Decline in Muscle Mass With Age in Women: A Longitudinal Study Using an Indirect Measure

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Muscle mass is known to decline with age, but only limited longitudinal data exist to quantify the rate of loss. Using 24-hour urine creatinine, corrected for the contribution of dietary meat intake, we assessed the change with age prospectively in 107 women who provided a minimum of 3 sets of measurements spanning an average interval of 11.9 years, centered around age 55 years. The rate of change in 24-hour urine creatinine at that age averaged −0.94%/yr (95% confidence interval [CI], −1.24% to −0.64%/yr; $P < .001$). Change in creatinine excretion was directly correlated with change in weight ($P < .01$), with those gaining weight tending to gain both lean and fat mass. The rate of change in creatinine excretion with age in our subjects is similar to that described in published cross-sectional studies on age-related change in total body potassium and in longitudinal studies using ^{40}K and dual-energy xray absorptiometry. Our study is the largest longitudinal study reported to date and provides, perhaps, a more secure basis for estimating muscle mass change with age than has been available heretofore.
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MUSCLE MASS is generally recognized to decline with age.[1] Most of the studies establishing this secular change are cross-sectional in character[1] and, until recently, the few longitudinal studies have generally been of short duration. Longitudinal studies are generally to be preferred since they eliminate birth cohort effects and other biases that can distort the magnitude of effects found cross-sectionally.

Urine creatinine excretion in otherwise healthy individuals is a reflection of muscle mass.[2] Accordingly, one would anticipate that within-subject change in creatinine excretion would reflect a corresponding change in muscle mass. Forbes et al have explicitly validated this expectation in a series of investigations in normal subjects, in subjects with muscle wasting disorders, and following treatment with androgens.[3–7] Recently, Forbes[8] reported his cumulative experience with ^{40}K counting in 15 men and 5 women measured over periods of 21 to 38 years, and Gallagher et al,[9] their experience in 24 men and 54 women, both elderly, studied for an average of 4.7 years. Here, we describe estimation of age-related muscle loss based on analysis of urine creatinine excretion data accumulated prospectively in a cohort of 107 women who ranged in age from 35 to 45 at study inception in 1967, many of whom had been followed for 25 years.

MATERIALS AND METHODS

Subjects

The cohort from which our sample was derived consisted of 191 Roman Catholic nuns who were studied from 1967 through 1992 as inpatients on a metabolic research unit. The subjects have been more extensively characterized elsewhere.[10] Most were studied several times at 5-year intervals. Together they contributed 507 individual study measurement sets to this analysis. The subjects each gave written consent to the project, and the Creighton University Institutional Review Board approved the project after it came into existence (well after the inauguration of this project). When routine work-up revealed concurrent medical conditions that might interfere with muscle metabolism during a particular study, the data for such a study were eliminated from this analysis. For purposes of the analysis of age-related change in creatinine excretion, we confine our attention to those subjects on whom we had a minimum of 3 measurements, generally at 5-year intervals. We also concentrated solely on those whose data spanned age 50 years. A total of 107 women contributed slope values of creatinine on age that met these criteria.

Analytical Methods

Data were all accumulated while subjects were inpatients on a metabolic research unit, with full collection of excreta. Urine creatinine was measured by the Jaffe reaction,[11] performed manually early in the course of the study and then using various automated methods later (within-assay precision < 3%). The values for urine creatinine represent the daily means of a continuous 8-day set of 24-hour urine collections accumulated while the subject was consuming a constant diet prepared to match self-selected prestudy protein intake. Body weight was measured on a beam balance scale in the morning before breakfast and after emptying the bladder. Meat protein during study was calculated from the weighed meat sources used for the constant inpatient diets, using published values from food tables applicable at the time of study. (Grossly visible fat and fascia were stripped away from the meat prior to preparation and weighing, so that source of variability is eliminated in this calculation.)

Data Handling and Statistical Analysis

Because ingested meat contributes to urinary creatinine excretion, and because meat intake might well change over time in the same individual, it is desirable to adjust the measured values of creatinine to remove the variable contribution coming from exogenous sources. This was done empirically by examining, first, the bivariate relationship between urinary creatinine and ingested meat protein. Figure 1 is a graph of this relationship, and shows clearly that creatinine excretion rises as meat protein intake rises.

However, a likely contributor to this rise would be the fact that large people both eat more and have more muscle mass as well. Hence, the simple bivariate regression coefficient is likely to exaggerate the effect of meat protein. To correct for this we adjusted the slope for height and weight, using a stepwise multiple linear regression model (SPSS for Windows, 10.1, SPSS, Chicago, IL) incorporating both meat protein and various body size and intake variables. Both height and weight entered the model with highly significant contributions. The coefficient of the meat protein term in the final model was +0.00580 (95% confidence interval [CI], 0.0044 to 0.0072), as contrasted with a value

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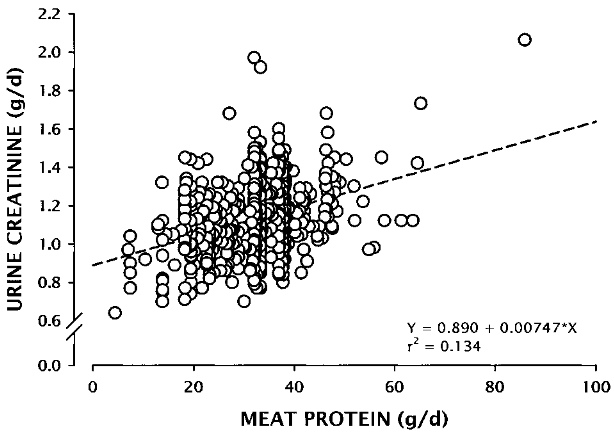

Fig 1. Scatterplot of 24-hour urine total creatinine excretion plotted against meat protein intake in 507 individual metabolic studies in 150 women.

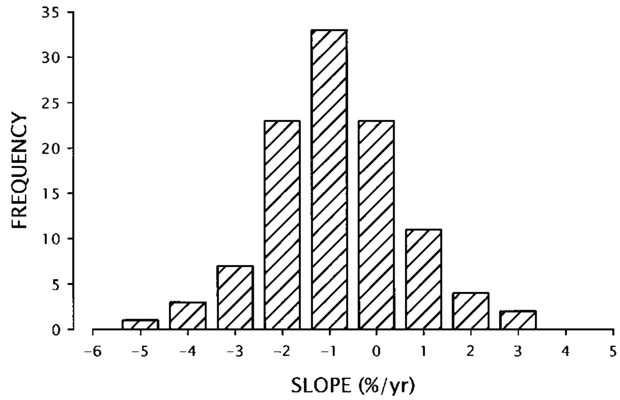

Fig 2. Frequency distribution of values for the slope of relative endogenous creatinine excretion (in %/yr) in 107 women. See text for details.

of +0.00747 for the slope of Fig 1. In brief, this value indicates that creatinine excretion rises by 5.8 mg for every gram of meat protein ingested. It was this value that was used to adjust downward the measured urine creatinine value so as to reflect creatinine coming solely from endogenous sources, as follows:

$$\mathrm{EndCr} = \mathrm{ExCr} - 0.0058 \cdot \text{meat protein},$$

where EndCr = creatinine of endogenous origin, and ExCr = total 24-hour excreted creatinine. (Creatinine and protein are in grams.)

In order to eliminate interindividual variability in creatinine excretion and muscle metabolism, each woman's values were normalized to the value obtained at the study performed closest to her age at 50 years, ie,

$$\mathrm{RelEndCr} = \mathrm{EndCr}(t)/\mathrm{EndCr}(50)$$

where RelEndCr = endogenous creatinine relative to age 50, EndCr(t) = endogenous creatinine at age t, and EndCr(50) = endogenous creatinine at age 50. Slopes were then computed through each woman's set of RelEndCr values, using standard Pearsonian regression methods and the SPSS statistical package.

RESULTS

Table 1 summarizes anthropomorphic and creatinine statistics for the 107 women contributing usable longitudinal data for this analysis. By design, the average age at the study providing the referent value for creatinine was about 50 years, and the average time span over which slopes were computed was 11.9 years (range, 7.8 to 16.1 years). The mean slope (±SD) was −0.94%/yr (±1.56). The 95% confidence limits around this estimate are −1.24% and −0.64%/yr. This slope value is highly significantly different from zero ($P < .001$) and indicates a decline in endogenous creatinine excretion (and hence in muscle mass as well) amounting to nearly 1%/yr. The distribution of slopes for the 107 individuals is shown in Fig 2. As can be seen, the distribution is symmetrical, and the variability from person to person doubtless reflects both real differences between individuals in change in muscle mass as well as analytical noise.

To explore one possible contributor to this distribution of values, we examined the correlation between weight change over the same period of observation and the creatinine slope. There was a statistically significant positive correlation ($P < .01$), with weight change accounting for 6.5% of the variation in creatinine slopes. The estimated creatinine slope at zero weight change was −1.1%/yr. In other words, those maintaining a steady weight were losing muscle and gaining a corresponding amount of fat.

Figure 3 plots the RelEndCr values observed in these women at approximately 5-year intervals, fitting the data to both linear (Fig 3A) and nonlinear (Fig 3B) models. The fit to both was extremely good ($r^2 > 0.96$). This presentation of the data is cross-sectional, but it is dependent on the analysis of the individual slopes, and its congruence with the slope data is shown by the fact that the tangent to the curve in Fig 3B at age 58 is −0.93%/yr, essentially the same as the mean slope (−0.94%/yr) across all 107 individuals.

Table 1. Pertinent Anthropomorphic and Outcome Variables in 107 Women Providing Longitudinal Data on 24-Hour Creatinine Excretion

Variable	Mean	SD	Range
Age at reference value (yr)*	50.0	1.4	47.5 to 52.4
Height (m)	1.625	0.057	1.49 to 1.79
BMI (kg/m^2)	23.8	3.7	17.8 to 38.2
Total creatinine (g)	1.150	0.163	0.773 to 1.597
Meat protein (g)	30.7	9.3	7.4 to 61.4
Endogenous creatinine (g)	0.972	0.158	0.584 to 1.391
Time span (yr)	11.9	2.5	7.8 to 16.1
Slope (%)	−0.94	1.56	−4.67 to +2.67

*Age at the study for which the value for creatinine was set to 1.0.

DISCUSSION

Ninety-eight percent of body creatine is in skeletal muscle[2] and a constant fraction of the body creatine pool is converted each day to creatinine.[2] Hence, it has long been recognized that daily creatinine excretion constitutes a good reflection of skeletal muscle mass. Forbes and Bruining,[7] in a series of 34 adults and children spanning a broad range of values for lean body mass (LBM) found a very tight correlation between LBM

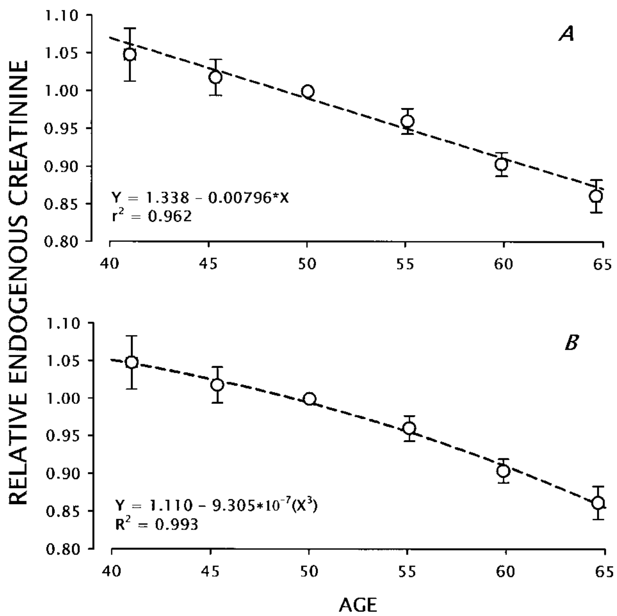

Fig 3. Plots of relative endogenous creatinine excretion values obtained at mean ages ranging from ~40 to ~65 years. (A) Linear fit and (B) nonlinear fit. (Error bars are 1 SEM.)

measured by ^{40}K counting and 24-hour creatinine excretion ($r^2 = 0.98$), with a coefficient for the creatinine term of 29.1 kg/g/d, meaning that each gram of creatinine excreted per day was derived from 29.1 kg LBM. In a later publication on this topic Forbes cites a similar, but somewhat lower value, 24.1 kg LBM/g creatinine.[12]

Despite this conceptually straightforward relationship, Lukaski downgrades creatinine as a measure of LBM because of low precision and accuracy.[2] These problems arise because of the variable (and usually uncontrolled) contribution of creatine and creatinine from ingested meat and the difficulty in obtaining fully complete 24-hour urine collections. The former can be particularly difficult in free-living subjects with changing diets, as several investigators[13,14] have shown that ingested creatine in meat is not immediately converted to creatinine, and that it takes several weeks for urine creatinine excretion to reach a new steady state after a change in meat intake.

However, both of these problems have been obviated in the present investigation. Study diets were constant, of known composition, and formulated to match prestudy protein intake. Additionally, urine creatinine was measured over an 8-day period on a metabolic unit proficient in complete specimen collection. The empirical correction factor we derived for meat (ie, 0.0058 g creatinine/g meat protein) is somewhat higher than that used by other investigators, in turn based on old published values for the creatine content of mammalian muscle meats.[15] Lykken et al,[13] for example, take a value of 0.00425 g/g, and some or all of the difference between that figure and ours may be due to the fact that our meat portions had all visible fat and fascia removed prior to weighing, a treatment that would remove nonmuscle tissue, and thus raise effective creatine concentration. Further, published values for muscle creatine make no provision for preformed creatinine in the meat, which would be promptly excreted and contribute directly to the daily urine creatinine excretion. Hence, we judge that our correction factor, which is actually not very different from those used by other investigators, is both plausible and preferable, particularly as it is based on the relationship actually observed in our subjects.

The concern of much of the literature in this field has been the accurate estimation of the various components of body composition in single measurements in individuals, rather than the estimation of secular trends within individuals. While it is generally conceded that muscle mass is lost with age, until recently available estimates had been based exclusively on relatively small samples and on cross-sectional data. Forbes,[8] reporting his cumulative experience, found a rate of change of −1.5 kg fat-free mass (FFM) per decade in measurements spanning 21 to 38 years obtained in a small sample of 20 individuals (15 men and 5 women). It was not possible in this analysis to estimate whether this rate of change was linear, or accelerated with advancing age.

Our longitudinal data complement these observations. They indicate a rate of loss of muscle mass in women at mid life of slightly less than 1%/yr. Assuming a muscle mass in our subjects in the range of 20 to 25 kg, our rate of loss calculates to 1.9 to 2.3 kg per decade, which is somewhat higher than Forbes' estimate. However, Forbes' studies were based on body ^{40}K content, which includes nonmuscle cellular organs that lose less mass with age (if at all). Hence, one could expect a somewhat lower value with his method. Also, the starting age in Forbes' study was as young as 24 years, an age when change in muscle mass may be close to zero, as suggested both in Fig 3B from our study and from the cross-sectional data of Cohn et al (see below).[17]

The study of Gallagher et al,[9] by contrast, was concentrated on individuals with average ages in the 8th decade of life. They observed losses of total appendicular muscle that, expressed per decade, amounted to about 2% in men and 1% in women, considerably lower than our values for *total* skeletal muscle loss.

In Fig 3 we chose to look beyond a linear fit (Fig 3A), despite a high value for r^2, simply because of its somewhat less plausible predictions outside the range of the values used to generate the fit. In fact, the data appear to exhibit a slight upward convexity and the curvilinear model used in Fig 3B not only provides a better value for r^2, but predicts only about a 10% difference in LBM between age 20 and age 50 (while the linear equation of Fig 3A predicts a difference nearly twice as large).

It may be of interest to compare these findings with the more abundant cross-sectional data. Much of the latter have been derived from the studies of Cohn et al at Brookhaven National Laboratory over the years.[16,17] In one of the more recent reports from this group of investigators,[17] data were presented for 134 adult men and women covering a 50-year age span. Women aged 75 years had about 30% less total body potassium (TBK) (and hence LBM) than women age 35, and men about 18% less. But the sample sizes at each age were small, the differences varied considerably by reference age, and no attempt was made to develop an aggregate pattern of loss using the data for all of the subjects.

In order to compare our data with theirs, we have converted their absolute TBK values to relative values at age 55 (their closest time point to our reference age) and combined the data for men and women. (While men and women have very different values for LBM and TBK, normalizing each set to the sex-specific value at a given age removes this sex difference, and in this case we found no significant difference between the sex-specific relative TBK values for 5 of the 6 age periods studied. Hence, combining the data for the 2 sexes seems reasonable.)

Figure 4A presents these data for normalized TBK values, with a regression line through them using the same type of model as used in Fig 3B. Here the upward convexity of the data points and goodness of fit to the model are apparent. Figure 4B compares the 2 curves (our longitudinal data and Cohn's cross-sectional data), showing graphically the general similarity of the 2 data sets. The small differences between the 2 curves may have several bases. First, the confidence limits of the parameters of the 2 regression equations overlap somewhat, and thus the apparent difference may not be real. Second, as already noted in discussing Forbes' data,[8] TBK measures nonmuscular and as well muscular tissues, and the slower rate of loss with age for nonmuscular organs will reduce the age-related slope for the composite to some extent. In any case, what both curves show is that muscle mass loss up to age 50 is small, but that rate of loss accelerates with age, reaching 1%/yr by the end of the 6th decade and increasing still further with advancing age. Finally, our finding of a somewhat greater rate of loss after age 60 than found by Cohn et al[17] may reflect the expected difference between longitudinal and cross-sectional studies, with the cross-sectional data, in this case, underestimating the true rate of loss.

While a decline in muscle mass, such as we and others have described, seems to characterize the population, it should not be concluded that such a loss is inevitable. Buskirk[18] described this loss with age as "a slow adaptation to a sedentary lifestyle." It is worth noting that many of our women exhibited positive

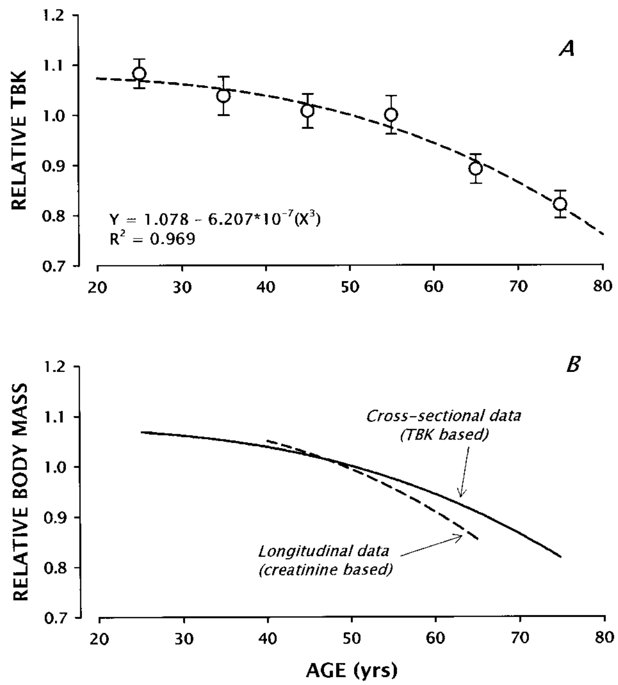

Fig 4. (A) Plot of the TBK data of Cohn et al[17] normalized to values at age 55, and fitted to the same type of nonlinear equation as used in Fig 3B. (Error bars are 1 SEM.) (B) Plot of 2 curves, the present study's longitudinal data and Cohn et al's[17] cross-sectional data, showing their general similarity.

slopes. Muscle mass has been described as lost in animals in captivity, but not in free-running animals.[19] Forbes[8] also concluded that loss was not inevitable. He noted that some of his subjects, like our women, actually gained FFM with age, and that this change was positively correlated with weight change. Like Forbes, we noted that there was significant muscle loss even at zero weight change, and therefore a counter balancing gain in body fat.

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