged 45–59 years) in Wales [10], it is unlikely to be explained by the Telecom worker's occupation or geographic origin.

These cross-sectional observations do not indicate whether decreased testosterone causes insulin to increase or vice versa. The increase of plasma insulin with age is thought to be a physiologic marker of insulin resistance [20–24]. The early appearance of decreased insulin sensitivity, beginning as early as 30 years of age [20], could be the cause or the consequence of the parallel age-related decrease of plasma testosterone levels in men [1, 2]. The hypothesis that hyperinsulinaemia is the initial event,

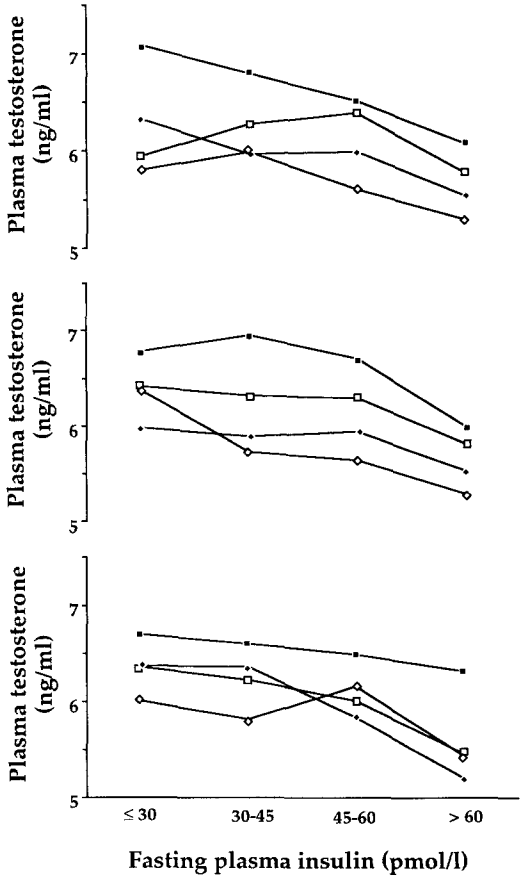

Fig. 1. Age-adjusted plasma testosterone according to values of fasting plasma insulin, by quartiles of body mass index (upper panel), subscapular skinfold (middle panel) and fasting plasma glucose (lower panel). 1st quartile-■-; 2nd quartile-□-; 3rd quartile-◆-; 4th quartile-◇-

preceding the decrease in plasma testosterone, is partly supported by the reduction of adrenal androgen production during hyperinsulinaemic-euglycaemic clamp in normal men; testosterone evolution was unfortunately not reported [33]. Moreover, the observation of decreased free and total testosterone levels in older adult men with diabetes [34, 35] can be considered to indirectly support that hypothesis.

The pharmacologic models showing an induction of impaired glucose tolerance and hyperinsulinaemia by the administration of androgens to male athletes [36] or to patients with aplastic anaemia [37] are far from physiologic conditions and therefore cannot be used as proof of the connection between testosterone and insulin in healthy men. The hypothesis that a low level of plasma testosterone is the primary modification, followed by hyperinsulinaemia, is suggested by the human model of Klinefelter's syndrome where dysgenesia-related hypogonadism is often accompanied by an impaired glucose tolerance, or even by diabetes, due to insulin resistance [38]. The observation that lower levels of gonadal and adrenal androgens were associated with increasing levels of waist-hip-ratio several years later in men of the Rancho Bernardo survey [39], also suggests that low androgens promote abdominal obesity. Abdominal obesity is, in turn, a well-known predictor of hyperinsulinaemia and diabetes [40].

Interestingly, the inverse association between androgens and insulin in men is the reverse of the better-known direct association between androgens and insulin seen in women. Thus, there is a positive correlation between testosterone and insulin levels (basal as well as glucose-stimulated) in women with polycystic ovary syndrome [41]. Moreover, non-diabetic women with central obesity typically have hyperinsulinaemia, insulin resistance and androgen excess [42–44].

Although this cross-sectional study does not permit direct inference about causality or a temporal relationship between testosterone, insulin, BMI and body fat distribution in men, the present data support the need for further studies in this field which could be of importance in the understanding of the relationship between diabetes and cardiovascular diseases.

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References

1. Simon D, Preziosi P, Barrett-Connor E et al. (1992) The influence of aging on plasma sex hormones in men: the Telecom Study. Am J Epidemiol (in press)
2. Nahoul K, Roger M (1990) Age-related decline of plasma bioavailable testosterone in adult men. J Steroid Biochem 35: 293–299
3. Kley HK, Solbach HG, McKinnan JC, Krüskemper HL (1979) Testosterone decrease and oestrogen increase in male patients with obesity. Acta Endocrinol 91: 553–563
4. Hill P, Wynder E, Garbaczewski L, Garnes H, Walker ARP, Helman P (1980) Plasma hormones and lipids in men at different risk for coronary heart disease. Am J Clin Nutr 33: 1010–1018
5. Kley HK, Edelman P, Kruskemper HL (1980) Relationship of plasma sex hormones to different parameters of obesity in male subjects. Metabolism 29: 1041–1046
6. Heller RF, Meade TW, Haines AP, Stirling Y, Miller NE, Lewis B (1982) Interrelationships between factor VII, serum testosterone and plasma lipoproteins. Thromb Res 28: 423–427
7. Dai WS, Gutai JP, Kuller LH, LaPorte RE, Falvo-Gerard L, Caggiula A (1984) Relation between plasma high density lipoprotein cholesterol and sex hormone concentrations in men. Am J Cardiol 53: 1259–1263
8. Breier Ch, Mühlberger V, Drexel H, Herold M, Lisch HJ, Knapp E (1985) Essential role of post-heparin lipoprotein lipase activity and of plasma testosterone in coronary artery disease. Lancet I: 1242–1244
9. Bennet A, Sie P, Caron P et al. (1987) Plasma fibrinolytic activity in a group of hypogonadic men. Scand J Clin Lab Invest 47: 23–27
10. Lichtenstein MJ, Yarnell JWG, Elwood PC et al. (1987) Sex hormones, insulin, lipids, and prevalent ischemic heart disease. Am J Epidemiol 126: 647–657
11. Stefanick ML, Williams PT, Krauss RM, Terry RB, Vranizan KM, Wood PD (1987) Relationships of plasma estradiol, testosterone, and sex hormone-binding globulin with lipoproteins, apolipoproteins, and high density lipoprotein subfractions in men. J Clin Endocrinol Metab 64: 723–729
12. Swartz CM, Young MA (1987) Low serum testosterone and myocardial infarction in geriatric male inpatients. J Am Geriatr Soc 35: 39–44
13. Bonithon-Kopp C, Scarabin PY, Bara L, Castanier M, Jacqueson A, Roger M (1988) Relationship between sex hormones and haemostatic factors in healthy middle-aged men. Atherosclerosis 71: 71–76
14. Khaw KT, Barrett-Connor E (1988) Blood pressure and endogenous testosterone in men: an inverse relationship. J Hypertens 6: 329–332
15. Barrett-Connor E, Khaw KT (1988) Endogenous sex hormones and cardiovascular disease in men. A prospective population-based study. Circulation 78: 539–545
16. Hugues GS, Mathur RS, Margolius HS (1989) Sex steroid hormones are altered in essential hypertension. J Hypertens 7: 181–187
17. Zumoff B, Strain GW, Miller LK et al. (1990) Plasma free and non-sex-hormone-binding-globulin bound testosterone are decreased in obese men in proportion to their degree of obesity. J Clin Endocrinol Metab 71: 929–931
18. Freedman DS, O'Brien TR, Flanders WD, De Stefano F, Barboriak JJ (1991) Relation of serum testosterone levels to high density lipoprotein cholesterol and other characteristics in men. Arteriosclerosis 11: 307–315
19. Khaw KT, Barrett-Connor E (1991) Endogenous sex hormones, high density lipoprotein cholesterol, and other lipoprotein fractions in men. Arteriosclerosis 11: 489–494
20. De Fronzo RA (1981) Glucose intolerance and aging. Diab Care 4: 493–501
21. Fink RI, Kolterman OG, Griffin J, Olefsky JM (1983) Mechanisms of insulin resistance in aging. J Clin Invest 71: 1523–1535
22. Rowe JW, Minaker KL, Pallotta JA, Flier JS (1983) Characterization of the insulin resistance of aging. J Clin Invest 71: 1581–1587
23. Bolinder J, Östman J, Arner P (1983) Influence of aging on insulin receptor binding and metabolic effects of insulin on human adipose tissue. Diabetes 32: 959–964
24. Jackson RA (1990) Mechanisms of age-related glucose intolerance. Diab Care 13 (Suppl 2): 9–19
25. Pyörälä K (1979) Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. Diab Care 2: 131–141

26. Welborn TA, Wearne K (1979) Coronary heart disease incidence and cardiovascular mortality in Busselton with reference to glucose and insulin concentrations. Diab Care 2: 154–160
27. Ducimetière P, Eschwège E, Papoz L, Richard JL, Claude JR, Rosselin G (1980) Relationship of plasma insulin levels to the incidence of myocardial infarction and coronary heart disease mortality in a middle-aged population. Diabetologia 19: 205–210
28. Scholler R, Nahoul K, Castanier M, Rotman J, Salat-Baroux J (1984) Testicular secretion of conjugated and unconjugated steroids in normal adults and in patients with varicocele. J Steroid Biochem 20: 203–215
29. Roger M, Nahoul K, Toublanc JE, Castanier M, Canlorbe P, Job JC (1979) Les androgènes plasmatiques chez le garçon de la naissance à l'adolescence. Ann Pediatr 26: 239–245
30. Hoffmeister H, Junge B (1970) Über die Haltbarkeit von Serumbestandteilen und die Zuverlässigkeit ihrer Bestimmung im Autoanalyser SMA 12-30-Survey. II. Z Klin Chem Klin Biochem 8: 613–617
31. SAS Institute (1985). SAS user's guide: statistics, version 5. SAS Institute Inc, Cary
32. Dai WS, Kuller LH, La Porte RE et al. (1981) The epidemiology of plasma testosterone levels in middle-aged men. Am J Epidemiol 114: 804–816
33. Nestler JE, Usiskin KS, Barlascini CO, Welty DF, Clore JN, Blackward WG (1989) Suppression of serum dehydroepiandrosterone sulfate levels by insulin: an evaluation of possible mechanisms. J Clin Endocrinol Metab 69: 1040–1046
34. Ando S, Rubens R, Rottiers R (1984) Androgen plasma levels in male diabetics. J Endocrinol Invest 7: 21–24
35. Barrett-Connor E, Khaw KT, Yen SSC (1990) Endogenous sex hormone levels in older adult men with diabetes mellitus. Am J Epidemiol 132: 895–901
36. Cohen JC, Hickman R (1987) Insulin resistance and diminished glucose tolerance in powerlifters ingesting anabolic steroids. J Clin Endocrinol Metab 64: 960–963
37. Woodward TL, Burghen GA, Kitabchi AE, Wilimas JA (1981) Glucose intolerance and insulin resistance in anaplastic anemia treated with oxymetholone. J Clin Endocrinol Metab 53: 905–908
38. Geffner ME, Kaplan SA, Bersch N, Lippe BM, Bergman RN, Golde DW (1985) Insulin resistance in Klinefelter's syndrome. Clin Res 33: 128A (Abstract)
39. Khaw KT, Barrett-Connor E (1992) Lower endogenous androgens predict central adiposity in men. Ann Epidemiol (in press)
40. Larsson B, Svärdsudd K, Welin L, Wilhelmsen L, Björntorp P, Tibblin G (1984) Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: 13 year follow-up of the participants in the study of men born in 1913. Br Med J 288: 1401–1404
41. Buffington CK, Givens JR, Kitabchi AE (1991) Opposing actions of dehydroepiandrosterone and testosterone on insulin sensitivity. Diabetes 40: 693–700
42. Kissebah AH, Vydelingum N, Murray R et al. (1982) Relation of body fat distribution to metabolic complications of obesity. J Clin Endocrinol Metab 54: 254–260
43. Evans DJ, Hoffmann RG, Kalkhoff RK, Kissebah AH (1983) Relationship of androgenic activity to body fat topography, fat cell morphology, and metabolic aberrations in premenopausal women. J Clin Endocrinol Metab 57: 304–310
44. Peiris AN, Sothmann MS, Hennes MI et al. (1989) Relative contribution of obesity and body fat distribution to alterations in glucose insulin homeostasis: predictive values of selected indices in premenopausal women. Am J Clin Nutr 49: 758–764

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