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Seasonal Variation of Testosterone and Waist to Hip Ratio in Men: The Tromsø Study

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Studies of seasonal variation in male testosterone levels show contradictory results. We report here a cross-sectional study of the seasonal variation in total and free testosterone, LH, and SHBG levels in 1548 men living in north Norway, a population exposed to a wide seasonal variation in temperature and daylight. Total testosterone showed a bimodal seasonal variation ($P < 0.001$) with a small peak in February, the nadir in June, and a more prominent peak in October and November. Free testosterone also showed a significant seasonal pattern ($P < 0.001$), with the peak in December and the nadir in August. These patterns persisted after adjusting for age and waist to hip ratio ($P < 0.001$). Lowest testosterone levels occurred in months with the highest temperatures and longest hours of daylight. Waist to hip ratio paralleled the change in daylight and temperature, with the highest values during the summer and was thus inversely related to the seasonal testosterone variation. The variations in hormone levels were large, with a 31% difference between the lowest and highest monthly mean level of free testosterone. Prospective studies are needed to establish the direction of the association and its etiology. (*J Clin Endocrinol Metab* 88: 3099–3104, 2003)

DURING THE LAST decade, there has been an increasing interest in the age-related decrease in endogenous testosterone levels in men. Lower levels of testosterone have been associated with cardiovascular risk factors (1–6) and, in one recent study, atherosclerosis (7). Thus, it is important to better understand the variations of men's endogenous testosterone levels.

Although there is agreement about a diurnal rhythm of testosterone in men that diminishes with increasing age (8, 9), studies of seasonal testosterone variation have shown contradictory results (Table 1). Seasonal variation was reported in cross-sectional studies by Bellastella *et al.* (10), Nicolau *et al.* (11), Reinberg *et al.* (12), Dabbs (13), and Perry *et al.* (14) but not by Dai *et al.* (15) and Abbaticchio *et al.* (16). Longitudinal studies have also shown inconsistent patterns (17–24). None of these studies examined body weight and fat distribution in relation to seasonal variation and endogenous sex hormone levels.

We report here a cross-sectional study of the seasonal variation in total and free testosterone, LH, and SHBG levels in men living in north Norway, a population exposed to wide seasonal variation in temperature and daylight. We also report the association of hormone levels with seasonal variation in temperature, hours of daylight, body mass index (BMI), and waist to hip ratio (WHR).

Subjects and Methods

Study population

From 1994 to 1995, all inhabitants aged 25 yr or older living in the municipality of Tromsø were invited to participate in the fourth survey of the Tromsø Study, a population-based multipurpose study. This fourth survey consisted of two screening visits held 4–12 wk apart. All subjects between 55 and 74 yr, random 5% samples of subjects in the other age groups, and a group of men aged 40–54 yr selected on the basis of high total cholesterol and/or low high-density lipoprotein cholesterol were invited to the second visit for a more extensive examination. A total of 6891 subjects (3393 men) attended the second visit, representing 79% of the eligible population. Serum samples were drawn for future analysis of sex hormones in a random sample of 1605 men, of whom 1565 had sufficient serum for hormone assays. Of these, eight men whose total testosterone levels were below the assay detection level and seven men whose SHBG levels were above the assay reference range were excluded. Two men using testosterone treatment were also excluded, leaving 1548 men to participate in this study. The 166 men from the high-risk men sample were included, after confirming that the inclusion of these men did not change the results.

Questionnaires

The letter of invitation included a questionnaire on the number of cigarettes smoked per day; hours of easy and vigorous physical activity per week; and number of glasses of beer, wine, and hard liquor drunk per 2 wk. From this questionnaire a physical activity score was made by adding together the hours of easy and vigorous physical activity, giving the hours with vigorous activity double weight. An alcohol intake score of beer, wine, and hard liquor was also created (assuming an equal amount of alcohol in one glass of each type).

A second questionnaire on medical history and past and present medication was filled out at home and returned by mail.

Measurements

The study period was from September 1994 to September 1995; no samples were taken in July because of the general vacation in Norway. Blood was drawn in nonfasting men between 0800 and 1600 h. Serum samples were stored at −70 C until they were first thawed for analyses of sex hormones in 2001, after an average of 6.5 yr.

Determination of total testosterone, LH, and SHBG was performed on Immulite 2000 (Diagnostic Products, Los Angeles, CA). The coefficients of variation (CV) were based on assays of pooled human sera in two

Abbreviations: BMI, Body mass index; CV, coefficient(s) of variation; WHR, waist to hip ratio.

TABLE 1. Publications on seasonal variation of testosterone

	n	Age range	Blood sample: time and frequency	Location	Design	Findings
Seasonal variation						
Reinberg *et al.* 1975 (17)	5	26–31	24 h mean, every 2nd month	Paris, France	Longitudinal	Peak in October
Smals *et al.* 1976 (18)	15	24–45	0900 h, every 3rd month	Nijmegen, The Netherlands	Longitudinal	Peak, summer through fall
Bellastella *et al.* 1982 (10)	40	6–12	0800 h	Napoli, Italy	Cross-sectional	Peak in July
Nicolau *et al.* 1984 (19)	14	73 ± 7 (mean ± SD)	24 h mean, every 3rd month	Bucharest, Romania	Longitudinal	Peak, summer through fall
Nicolau *et al.* 1985 (11)	63	51–80+	24 h mean	Bucharest, Romania	Cross-sectional	Peak, summer through fall
Reinberg *et al.* 1988 (12)	207			Houston, Texas	Cross-sectional	Peak in November
Dabbs 1990 (13)	4462	32–44	0800 h	Subjects came from all parts of United States	Cross-sectional	Peak in December
Meriggiola *et al.* 1996 (20)	16	19–42	Afternoon, monthly	Seattle, Washington	Longitudinal	Peak in June
Valero-Polti *et al.* 1998 (21)	20	26–47	0800–0930 h, monthly	Barcelona, Spain	Longitudinal	Peak in May
Perry *et al.* 2000 (14)	65	70–102	0800–1200 h	St. Louis, Missouri	Cross-sectional	Peak in March
No seasonal variation						
Baker *et al.* 1976 (22)	26		Any time of day, every 3rd month	Melbourne, Australia	Longitudinal	
Dai *et al.* 1981 (15)	243	39–61	0900–1000 h	Pittsburgh, Pennsylvania	Cross-sectional	
Martikainen *et al.* 1985 (23)	24	21–41	1000–1200 h, monthly	Oulu, Finland	Longitudinal	
Abbaticchio *et al.* 1987 (16)	248	28.9 ± 7.5 (mean ± SD)		Bari, Italy	Cross-sectional	
Maes *et al.* 1997 (24)	13		0800 h, monthly	Antwerp, Belgium	Longitudinal	

hormone levels run in parallel each day. The intra- and interassay CV for total testosterone were 3.5% and 5%, respectively, at a concentration above 1.0 nmol/liter and 12% and 20%, respectively, in the range 0.1 (limit of detection) to 1.0 nmol/liter. For the determination of LH the intra- and interassay CV and sensitivity were 5%, 8%, and 0.7 IU/liter, respectively, and for SHBG 3.5%, 6%, and 1.0 nmol/liter, respectively. The assays were run daily in batches of 100 samples, and, to reduce any potential influence of interassay variation on the results, the assays were carried out on randomly selected samples.

Three men had LH levels below the detection limit. Undetectable levels were converted to values midway between zero and the assay sensitivity level for analysis. Free testosterone values were calculated from total testosterone and SHBG using the Vermeulen formula (25).

Height, weight, and waist and hip girth were measured with participants wearing light clothing without shoes; waist was measured at the umbilical line and hip girth at the widest circumference according to protocol. BMI [weight (kilograms)/height^2 (square meters)] and WHR were calculated.

The meteorological station in Tromsø provided records of the mean monthly temperature in Tromsø for the study period.

Statistical analyses

SPSS Base 10.1 for Windows User's Guide (SPSS, Inc., Chicago, IL) was used for all analyses. LH levels were skewed and log transformed for the analytical calculations, and levels of the other hormones and covariates were normally distributed. Seasonality was examined by comparing monthly means by ANOVA. *Post hoc* testing with the least significant differences method was performed to identify monthly means significantly different from the peak value. Analysis of covariance was used to calculate adjusted means of the sex hormones, BMI, and WHR. Age and WHR adjustment was done by using age and WHR as continuous variables and covariates in the models. Because we lacked samples from the month of July, for presentation purposes July values were calculated by adding mean levels from June and August divided by two. However, leaving July out of the presentation did not change the observed associations. All statistical tests were two tailed, with statistical significance defined as $P < 0.05$.

Ethics

The Tromsø regional ethics committee approved the study, and all subjects gave their written informed consent.

Results

As shown in Table 2, the mean age of the study population was 60 yr, the mean BMI was 26.1, and the mean WHR was 0.92. More than two thirds did not smoke, and 35% reported to use alcohol less than once in a 2-wk period. The majority were relatively sedentary, with 58% in the lowest physical activity group. Table 2 also shows mean levels of total testosterone (13.2 nmol/liter), free testosterone (205 pmol/liter), LH (5.5 IU/liter), and SHBG (51.6 nmol/liter).

Mean and 95% confidence interval for total and free testosterone, LH, and SHBG levels for each month are shown in Fig. 1. Total testosterone (Fig. 1) shows a bimodal curve with a small peak in February (13.4 nmol/liter), the nadir in June (12.0 nmol/liter), and a more prominent peak in October and November (14.3 nmol/liter). The seasonal variation was significant ($P < 0.001$) and persisted after adjusting for age and BMI or age and WHR ($P < 0.001$) (data not shown). Free testosterone (Fig. 1) also showed a significant seasonal pattern with the peak in December (230 pmol/liter) and the nadir in August (176 pmol/liter); results were not materially changed after adjusting for age and WHR ($P < 0.001$). From the lowest to the highest monthly mean, there

TABLE 2. Characteristics of the 1548 Tromsø men

Age (yr)	60.3 ± 10.0
Anthropometric characteristics	
Height (cm)	175.2 ± 6.9
Weight (kg)	80.3 ± 12.1
BMI (kg/m^2)	26.1 ± 3.4
Waist/hip ratio	0.92 ± 0.06
Hormone levels	
Total testosterone (nmol/liter)	13.2 ± 5.1
Free testosterone (pmol/liter)	205 ± 75
LH (IU/liter)	5.5 ± 4.1
SHBG (nmol/liter)	51.6 ± 22.3
Lifestyle factors	
Smoking—cigarettes/day—none/1–10/11 or more (%)	67/26/7
Alcohol—units/2 wk—none/1–14/15 or more (%)	35/58/7
Physical activity score—0–3/4–6/7–9 (%)	58/23/19

Alcohol units, one glass of beer, wine, or hard liquor (assuming an equal amount of alcohol in one glass of each type); physical activity score, 0–3 is equivalent with occasional or regular easy activity, whereas 7–9 would involve vigorous exercise on a weekly basis; Values are mean ± SD for age, anthropometric factors, and hormone levels.

was a difference of approximately 19% for total and 31% for free testosterone.

We found an inverse and significant association between time of venipuncture and both total and free testosterone, but adjusting for venipuncture time did not change the seasonal variation of total and free testosterone (data not shown).

This study included all men with or without chronic diseases. Excluding men with a history of cardiovascular disease and men using medication for hypertension or heart disease did not change the observed seasonal variation in testosterone (data not shown).

For LH there was a significant annual variation ($P = 0.016$) with peaks in April and October (Fig. 1), a pattern that became stronger after adjusting for age and WHR ($P = 0.007$). As shown in Fig. 1, seasonal variation was also found for SHBG ($P < 0.001$), with a peak in October and nadir in December through January, a pattern that was present before and after adjusting for age and WHR. No seasonal relationship between SHBG and total testosterone was seen, and adjusting for SHBG did not change the observed seasonal variation in testosterone.

BMI did not show any seasonal variation (data not shown). In contrast, WHR showed a significant variation during the year ($P = 0.018$) independent of age and reported physical activity ($P = 0.018$). WHR was inversely associated with total testosterone levels ($P < 0.001$), as shown in Fig. 2A. The close relationship between WHR and testosterone is illustrated by inverting the axis of WHR, shown in Fig. 2B. No seasonal relationship between WHR and SHBG was seen.

Self-reported smoking habits and physical activity were constant throughout the year. A variation in the consumption of alcohol was found ($P < 0.001$) with a peak in May (data not shown). Adjusting for alcohol did not change the seasonal pattern in testosterone or SHBG.

There were inverse seasonal relationships between hours of daylight and total testosterone and mean monthly temperature and total testosterone. Thus, the lowest testosterone levels are seen in months with the highest temperatures and

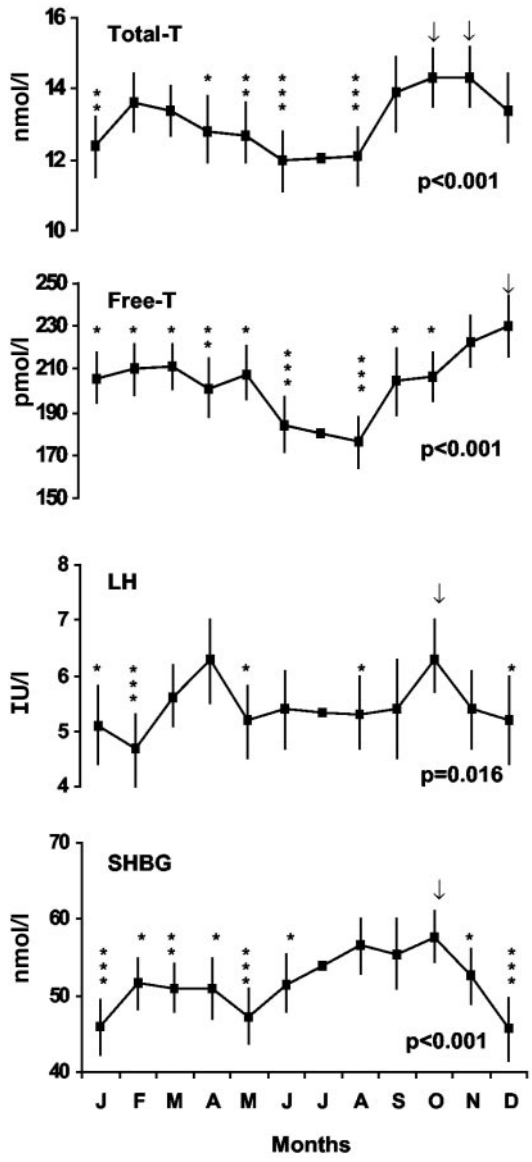

FIG. 1. Mean and 95% confidence interval serum concentration of total testosterone (Total-T) (nmol/liter), free testosterone (Free-T) (pmol/liter), LH (IU/liter), and SHBG (nmol/liter) by month and *P* value for the distribution. Significant differences from the peak value (↓), *, $P < 0.05$; **, $P < 0.01$; or ***, $P < 0.001$.

longest hours of daylight. These associations are illustrated in Fig. 2C, with the axis of total testosterone inverted. It is also obvious from examination of the pattern shown in Fig. 2, B and C that WHR varied with hours of daylight and mean monthly temperature.

Discussion

Total testosterone in Tromsø men showed a seasonal bimodal distribution, with the lowest levels in summer (June through August), a small peak in the late winter, and a higher, more prominent peak in the fall. The seasonal pattern for the free testosterone nadir was similar, with the lowest levels in summer and the peak somewhat later in December. The largest cross-sectional study, which analyzed archival

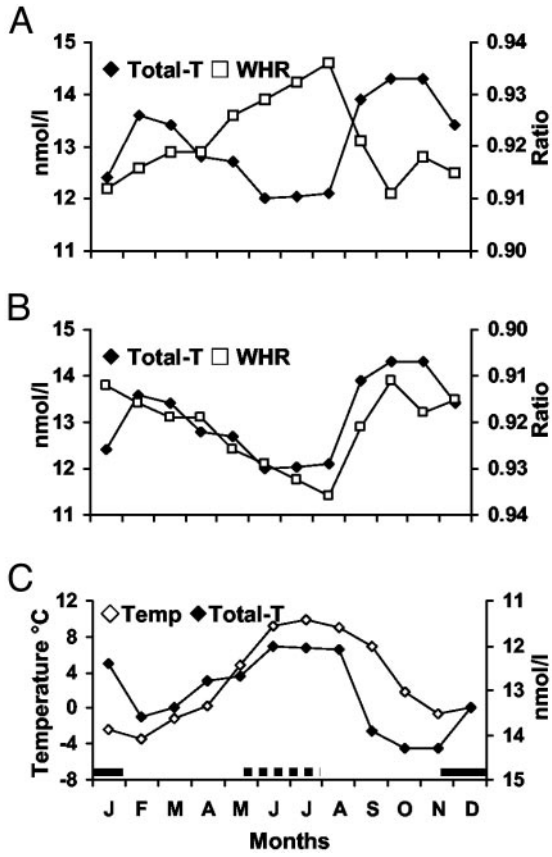

FIG. 2. Mean serum concentration of total testosterone (◆, total-T) (A) (nmol/liter) and mean WHR (□) by month and with reversed WHR axis (B). Mean monthly temperature degrees C (◇, temp) (C) in Tromsø and inverted levels of total testosterone (◆, total-T) (nmol/liter). From November 23 to January 21 (*bold black lines*), the sun is below the horizon, and it does not set from May 23 to July 23 (*bold dotted line*).

data from 4462 U.S. military veterans aged 32–44 yr, reported a seasonal peak of total testosterone in December (13). The highest levels of total testosterone in October and November found in the Tromsø cohort are in agreement with a cross-sectional study from Texas of 207 subjects (participants in a prevasectomy study), in which a peak of testosterone was found in November (12); in this study the pattern over the year was also presumed to be bimodal. In a longitudinal study of five Parisian men, the same investigators reported a peak in total testosterone levels in October (17). In contrast, Meriggiola *et al.* (20) reported the highest levels of testosterone in May and June based on a longitudinal study of 16 healthy men. A summer peak was reported by others (11, 18, 19, 21); in three of these studies, the high values continued into the fall (11, 18, 19).

Ten of the 15 published papers on seasonal variation of testosterone in men found significant variation (Table 1). The majority studied younger men: only three examined older men (11, 14, 19). In two studies (11, 13), separate analyses of different age groups did not change the results. In our study, results were unchanged when adjusted for age.

Although seasonality seems to be almost universal, its timing varies, possibly reflecting an effect of the duration of daylight or temperature on the reproductive system (26). Tromsø is located at 70 degrees north with extreme variation in daylight exposure: The sun is below the horizon from November 23 to January 21 and does not set from May 23 to July 23. However, in a longitudinal study of 24 men from north Finland, where the length of day in summer and midwinter is comparable to Tromsø, testosterone levels showed only a small and nonsignificant increase in July (23). In the Finnish study, melatonin showed seasonal variation, with peaks in May and December. However, no association between melatonin and testosterone levels was found, making variation in daylight unattractive as an explanation for the testosterone variation. Furthermore, long-term melatonin administration did not alter pituitary-gonadal hormone secretion in a study of normal men (27). Melatonin was not measured in the present study.

We explored the possibility that LH could be responsible for the seasonal pattern of testosterone, and a small seasonal variation for LH was observed in Tromsø. This observation is in agreement with five other studies (11, 12, 19, 20, 23) that also found an annual LH variation and in contrast to one study in which no annual variation was found (21). In one study, a temporal relationship between the peaks of LH and testosterone was suggested (20), which, however, was not the case in the Tromsø study.

Because SHBG is positively associated with testosterone and negatively associated with WHR, the seasonal variations of SHBG could also affect the seasonal variations in both testosterone and WHR. However, adjusting for SHBG in our analysis did not materially change the seasonal pattern of total testosterone, and no seasonal association between SHBG and total testosterone levels or SHBG levels and WHR was found.

We found only one study of the seasonal variation of SHBG; in this longitudinal study of 20 healthy young men from Barcelona, seasonal variation was found with peak values in January (21). In Tromsø the highest SHBG levels were observed in the fall, and the lowest levels were found in December and January. Compared with Tromsø, the peaks and nadirs are almost inverted. We have no explanation for the diverging results other than the difficulty of comparing findings in a small longitudinal study of young men with our study of men from a general population.

Our finding of an inverse seasonal relation between total testosterone and temperature is novel because temperature data were not reported in any of the other published studies (Table 1). The climate in Tromsø is arctic, but winters are not extremely cold because of the Gulf Stream. In regions with cold winters and moderate summers, the number of human conceptions has been reported to correlate positively with temperature (26). This observation does not necessarily mean that higher testosterone levels are present, and testosterone levels were not associated with conception success in an investigation of how to select sperm donors (28). We do not suggest that changes in temperature are responsible for the seasonal variation of testosterone. Temperature variation could, however, be responsible for change in diet and physical activity leading to changes in WHR.

We postulated that the observed seasonal variations in both total and free testosterone observed in Tromsø could be

due to seasonal changes in weight or physical activity. We found no annual variation in self-reported physical activity or measured BMI but a strong seasonal variation in WHR. Variation in WHR represents variation in abdominal fat mass and is supported by a study showing body fat to be highest in the summer and early fall (29). Zahorska-Markiewicz and Markiewicz (29) suggest that fat is the main fuel oxidized in the winter, and a lower utilization of fat as a metabolic substrate in summer could account for accumulation of body fat. Visceral fat is readily mobilized by lifestyle changes in energy balance (30), making seasonal variation in WHR a plausible explanation for the observed inverse relationship between testosterone levels and WHR during the year. This explanation is compatible with other reports that increased abdominal obesity is associated with low circulating testosterone levels in men (31). However, the opposite seems more plausible because lower testosterone have been reported to predict central obesity (32), and clinical trials have shown that replacement doses of testosterone increase muscle mass and decrease fat mass in men with low testosterone levels (33–37), suggesting that lower testosterone levels may play a causal role in the visceral fat accumulation.

Alcohol intake, particularly heavy consumption of beer, is known to cause a "beer belly," but adjusting for alcohol did not change the seasonal pattern of WHR. There was a seasonal variation in reported alcohol intake, and residual confounding cannot be excluded. Reported physical activity did not vary during the year and did not explain the variation in WHR. Physical activity is not precisely estimated from self-report, however, and misclassification could have obscured these associations.

This is the largest population-based study of the seasonal variation of sex hormones, but it does have several limitations. Hormone levels were based on a single sample, drawn between 0800 and 1600 h. Preferably, samples should have been drawn in the morning to avoid the diurnal variation. Sample hour was associated with both total and free testosterone, with the expected higher levels of testosterone in the morning. Adjusting for time of venipuncture did not change any of the associations or lack of associations, however. Serum samples were frozen at −70 C for approximately 6.5 yr, with hormone levels measured when samples were thawed for the first time. Levels of steroid hormones have been shown to be relatively stable in frozen serum (38, 39). SHBG is stable in short-term frozen storage (39), but one study (40) reported reduced levels after longer storage. However, this factor would not be expected to alter the ordinal associations for the observed levels. We did not measure free testosterone, but the calculation we used was recently evaluated by three different investigators and found to be a simple and reliable index of free testosterone (25, 41, 42). Calculation of free testosterone by this algorithm is based in part on SHBG concentrations, which also showed a seasonal variation. Laboratory drift is not likely to explain seasonal variation in sex hormones because all hormones were assayed at the same time in the same laboratory. Obviously, laboratory drift would not explain seasonal variation in WHR.

The most important limitation, of course, is that the results are cross-sectional, and the direction of the associations cannot be determined. Thus, low testosterone could lead to a higher WHR or vice versa; whatever is true, temperature or some associated characteristic seem to change testosterone and WHR. It seems unlikely that self-selection of older and less healthy individuals (with lower testosterone levels) to attend the clinic in the summer would explain these results because seasonal patterns persisted almost unchanged in analyses adjusted for age and chronic disease.

Finally, these finding in north Norway might not relate to men living in geographical areas with less extreme seasonal variation.

In conclusion, Tromsø study data show strong evidence of a seasonal variation of total and free testosterone and note a parallel inverse seasonal variation in WHR and temperature not previously described. Prospective studies are needed to establish the direction of the association and its etiology. These findings are clinically important because free testosterone levels may vary as much as 31% by season, and studies of change after medical and lifestyle intervention must consider seasonal variation of hormones before concluding that levels have increased or decreased over time.

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