Different Limit to the Body's Ability of Increasing Fat-Free Mass

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It is a common understanding that fat-free mass (FFM) increases with body weight. However, limited information is available as to the relationship between weight increase and changes in body composition. We performed the present study to determine quantitatively the relationship between body composition, total body weight, age, and sex. Body composition data were obtained by isotopic dilution on 273 subjects ranging in body mass index (BMI) from about 13 to 70 kg/m^2. Adipose free tissue (AFT) was modeled as a nonlinear, increase-limited function of body weight. Model parameters were evaluated as functions of sex, age, and height. The relationship between AFT and body weight was very well approximated by means of the nonlinear model (R^2 = .95), with maximal AFT being determined by both sex and height and with AFT growth rate determined only by sex. AFT clearly shows a nonlinear behavior, tending to increase less and less with progressively increasing body weight. With the proposed model, an asymptotic maximal AFT may be postulated. The organism seems to have an intrinsic limitation to how much skeletal muscle development may take place to accommodate the necessities of an ever-increasing load. These limits are different between the sexes, with women tending to approach more rapidly than men a lower maximal AFT for the same height.

SINCE THE DAWN of the scientific era[1] and up to the present,[2,3] a great deal of attention has been focused on the relationship between body proportions and sustainable weight in several zoological species. The problem of determining the size limits in living human subjects, however, has not been the object of an equal amount of study, even though from a clinical and social health point of view, its importance to us is far greater.

With the development of higher mental functions, which allows the formulation of abstract concepts and the control of spontaneous reactions, man has progressively lost some instincts, such as eating as an immediate response to hunger or drinking exclusively as a reflex to thirst. Changes in living models along with the abundance of food, offered in a variety of appetizing preparations, encouraged these changes. Nowadays, the percentage of obese individuals is progressively increasing,[4,5] creating major problems for society's organization, principally as a result of an increased cost of health care.

It is a common understanding that obese subjects, besides having a larger fat mass (FM) when compared with normal weighting people, also show an increased fat-free mass (FFM). This concept is based on the consideration that in order to carry a larger FM, an increasingly larger FFM is required. However, the characteristics according to which FFM increases with progressive weight increase have not been well elucidated. To our knowledge, the only data reported in the literature are those of Forbes[3] on 164 adolescent and adult women presenting with a wide range of weight. A related question concerns the relationship between FM and FFM in a population of both men and women.

Body composition was studied in a large sample of adult healthy subjects (n = 273) with body mass index (BMI) ranging from 12.9 to 69.9 kg/m^2, and a mathematical model was applied to interpret the interrelations between weight and adipose free tissue (AFT), taking into account age, sex, and height as possible major determinants.

MATERIALS AND METHODS

Subjects

Two hundred seventy-three Caucasian subjects (147 women and 126 men, between 15 and 74 years of age; median, 34 years), consecutively admitted to the outpatient clinic of Metabolic Diseases of the Catholic University School of Medicine in Rome in the last 2 years, were recruited for the study. Fifty-two subjects randomly taken from the whole sample underwent muscle biopsy on the vastus lateralis on a different day from that of the study of body composition in order to measure the lipid content of the skeletal muscle specimen.

The subjects studied were clinically euthyroid, had no evidence of renal, cardiac, or hepatic dysfunction and were not treated with drugs affecting energy metabolism. All examined women were studied in the follicular phase of the menstrual cycle. All subjects enrolled were studied under stable conditions on a free diet. Diagnoses of anorexia nervosa were made according to the criteria of Diagnostic and Statistical Manual (DSM)-IV-R.

The studied sample included normal subjects (BMI for men between 20.7 and 27.8, for women between 19.1 and 27.3), anorexic patients, and several degrees of obesity ranging from simple overweight to morbid obesity (BMI > 40). None of them was an athlete or was regularly involved in a program of physical exercises.

The study protocol followed the guidelines of the hospital Ethics Committee, and all subjects gave their written informed consent.

Experimental Protocol

Body composition. Body weight was measured to the nearest 0.1 kg by a beam scale. Body composition was estimated on the basis of the total body water (TBW) measured by isotopic dilution giving 80 μCi of tritiated water (100 mCi/mL) in 5 mL of saline solution as an intravenous bolus injection. The disintegrations per minute (dpm) were counted in duplicate on 0.5 mL of plasma for each sample by means of a Canberra-Packard, Model 1600TR (Canberra, CT) β-scintillation counter. The amount (in dpm) of the tritiated water bolus was divided by the average concentration of labelled water (dpm/mL) obtained at steady state in order to compute TBW as the apparent volume of

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distribution of labelled water. The plasma tritium values have been adjusted for dilution by plasma solids constant, namely TBW-dpm/g water-dpm/g plasma/0.94. The TBW values were divided by 0.73, to yield FFM.[6]

Skeletal muscle lipid analysis. Total skeletal muscle lipids were obtained as the sum of separately measured extracellular and intracellular lipids. A specimen of 200 mg was taken and immediately placed into a calcium-free Hank's solution added with EDTA and bubbled with O_2 95% and CO_2 5%. The sample was washed many times to remove the blood and to ligate the calcium ions. It was then immersed in a fresh Hank's solution added with collagenase type IV 50 mg and calcium ions and agitated in a Dubnoff water-bath maintained at 37°C until the tissue appeared soft. At this point, the specimen was gently removed, cells were brushed with a blunted spatula, filtered, suspended in phosphate-buffered saline (PBS) and centrifuged 2 times at 50 $\times$ g for 2 minutes.

Lipids were extracted twice with 8 vol of chloroform-methanol (2:1, vol/vol) from the acellular material stirring the solutions at 60°C for 15 minutes. The combined extracts were dried in a GyroVap apparatus (GV1; Gio. DeVita, Rome, Italy) operating at 60°C, coupled with a vacuum pump and a gas trap (FTS Systems, Stone Ridge, NY). The dry weight of lipid extracts was obtained by weighing the sample tube before and after drying the extracts. The same procedure was applied to the separated muscle cells.

Mathematical modeling and statistics. Because the functional, effective FFM was of interest to the exclusion of possible intramuscular accumulations of fat, a relationship between body weight and percent intramuscular fat was derived on a subsample of 52 experimental subjects undergoing muscle biopsy. By deciding to correct or not for estimated intramuscular fat, 2 approaches may be followed. If we considered that, regarding muscle, water content is only related to the content of effective fat-free tissue (in other words, that intramuscular fat deposits are not associated with any amount of water), then using the classic hydration coefficient (0.732) would yield the correct amount of effective fat free tissue. If, on the other hand, intramuscular fat deposits were associated with some amount of water (blood-filled capillaries, interstitial fluid, etc), we would overestimate the effective FFM. The FFM routinely computed using the 0.732 hydration coefficient (FFM_1) represents therefore an upper bound of the real FFM.

Conversely, if we supposed that intramuscular fat has the same hydration coefficient as functional muscle tissue, we could correct for the amount of intramuscular fat using the relation:

$$FFM_2 = \frac{TBW}{0.732} - 0.4 \cdot \frac{k_F}{100} \cdot \text{weight}$$

in which we assume that skeletal muscle mass accounts for 40%[7] of body weight and in which k_F is the estimated percent content of intramuscular fat in the subject (see Results for derivation). This correction is likely to be excessive because of 2 factors: first, the hydration coefficient of fat is likely to be much smaller than the hydration coefficient of fat-free muscle tissue. Second, in an obese individual, the contribution of muscle to the total body weight is likely to be less than the 40% measured in lean individuals. The computed FFM_2 represents therefore a lower bound of the effective, functional FFM. So, given

$$FM = BW - FFM_1 \text{ (BW = body weight)}$$

a further correction can then be computed taking into account that about 20% of the FM is composed of water, cytosolic material, and other supporting tissues containing proteins,[8]

$$FFM_3 = FFM_2 + 0.2FM\,.$$

In the following, we will generically refer to FFM_3 (the corrected FFM) as AFT.

If these 3 estimates of FFM (FFM_1, FFM_2, and AFT) exhibit a similarly increment with weight, we may conclude that the actual FFM, lying in between, will also increase with weight in the same way.

Because FFM is largely determined by the amount of bone and muscle in the body, it seems reasonable to suspect that it does not grow without bound as body weight increases. Rather, FFM ought to grow gradually less, with body weight increases being accounted for by fat deposition to a progressively larger degree. In order to check this hypothesis, the following nonlinear model of FFM increase was thought to be applicable, as a first approximation, to the recorded experimental data:

$$FFM = \frac{T_{max} \cdot \text{weight}}{K_{50} + \text{weight}}, \tag{1}$$

in which T_{max} is the maximum FFM attainable by the subject (ceiling FFM) and K_{50} is the body weight at which the subject attains 50% of his or her maximal FFM.

However, T_{max} and K_{50} are general functions of the sex, age, and height of the subject. It seems reasonable to suppose, for instance, that younger, taller males have a greater maximum developable FFM than older, shorter females. T_{max} and K_{50} could be represented by the following linear functions of sex, height, and age:

$$T_{max}\,[\text{kg}] = \alpha + \beta \cdot \text{sex} + \gamma \cdot \text{height [cm]} + \delta \cdot \text{age [years]}\,, \tag{2}$$

and

$$.K_{50}\,[\text{kg}] = \zeta + \eta \cdot \text{sex} + \theta \cdot \text{height [cm]} + \kappa \cdot \text{age [years]}\,. \tag{3}$$

The following complete nonlinear model was therefore fitted to the AFT data using backward stepwise regression to eliminate nonsignificant coefficients:

$$AFT\,[\text{kg}] = \frac{(\alpha+\beta \cdot \text{sex}+\gamma \cdot \text{height}+\delta \cdot \text{age}) \cdot \text{weight [kg]}}{\zeta+\eta \cdot \text{sex}+\theta \cdot \text{height}+\kappa \cdot \text{age}+\text{weight [kg]}}\,. \tag{4}$$

RESULTS

Figure 1 depicts the linear regression analysis between percent intramuscular fat and body weight on the sample of subjects who underwent muscle biopsy. The derived equation is:

$$\% \text{ intramuscular fat} = k_F \cdot \text{weight}$$

in which the intercept was not significantly different from zero and was therefore excluded from the regression equation, and

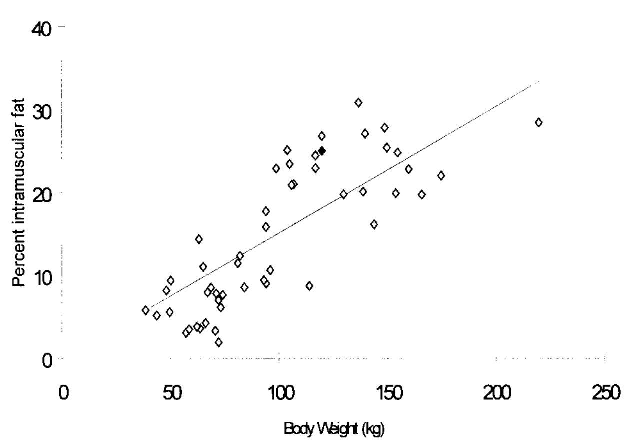

Fig 1. Relationship between body weight (in abscissa) and percent intramuscular fat. The solid regression line is also shown.

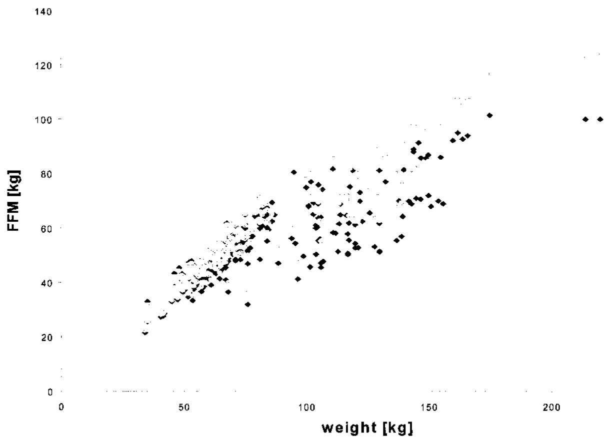

Fig 2. Scatter diagram of uncorrected FFM against body weight. The values follow an apparently nonlinear distribution. Males (●), females (◆).

in which k_F, the increase in percent intramuscular fat per kilogram increase in body weight, was $k_F = 0.152 \pm 0.007$.

Figure 2 shows the scatter diagrams of FFM versus body weight stratified by sex (men- circles, women- diamonds). Figure 3 shows the scatter diagrams of AFT versus body weight stratified by sex.

The correction gave rise to a distribution of points behaving similarly to and tending to a lower limit than the uncorrected points. Because the amount of correction effectively applicable is unknown and because the qualitative conclusions hold for corrected points if they hold for uncorrected points, in the continuation of the analysis, only the standard uncorrected determination of FFM is used.

In the model fitting of AFT data, none of the coefficients associated with age (δ and κ) were significant: apparently, given the experimental sample available to us, the effect of age is not discernible over and above the effects of sex and height. The coefficients α and θ were also not significantly different from zero. The final model considered was therefore the following:

$$\mathrm{AFT} = \frac{(\beta \cdot \mathrm{sex} + \gamma \cdot \mathrm{height})\ \mathrm{weight}}{\zeta + \eta \cdot \mathrm{sex} + \mathrm{weight}}. \tag{5}$$

For this model, parameter estimates, asymptotic standard errors, and an asymptotic correlation matrix (Tables 1 and 2) were obtained by ordinary least squares via a Levenberg-Marquardt algorithm. The model exhibited a good fit to the experimental data, with $R^2 = .95$.

The scatter of AFT versus body weight, depicted in Fig 3, shows distinct distributions for men and women. Figure 4 shows the theoretical increase in AFT with increasing weight in

Table 1. Parameter Estimates, Approximate SE, *t*, and Approximate Probability That Coefficient Equals Zero

Parameter	Estimate	Approximate SE	*t*	*P*
β	89.396405	14.70993	6.08	.0001
γ	1.012715	0.04799	21.10	.0001
ζ	162.256769	12.83175	12.64	.0001
η	98.270236	21.28914	4.62	.0001

Table 2. Asymptotic Correlation Matrix of the Parameter Estimates

	β	γ	ζ	η
β	1.0000	−0.5085	−0.5059	0.9930
γ	−0.5085	1.0000	0.9947	−0.5456
ζ	−0.5059	0.9947	1.0000	−0.5491
η	0.9930	−0.5456	−0.5491	1.0000

women of 151 and 171 cm in height, corresponding to 10 cm less and more than the 161 cm average height in our sample. Figure 5 shows the corresponding increase in AFT with weight in men 164 and 184 cm in height, average height being 174 cm in our sample.

For the purpose of comparison with previously published results,[3] we also computed separate linear regressions of FFM on log (FM) for females and males. The obtained equations were: FFM [kg] = 23.4 (±2.16) log (FM [kg]) + 15.9 (±3.07) for females ($R = .67$, all coefficients significant to $P < .001$) and FFM [kg] = 34.2 (±2.52) log (FM [kg]) + 17.9 (±3.32) for males ($R = .77$, all coefficients significant to $P < .001$).

For the purpose of comparison with standard measures of FFM, we also computed a unique linear regressions of AFT on FFM (no significant differences have been detected between men and women). The obtained equation was: AFT [kg] = 0.86 (FFM [kg]) + 5.99 ($R^2 = .95$ all coefficients significant to $P <$ .001).

DISCUSSION

From the reported results, it appears that the ceiling AFT attainable by a subject is a function of both sex (with males in our sample having a ceiling AFT about 89 kg greater than females for the same height) and height (for females, for example, ceiling AFT being about 1,012 g/cm of height or about 162 kg for a woman of 160 cm in height). The value of K_{50} indicates how progressively a subject tends towards his or her ceiling AFT as weight increases. This value was dependent only on sex being smaller in females. Males appear to increase their AFT, towards their (higher) theoretical ceiling value, in a

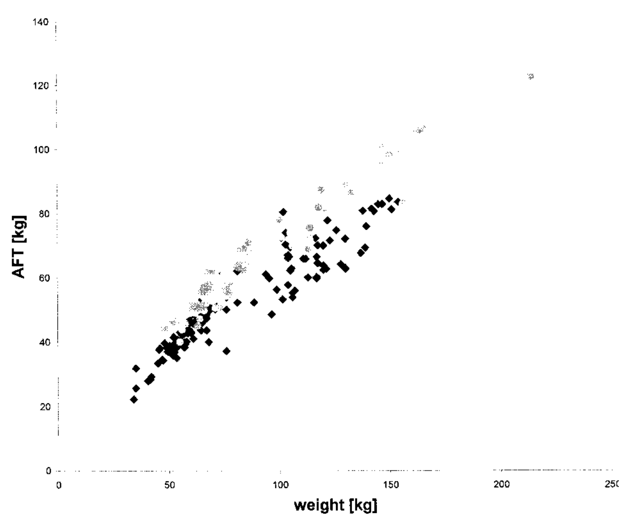

Fig 3. The scatter of AFT *v* weight shows distinct distributions for males (●) and females (◆).

slowly progressing fashion, reaching a given percent of ceiling AFT at higher weights than females. Females, conversely, appear to increase their AFT faster with initial gains in weight, but slow down increasing their AFT at relatively lower body weights than males.

Forbes[3] evaluated lean body mass (LBM) by ^{40}K counting in a sample of 164 women ranging in weight from underweight to varying degrees of obesity and ranging in age from 14 to 50 years. He demonstrated that the relationship between LBM and body fat was curvilinear. He chose to represent this nonlinearity by regressing LBM onto log-transformed body fat content (corresponding in the terminology of the present work to FFM and FM, respectively). We preferred to fit directly a nonlinear model for AFT in terms of weight, on one hand, because weight is the original independent variable generally measured (FM being usually obtained by subtraction of FFM from weight) and probably allows a more intuitive characterization of the subject; on the other hand, direct fitting of a nonlinear model, instead of linear fitting on a transformed variable, preserves the (supposedly normal) error distribution of the observations around the prediction. However, applying to our female data the same equation as in Forbes,[3] we obtain almost exactly the same results: the degree of concordance between our regression on all female data and the regression on grouped female data reported in Forbes[3] is actually surprisingly high. We also applied the same equation to the male subsample, obtaining actually rather different regression coefficients. To exemplify, while Forbes[3] computed that a doubling or halving of body fat content corresponds to a variation of LBM of 7.2 kg, our corresponding figure for females is 7.04 kg, while for males, it is 10.30 kg. This is yet another confirmation that males appear to vary more their FFM than females in response to similar variations of FM. In another study,[9] FFMs of obese subjects were found to be similar to normal individuals of the same sex, height, and age; however, the more limited range of BMI in the population investigated in Edmonds et al[9] may at least partially account for these differences.

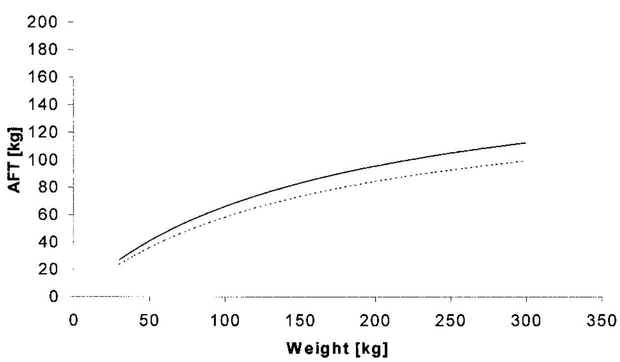

Fig 4. Theoretical predicted increase of AFT with weight in women of respectively 171 (upper curve) and 151 (lower curve) cm height.

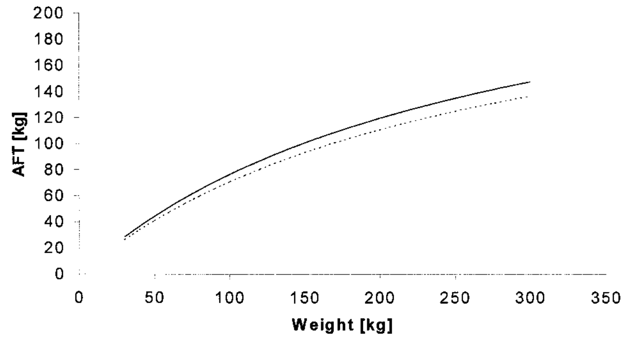

Fig 5. Theoretical predicted increase of AFT with weight in men of respectively 184 (upper curve) and 164 (lower curve) cm height.

FFM in adults is over one half skeletal muscle.[10] A higher than normal FFM in obese subjects can be explained either by the hypothesis that skeletal muscles progressively hypertrophy to adapt to an ever-increasing load, but that this adaptive capacity is limited and progressively fails, while FM continues to increase, or by the hypothesis that larger musculoskeletal frames may more easily accommodate larger FMs. We remark that, according to the present model, because FM is just the difference between weight and FFM, it will increase proportionally less at low weights and increase at ratios nearer and nearer to 1 kg FM/kg weight increase as weight grows large. Of course, the above observations do not apply to athletes or muscular men who are known to have a large body weight related to elevated FFMs.[11] Furthermore, the data found for our study population might be applied to other populations, although it remains to be verified.

The derived relationship between changes in AFT and changes in weight allows a prediction to be made about the likely changes of body composition after weight loss or gain in a general population of both sexes.

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