Endocrine and Clinical Correlates of Myostatin Serum Concentration in Men—the STRAMBO Study

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Context: Myostatin is expressed mainly in skeletal muscle cells and acts as an inhibitor of muscle growth and differentiation. However, data on the determinants of serum myostatin concentrations in humans are limited.

Objective: The aim of the study was to assess the correlates of serum myostatin concentrations in men.

Design: We conducted a cross-sectional analysis of the STRAMBO cohort.

Setting: Men holding private health insurance coverage with Mutuelle de Travailleurs de la Région Lyonnaise were included in the study.

Participants: A total of 1121 male volunteers aged 20–87 yr participated in the study.

Interventions: Nonfasting blood samples were collected.

Main Outcome Measures: We measured the association of the investigated variables with circulating myostatin levels.

Results: Serum myostatin levels increased slightly with age until 57 yr and then decreased. Circulating myostatin levels showed circannual variation, with the highest concentration in spring. In men older than 57 yr, serum myostatin levels decreased across increasing quartiles of body mass index and of total central and peripheral fat mass ($P < 0.05$ to < 0.001). Serum myostatin levels were positively correlated with serum levels of 25-hydroxycholecalciferol (25OHD), even after adjustment for season. Average myostatin levels were 0.47 SD higher in men with 25OHD above 40 ng/ml, compared with those with 25OHD below 20 ng/ml ($P < 0.05$). Current smokers had lower myostatin concentration. Neither current physical activity nor serum levels of PTH, testosterone, and 17β-estradiol were associated with myostatin concentrations.

Conclusions: In men, circulating myostatin levels show seasonal changes and are associated with age, body mass index, fat mass, smoking, and 25OHD levels. **(*J Clin Endocrinol Metab* 97: 3700–3708, 2012)**

Myostatin (growth-differentiation factor 8) is a member of the TGF-β superfamily (1). It is expressed mainly in muscle. Myostatin inhibits muscle growth (2). Loss-of-function mutations in its gene are associated with high muscle mass phenotype. A boy with a point mutation in the myostatin gene lacked mature myostatin protein,

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Abbreviations: AFTC, Apparent free testosterone concentration; ASM, appendicular skeletal muscle mass; BMI, body mass index; CV, coefficient of variation; GFR, glomerular filtration rate; 25OHD, 25-hydroxyvitamin D; RASM, relative ASM index.

resulting in very high muscle mass and strength, but no extramuscular abnormalities (3).

Clinical studies on serum myostatin and its clinical correlates are limited. They are focused on specific interventions or diseases such as intensive physical training, heart failure, chronic obstructive pulmonary disease, severe obesity, HIV infection, end stage liver disease, or stroke (4–10). Few studies have evaluated serum myostatin levels in the general population, normal values, age-related changes, or physiological correlates. Moreover, they were made in small and nonrepresentative groups. Data on the age-related differences in serum myostatin levels are limited and inconsistent (11, 12). Myostatin expression was increased in the skeletal muscle cells in heavy smokers and in chronic alcoholics (13, 14). Serum myostatin levels were inversely correlated with fat-free mass in young healthy men and HIV-infected men (15). By contrast, serum myostatin was not correlated with quadriceps muscle mass and strength in men from the general population (11). Myostatin expression was elevated in extremely obese women (4) and decreased in obese patients after biliopancreatic diversion (16). Susceptibility to obesity varied according to myostatin gene polymorphisms (17). However, these results cannot be compared for methodological reasons because they assessed myostatin mRNA *vs.* protein and used various immunoassays detecting different forms of myostatin.

To our knowledge, seasonal variation of serum myostatin levels and their associations with age, lifestyle, hormones, muscle mass, and strength in the general population have not been thoroughly studied. Therefore, our aim was to investigate these associations cross-sectionally in a cohort of men aged 20 to 87.

Subjects and Methods

Participants

The STRAMBO study is a single-center, prospective cohort study of skeletal fragility and its determinants in men performed as a collaboration between Institut National de la Santé et de la Recherche Médicale and Mutuelle de Travailleurs de la Région Lyonnaise (MTRL) (18). The study was approved by the local ethics committee and is conducted in agreement with the Helsinki Declaration of 1975 and 1983. Letters inviting participation in the study were sent to a randomly selected sample of men aged 20–87 from the MTRL lists living in greater Lyon. During the recruitment (2006–2008), 1169 men provided informed consent to participate. This analysis was performed in 1121 men who completed bone densitometry and had valid measurements of the investigated parameters. All men able to provide informed consent, to answer the questionnaire, and to participate in the exams were included. No specific exclusion criteria were used.

Serum measurements

Nonfasting serum was collected at 1300 h and stored at −80 C. Myostatin was measured by a competitive ELISA (Immundiagnostik AG, Bensheim, Germany) based on polyclonal antibodies raised in a rabbit against recombinant human myostatin (19). The recombinant protein used for immunization is the full-length myostatin including the propeptide and the C-terminal mature myostatin protein. The antibodies detect all subunits and the protein dimer as validated by Western blot analysis and peptide mapping. Samples were measured using a microtiter plate reader (Fluostar Omega; BMG Labtech, Offenburg, Germany) at 450 nm against 620 nm as a reference. The detection limit is 0.8 ng/ml, and the dynamic range is 0 to 195 ng/ml. The intra- and interassay coefficients of variation (CV) were 10 and 15%, respectively. Sera from Holstein (normal myostatin) and Belgian Blue cows (premature stop codon of the myostatin gene at amino acid 287 of prepro-myostatin, resulting in truncated C terminus) were also analyzed. Belgian Blue lack a normal C terminus that is required for proper dimerization of the mature myostatin protein. Two bovine sera obtained from Holstein cows (controls) and one from a Belgian Blue cow were diluted 1:10, heated to 95 C for 5 min, and then separated under non-reducing conditions at 12 C by 10% SDS-PAGE. Western blot analysis using our antibody revealed two bands in Holstein cattle, a 25-kDa band representing the dimer of mature myostatin, and a 50-kDa band, the monomeric full-length pro-myostatin (Fig. 1). Consistent with the absence of the mature myostatin protein, Belgian Blue serum lacked the 25-kDa band but revealed a 40-kDa band, most likely corresponding to the truncated pro-myostatin.

Serum 25OHD was measured with a RIA after acetonitrile extraction (DiaSorin, Stillwater, MN) (20). This assay includes 25-hydroxyvitamin D_2 ($25OHD_2$) and 25-hydroxyvitamin D_3 ($25OHD_3$) and cross-reacts with $24,25(OH)_2D_2$, $24,25(OH)_2D_3$, $25,26(OH)_2D_2$, and $25,26(OH)_2D_3$, but not with the C-3 epimers (3-epi-$25OHD_2$, 3-epi-$25OHD_3$) (21, 22). The detection limit was 3 ng/ml. Intraassay CV was 5–7% for the levels of 10–50

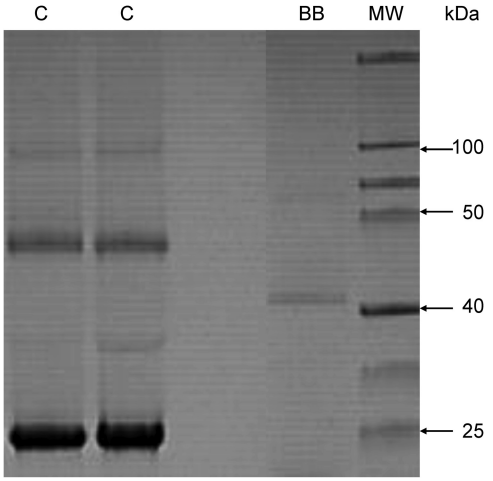

FIG. 1. Western blot analysis of bovine serum from two Holstein cows serving as controls (C) and one Belgian Blue cow (BB) using the polyclonal myostatin antibody from the immunoassay used in this study. In the Holstein cattle, a 25-kDa band, which represents the dimer of mature myostatin, and a 50-kDa band, the monomeric full-length pro-myostatin, were detected. Belgian Blue cows lacked the 25-kDa band (corresponding to mature myostatin), but revealed a 40-kDa band (corresponding to the truncated pro-myostatin). MW, Molecular weight marker.

ng/ml. Interassay CV were 9–11%. Serum PTH was measured by a human-specific two-site immunochemiluminescence assay (ELECSYS; Roche Diagnostics, Mannheim, Germany) (23). The detection limit was 3 pg/ml. The intra- and interassay CV were less than 5%. Serum testosterone was measured by tritiated RIA after diethyl ether extraction (24). Serum 17β-estradiol was measured using an ultrasensitive RIA (Cisbio-International, Gif sur Yvette, France) (25). SHBG was measured by RIA with an intraassay CV of 3.8% and an interassay CV of 4.6% for 35 nmol/liter (Cisbio-International) (24). Apparent free testosterone concentration (AFTC) and bioavailable 17β-estradiol level were calculated as described (26, 27).

Lifestyle and comorbidities

Men completed an interviewer-administered questionnaire. Lifestyle, history of falls, medical history, and medication use were self-reported without formal ascertainment. Assessment of smoking habits included categorization (current, former, never smoker), duration, number of cigarettes per day, and for former smokers, time since cessation. Current alcohol intake was assessed by average weekly amount of wine, beer, and liquors. Current caffeine intake was assessed by average weekly number of coffee cups. Calcium intake was estimated using a food-frequency questionnaire adapted to French alimentary habits (28). Leisure physical activity was calculated on the basis of the overall amount of time spent gardening and doing the housework. Current leisure sport physical activity was assessed on the basis of the overall amount of time spent practicing sport activities. Seasonal activities were averaged on the entire year. Occupational physical activity was evaluated according to a self-reported four-level scale (low, moderate, relatively high, and high) corresponding to the longest period during the professional activity. Participants self-reported chronic diseases (ischemic heart disease, diabetes mellitus, hypertension, Parkinson's disease, history of stroke), their duration, and treatment. Comorbidities were not ascertained. Weight and height were measured in light clothes without shoes using standard clinical equipment.

Measurement of appendicular skeletal muscle mass (ASM)

Body composition was assessed by dual-energy x-ray absorptiometry using Hologic Discovery A device (Hologic, Waltham, MA). The software provides values for lean mass, fat mass, and bone mineral content for the whole body and specific regions. ASM was calculated as the sum of lean soft tissue in the right and left arms and legs. The limbs were isolated from the trunk using manually adjusted computer-generated lines. The legs and arms were defined as the soft tissue extending from a line drawn through and perpendicular to the axis of the femoral neck and angled with the pelvic brim to the phalange tips and as the soft tissue extending from the center of the arm socket to the phalange tips, respectively. Relative ASM index (RASM) was calculated as ASM/body height^2 (kg/m^2).

Measurement of grip strength and physical performance

The grip strength was measured by a handheld sphygmomanometer (Martin Vigorimeter) on the dominant hand using the largest rubber bulb for all men (29). The mean of these three measures was used in statistical analyses. Physical tests of muscle strength and balance and the categorization of the results have been previously described (30).

Statistical analysis

Statistical analyses were performed using the SAS 9.1 software (SAS, Cary, NC). Data are presented as the mean ± SD or median and interquartile range. Variables with non-Gaussian distribution were log-transformed. Relation of myostatin levels with age was modeled by the PROC LOESS (Automatic Smoothing Parameter Selection). The AICC (corrected Akaike information criterion) *vs.* smoothing parameter plot was used to ensure that the smoothing parameter value corresponded to the global minimum of the AICC criterion. Associations between linear variables were assessed by simple and partial Pearson's correlation coefficient and by linear regression. The sensitivity analysis was performed to check the validity of the split point. We chose split point every other year between 50 and 70 yr of age and compared correlation and regression coefficients before and after the split point.

The association between class variables and log-myostatin was assessed by backward analysis of covariance. The initial model included age, weight (or fat, lean, RASM), height, time spent outdoors, 25OHD, PTH, testosterone, 17β-estradiol, glomerular filtration rate (GFR), calcium intake (continuous), current smoking (yes/no), comorbidities (ischemic heart disease, hypertension, diabetes: yes/no), alcohol intake (quartiles), and month of blood collection. The variables with $P < 0.15$ were retained in the final model. For the assessment of the seasonal changes, the final model was adjusted for age and 25OHD (and fat in men aged >60 yr). Associations between continuous variables were assessed using multivariate linear regression including confounding variables: age, weight (or RASM), hormones, comorbidities, physical activity, and grip strength. The association of serum myostatin levels and smoking or alcohol intake was assessed by analysis of covariance. Smoking was defined as classes, and the final model was adjusted for age, 25OHD, month, alcohol intake, and height. Average weekly alcohol intake was divided into quartiles. The associations of hormonal levels with serum myostatin was assessed by analysis of covariance using various thresholds (quartiles, thresholds based on mean and SD in young men). For 25OHD, traditional thresholds (20, 30, and 40 ng/ml) were studied. The final model was adjusted for age, fat, month, smoking, alcohol intake, time spent outdoors, GFR, and hypertension. For the association between myostatin levels and month of blood collection, the highest coefficient of determination was obtained by the third degree polynomial model. For the calculation of the partial correlation coefficients corrected for month, we used the differences between log-transformed myostatin levels and the expected values which were calculated using the regression equation.

Results

Age-related changes

Table 1 presents average serum myostatin levels and SD values in 10-yr age groups. The association between myostatin and age showed a split point at the age of 57 (Fig. 2). Serum myostatin levels correlated positively with age in 296 men age 57 or younger ($r = 0.11$; $P < 0.05$) and

TABLE 1. Reference normal values of the serum concentration of myostatin in men according to age group

Age range (yr)	Myostatin (ng/ml)
20 to 30 (n = 76)	30.5 ± 9.5
>30 to 40 (n = 69)	26.7 ± 10.6
>40 to 50 (n = 88)	28.3 ± 10.1
>50 to 60 (n = 91)	32.7 ± 10.1
>60 to 70 (n = 314)	30.6 ± 11.9
>70 to 80 (n = 345)	28.4 ± 12.3
>80 (n = 121)	28.9 ± 10.4

Data are expressed as mean ± SD. The arithmetical mean and SD were calculated from 1121 samples on the basis of the log-transformed values after exclusion of 17 outliers.

increased 0.10 SD per decade. In 825 men aged more than 57, serum myostatin decreased with age ($r = -0.07$; 0.09 SD per decade; $P < 0.05$). The correlation and regression coefficients differed between the groups ($P < 0.01$). Other thresholds between 50 and 70 yr of age provided lower correlation and regression coefficients and weaker differences in the coefficients between younger and older men. Thus, on the basis of these analyses (LOESS curve, correlation, regression), we made the analyses separately in men aged 57 or less and those older than 57.

Men aged 57 yr or less

Average age was 39 yr (Table 2). Eighteen men self-reported hypertension, 11 diabetes mellitus, and 23% were current smokers. Serum myostatin levels showed seasonal variation with higher values during the spring ($P < 0.05$) (Fig. 3A). Serum myostatin levels were not associated with other parameters (smoking, alcohol consumption, current and former physical activity, muscle mass, grip strength, 25OHD, PTH, testosterone, AFTC, total and bioavailable 17β-estradiol) regardless of the statistical approach.

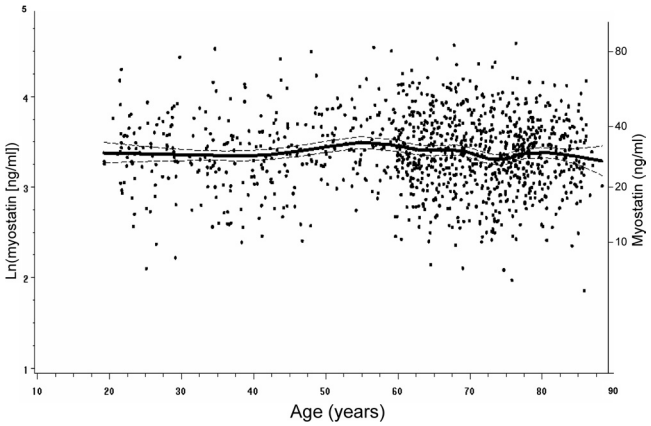

FIG. 2. Association between the serum myostatin levels and age in 1121 men aged 20 to 87 assessed by the LOESS procedure. Left y-axis presents the logarithm of the serum myostatin concentration (ng/ml). Right y-axis presents the absolute values of the serum myostatin concentration on a logarithmic scale. The *solid line* presents the model and the *dotted lines* present the 95% confidence interval.

Men aged more than 57—physical activity, muscle mass, and force

The average age was 72 yr. Serum myostatin levels correlated negatively with body mass index (BMI) (partial $r = -0.08$; $P < 0.05$). In the multivariable models, serum myostatin decreased across increasing BMI quartiles ($P < 0.05$) and was 3% (0.24 SD; $P < 0.05$) lower in the highest BMI quartile (>29.7 kg/m^2) compared with the lowest one (<25.1 kg/m^2).

After adjustment for confounders, serum myostatin correlated negatively with total, central, and peripheral fat mass ($r = -0.11$; $P < 0.005$) and decreased across the increasing quartiles of fat mass ($P < 0.005$ to < 0.001) (Fig. 4A). In the highest quartile of total fat, serum myostatin was 4.4% lower (0.36 SD; $P < 0.005$) *vs.* the lowest quartile. Multi-adjusted differences in the serum myostatin levels between the extreme quartiles were 8.4% (0.50 SD; $P < 0.005$) for central fat and 3.8% (0.32 SD; $P < 0.01$) for peripheral fat.

Serum myostatin was not associated with the intensity of physical activity, regardless of its type (current leisure and former competitive sport activity, time spent outdoors, occupational physical activity) and regardless of the statistical approach ($P > 0.3$). Moreover, serum myostatin level was not associated with RASM, grip strength, a history of falls, or physical performance regardless of the statistical approach (continuous, various thresholds) ($P > 0.25$).

Men aged more than 57—smoking and alcohol intake

Current smokers had lower serum myostatin levels than nonsmokers (median, 24.9 *vs.* 30.1 mg/liter; $P < 0.005$). The difference was significant in multivariable models ($P < 0.005$). Serum myostatin levels did not differ between men who smoked more than eight cigarettes daily (median) and those who smoked less (31). Serum myostatin levels did not differ between former smokers and men who never smoked. Serum myostatin levels were not associated with alcohol intake after adjustment for confounders ($P > 0.12$).

Men aged more than 57—25OHD, PTH, calcium intake, and season

Serum myostatin and 25OHD levels correlated positively in 804 men who did not take vitamin D supplements ($r = 0.13$; $P < 0.001$). Serum myostatin levels increased by 0.13 SD per 10 ng/ml of 25OHD. Serum myostatin displayed a circannual variability with the highest levels dur-

TABLE 2. Descriptive analysis of the STRAMBO cohort

	Men aged ≤57 yr	Men aged >57 yr
n	296	825
Age (yr)	39 ± 11	72 ± 8
Body weight (kg)	79 ± 12	78 ± 11
Body height (cm)	176 ± 7	168 ± 6
BMI (kg/m^2)	25.6 ± 3.8	27.6 ± 3.6
Smoking habits, n (%)		
Current	69 (23.4)	55 (6.7)
Former	76 (25.8)	511 (61.9)
Never	151 (50.8)	259 (31.4)
Alcohol intake (g/wk)[a]	46 [0; 107]	110 [16; 236]
Coffee (cups/wk)[a]	14 [3; 25]	10 [5; 18]
Calcium intake (mg/d)	810 ± 308	767 ± 245
Time spent outdoors (h/wk)[a]	6 [4; 9]	7 [4; 11]
Leisure physical activity (h/wk)[a]	2 [1; 4]	3 [0; 8]
Leisure sport activity (h/wk)[a]	2 [1; 3]	1 [0; 2]
Occupational physical activity, n (%)		
Low	95 (32.4)	188 (22.8)
Moderate	92 (31.1)	239 (29.0)
Relatively high	82 (27.7)	224 (27.2)
High	26 (8.9)	174 (21.1)
Self-reported diseases, n (%)		
Ischemic heart disease	3 (1.0)	126 (15.3)
Hypertension	10 (3.4)	333 (40.4)
Diabetes mellitus	9 (3.0)	106 (12.9)
History of stroke	1 (0.3)	31 (3.8)
Lean body mass (kg)	62.4 ± 7.4	58.3 ± 6.8
RASM (kg/m^2)	8.52 ± 0.99	8.22 ± 0.86
Muscle force (kPa)	94.2 ± 19.7	69.7 ± 17.0
Fat body mass (kg)	16.5 ± 6.1	19.8 ± 6.2
Myostatin (ng/ml)[a]	32.2 ± 13.9	31.8 ± 13.4
	30.3 [23.5; 38.4]	29.3 [22.6; 38.6]
Calcium (mmol/liter)	2.41 ± 0.14	2.36 ± 0.17
Phosphate (mmol/liter)	1.12 ± 0.15	1.05 ± 0.16
25OHD (ng/ml)	23.8 ± 10.9	21.9 ± 9.6
PTH (pg/ml)[a]	36 [28; 44]	44 [34: 57]
Testosterone (nmol/liter)	12.9 ± 5.0	11.7 ± 5.2
AFTC (pmol/liter)	311 ± 106	239 ± 91
17β-estradiol (pmol/liter)	51.5 ± 19.2	52.9 ± 20.5
Bioavailable 17β-estradiol (pmol/liter)	40.6 ± 16.3	37.5 ± 15.1
SHBG (nmol/liter)	29.8 ± 13.3	43.1 ± 21.2
C-reactive protein (mg/liter)[a]	1.07 [0.51; 1.97]	1.67 [0.85; 3.28]

Data are presented as mean ± SD, unless specified otherwise.

[a] Variables with non-Gaussian distribution are presented as median [first quartile; third quartile].

ing the spring ($P < 0.001$). Both season and 25OHD contributed to the variability of myostatin levels ($P < 0.001$ and $P < 0.05$). After adjustment for confounders including month and BMI (or total fat mass), serum myostatin levels increased across the 25OHD groups (p for trend <0.05), with the highest values in men with 25OHD greater than 40 ng/ml (Fig. 4B). Average myostatin levels were 0.47 SD higher in men with 25OHD above 40 ng/ml *vs.* men with 25OHD below 20 ng/ml ($P < 0.05$). The circannual variability of myostatin levels remained significant after adjustment for confounders including 25OHD ($P < 0.001$) (Fig. 3B). Average myostatin level was 0.48 SD higher in the spring compared with the autumn ($P < 0.005$).

PTH levels had a circannual variation ($P < 0.001$) with 0.41 SD higher levels ($P < 0.001$) in the winter compared with summer months. Serum myostatin levels were not associated with serum PTH levels regardless of statistical approach. Adjustment for PTH did not influence the association of myostatin with 25OHD or season.

Serum levels of testosterone (total and AFTC), 17β-estradiol (total and bioavailable), GFR, and calcium intake were not associated with the myostatin concentration regardless of the statistical approach.

Discussion

In men, serum myostatin slightly increased until the age of 57, then decreased. Before and after the age of 57, serum myostatin levels were higher in spring than in autumn. In

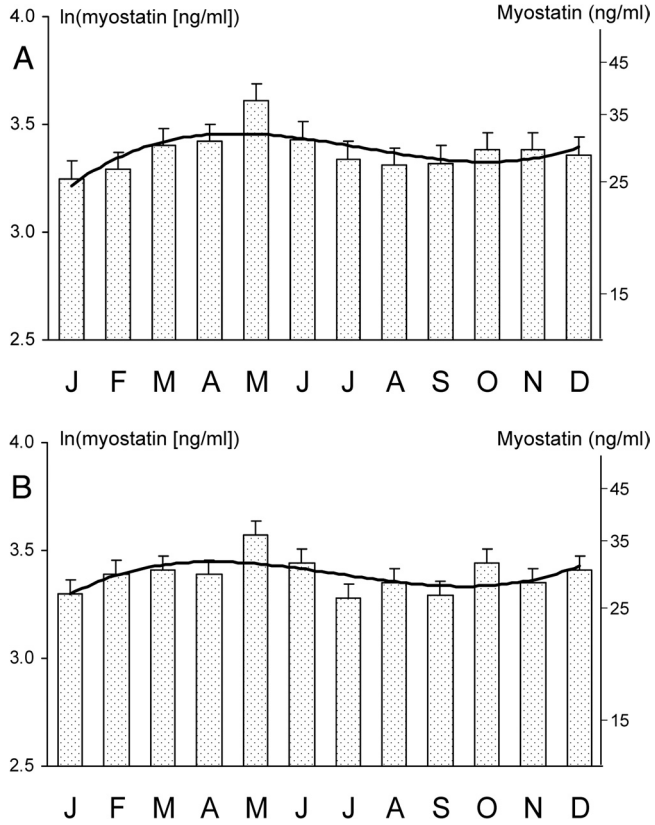

FIG. 3. Average values of log-transformed myostatin levels according to the month of blood collection [January (J) to December (D)]. *Bars* correspond to mean ± SEM, *continuous line* corresponds to third degree polynomial model. A, 296 men aged ≤57 yr; B, 825 men aged >57 yr. Left y-axis presents the logarithm of the serum myostatin concentration (ng/ml). Right y-axis presents the absolute values of the serum myostatin concentration on a logarithmic scale.

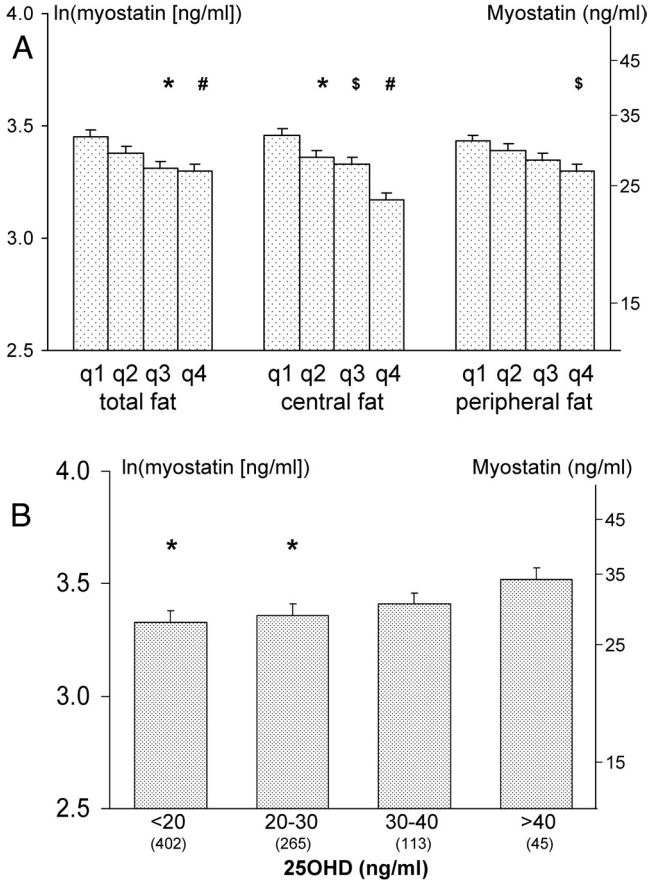

FIG. 4. A, Association of serum myostatin concentration with total, central, and peripheral fat mass. q1, q2, q3, and q4—first (lowest), second, third, and fourth (highest) quartiles, respectively. Thresholds of the quartiles: total fat mass (15.45, 19.10, and 22.99 kg), central fat mass (7.82, 10.39, and 12.75 kg), peripheral fat mass (7.24, 8.73, and 10.57 kg). *, $P < 0.05$; $, $P < 0.01$; #, $P < 0.005$ *vs.* the lowest quartile. B, Association between myostatin and 25OHD levels in men aged 57. *Numbers in brackets* represent the n-number. *, $P < 0.05$ *vs* men with 25OHD >40 ng/ml. Data are presented as multi-adjusted means and SEM. Left y-axis presents the logarithm of the serum myostatin concentration (ng/ml). Right y-axis presents the absolute values of the serum myostatin concentration on a logarithmic scale.

men older than 57 yr, current smoking, and lower 25OHD concentrations were associated independently of each other with lower serum myostatin levels.

The correlation coefficients between myostatin levels and age were weak but significant in this large cohort. Previous data on the age-related differences are discordant (11, 12); however, they were obtained in smaller groups.

We used an immunoassay that measures all forms of myostatin and does not distinguish between the active C-terminal dimer of myostatin and the N-terminal propeptide. Therefore, the myostatin levels measured in our study are higher than those found using immunoassays more specific for the active C-terminal dimer (11, 12). The levels that we measured may reflect synthesis and secretion rate of myostatin. However, they do not assess biological activity of myostatin.

A caveat in the interpretation of circulating myostatin is that myostatin, which inhibits muscle growth, is synthesized by the muscle itself. Thus, the negative correlation between serum myostatin levels and age may reflect age-related local decrease in muscle mass, effect of age-related factors, or the compensatory mechanism attempting to limit the decrease in muscle mass. Moreover, because myostatin exerts its biological activity locally in the muscle, the circulating levels of myostatin do not necessarily reflect its local amount in different tissues.

A significant circannual variation of the serum myostatin levels with higher levels in spring was found in both younger and older men. This association is independent of the 25OHD levels. Seasonal variations of myostatin expression were described in animals; however, they varied according to the animal species and even according to the muscle. In small birds, myostatin mRNA expression in the pectoralis muscle is lower in winter, which coincides with higher muscle mass (32). This represents adaptation to cold weather in small birds in which shivering of larger muscles is a source of heat and improves the thermoregulation. In ground squirrels, myostatin mRNA expression

decreases during winter hibernation in muscles that are necessary for respiration (33). By contrast, myostatin did not decrease in skeletal muscles undergoing atrophy, which may decrease oxygen intake. The speculation about the role of myostatin in the adaptive mechanisms is attractive, but data obtained in other animals are less clear (34, 35). In addition, data obtained in small animals, which have very high body surface compared with body mass, cannot be extrapolated to humans.

In older men, serum 25OHD and myostatin levels were correlated positively. Data on the effect of vitamin D on myostatin expression are limited. In a mouse myoblast cell line, 1α,25-dihydroxycholecalciferol (100 nM) decreased myostatin mRNA expression by 90% (36). In aged female rats (27 months), 1α-hydroxycholecalciferol (0.1 μg/kg) did not affect myostatin mRNA expression (37). However, these data were obtained using a high dose of active vitamin D form and cannot be compared with the relation between circulating 25OHD and myostatin. Moreover, positive association of 25OHD with muscle mass and strength was described in some (38, 39) but not all (40, 41) studies. Thus, the mechanism of the association between vitamin D and myostatin and its potential physiological role remains elusive.

We found a negative relation of myostatin with fat mass. In mice on a high-fat diet, myostatin inhibition increased energy expenditure and insulin sensitivity and also decreased weight gain and fat deposits in liver and muscle (42, 43). These data show a major role of myostatin in the regulation of energy and fat metabolism. However, clinical data on myostatin in obesity are scanty. Single nucleotide polymorphisms of the myostatin gene are associated with differences in body weight (17). In morbidly obese women (BMI >50 kg/m^2), myostatin levels in muscle and plasma were elevated (4, 45). In obese patients, serum myostatin decreased after gastric or biliopancreatic surgery (16, 45). By contrast, in obese adolescents, a training program induced weight loss that was associated with higher myostatin levels (46). Further studies are needed to elucidate the role of myostatin in weight control.

We found no significant association between self-reported current leisure physical activity and serum myostatin levels. Data on the association between physical activity and myostatin levels are discordant. Myostatin expression decreased after aerobic exercise in sedentary men aged 40–64 with hyperinsulinemia and in older women (7, 48). In young men, resistance training decreased myostatin expression and blood level in some (49, 50) but not all (51) studies. By contrast, physical activity training in obese children decreased body weight and increased serum myostatin (46). However, these studies are not comparable with ours. They present clinical experiments performed mainly in young subjects and using standardized protocols. Conversely, we studied men from the general population who self-reported various types of more or less regular leisure physical activity of light or moderate intensity. For seasonal activities, the time lag between the end of intensive activity and blood collection was not constant.

We did not find a relation between alcohol intake and myostatin levels. In rats, high doses of ethanol administered for 3–4 months increased myostatin mRNA content in muscle (44, 47). In chronic alcoholic individuals, especially in those with cardiomyopathy, myostatin content in cardiomyocytes was increased (14). However, these studies assessed myostatin and its mRNA in muscle and not in blood. Low alcohol intake in our study (median, 0.2 g/kg · d) cannot be compared with chronic alcoholics or with very high doses used in the experimental studies (16 g/kg · d for 4 months) (52). In some men, low alcohol intake may be the consequence of poor health status and of associated factors, *e.g.* poor mobility, medications, *etc.*

In our cohort, current smokers had lower myostatin levels than men who did not smoke. In eight long-term heavy smokers, myostatin expression in muscle biopsies was higher, whereas overall protein synthesis was lower in comparison with nonsmokers (52). Again, these data are not comparable with ours. First, we measured serum myostatin and not local expression. Second, the median tobacco consumption in the STRAMBO cohort was eight cigarettes per day, thus, much less than in the above study ($>$20 cigarettes/day) (31).

Our study has limitations. The cohort includes male volunteers who may be healthier than the general population, especially among older men. Lifestyle factors, such as smoking, alcohol intake, or physical activity were assessed by self-report. Several comorbidities that are associated with abnormal serum myostatin levels, *e.g.* heart failure, were not specifically assessed. Dual-energy x-ray absorptiometry may overestimate muscle mass, especially in older men. Blood was collected in the nonfasting state. In addition, it was not possible to measure other proteins that may bind to myostatin and influence its biological activity, *e.g.* follistatin, growth and differentiation factor-associated serum protein 1, or latent TGF-β binding protein 3.

Our study assessed the correlates of the serum myostatin levels in a large cohort of home-dwelling men. To the best of our knowledge, this is probably the largest study describing the endocrine and clinical correlates of circulating myostatin in men. We found higher myostatin levels during the spring. In addition, in men after 57 yr of age, higher fat mass, lower 25OHD levels, and current smoking are all independently associated with lower serum

myostatin concentrations. Our data need to be confirmed in other cohorts with a different ethnic and geographical background, and similar analyses should be performed in women. These studies should help us to understand biological mechanisms and clinical implications of our findings in metabolic and musculoskeletal diseases.

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