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UNRELIABLE USE OF STANDARD MUSCLE HYDRATION VALUE IN OBESITY

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Abstract

Intramuscle water content is assumed to be constant in humans independently of their anthropometric characteristics. To verify if this assumption is correct, intramuscle water, proteins, glycogen, and both total and intramyocytic triglycerides, were measured in 51 samples of rectus abdominis muscle obtained from 16 lean and 35 overweight and obese subjects (BMI cutoff 24.9 Kg/m^2). Data (referred to wet tissue) were analyzed by means of a composition model at the cellular level of the skeletal muscle (SM). The average SM water content was 76.3±3.3% in normal-weight individuals and 65.7±5.8% in obese subjects ($P < 0.0001$). Total triglycerides were 5.5±2.3% in controls, and 19.0±7.0 in obese subjects ($P < 0.0001$). The intramyocytic triglyceride fraction was also increased in obese subjects. The composition model provides an explanation for the negative correlation between total triglycerides and intramuscle water, and some of model parameters were determined from the data. In conclusion, although the hydration of fat-free SM mass may be unchanged in obese subjects, the hydration of *in toto* muscle mass decreases as its lipid content increases.

Key words: Muscle hydration, muscle triglycerydes, mathematical model

1. Introduction

Skeletal muscle (SM) accounts for up to 40% of body weight (36) and is a major determinant of whole body energy expenditure, even at rest, because of its large mass. Quantification of muscle mass is important for cross-sectional studies and when normalizing various physiological parameters such as muscle force production, metabolic rate of oxygen uptake, blood flow, and protein turnover. Furthermore, investigations into the causes of skeletal muscle loss with advancing age have stimulated a renewed interest in the quantification of this important component of body composition (3,5,10,17).

Whilst it is well established that myocytes represent the largest body reservoir of carbohydrates, in the form of glycogen, their role in lipid storage and metabolism is not completely understood. Skeletal muscle is thought to be the major site for removal of both non-esterified fatty acids (NEFA) and triglycerides (TG) from the circulatory stream, although the exact role and function of the intramyocytic TG pool has not been thoroughly ascertained. According to the few studies which have addressed this topic, the TG pool appears to be in dynamic and rapid equilibrium with substrate utilization and supply (7,24). In addition, high levels of either circulating NEFA (25,26,29-32) or TG (14,27) along with supranormal levels of intramyocytic TG (TG_m) (28) may play a pivotal role in the pathogenesis of insulin resistance in humans.

Electron micrographs reveal that the storage of TG_m in lipid droplets is less homogeneous than the storage of glycogen (1,9,18,19,40). However, attempts to quantify the TG_m content in skeletal muscle biopsies have been so far technically inadequate due to contamination with adipose tissue lipids (11,33,47). On the other hand, the small specimens ($75-100$ mg) of needle biopsy muscle tissue available for testing make it difficult to perform the analysis of TG_m in duplicate or in triplicate, thus explaining the large coefficients of variation (20 to 50%) observed (47). Recently, the use of ^1H magnetic resonance spectroscopy has been validated to quantitatively differentiate between adipocyte and intracellular TG_m stores in both animals and humans (38). However, in spite of the excellent results obtained, this technique is still too advanced and expensive to be used for large population studies.

Another related problem is the intramuscle water content, which is assumed to be constant in humans independently of their anthropometric characteristics and is derived from the data of the so called "Reference Man" (36). For instance, the ratio of tissue water to lean tissue mass was assumed to be constant (and equal to 0.81 in SM) in the analysis of data of ^1H magnetic resonance spectroscopy (38). This point is a relevant one in the assessment of body composition, since it is on the above data as well as on animal studies (8,15) that the mean whole body cellular hydration is assumed to be equal to 0.70 (43). This factor is used in the fat-free body mass hydration model recently proposed (43) as the most reliable method for the calculation of the ratio of total body water to fat free mass, and also serves as the basis of the dual-energy X-ray absorptiometry body composition model.

In order to clarify the hydration level of SM across subjects differing in body weight, a large sample of SM tissue, taken during abdominal surgical operations in subjects with body mass index (BMI) ranging between 17.1 and 66.7 kg/m^2, was chemically analyzed. An improved technique to avoid contamination of muscle fibers by adipocyte lipids, and employing a large amount of skeletal muscle tissue to obtain a low within-biopsy variability, was used. Data were analyzed by means of a composition model at the SM cellular level and estimates of model parameters were obtained.

2. Materials and Methods

Subjects

Subjects were 51 non-diabetic patients, 26 males and 25 females, who underwent abdominal surgery for colecystectomy, due to the presence of cholesterol gallstones (21 patients) or biliopancreatic diversion for morbid obesity (30 patients). The subjects were divided into a lean (control) group and a group including overweight and obese subjects, denoted in the present paper as the obese group. Subjects with BMI larger than 24.9 kg/m^2 were included in the obese group, so some of the gallstone patients were classified as obese. The average age was 52.2±11.2 years in lean subjects (n=16) and 45.1±15.6 years in obese subjects (n=35); weight was 59.9±11.0 vs. 117.7±35.2 kg and BMI was 21.8±2.4 vs. 43.0±11.3 kg/m^2 ($P < 0.001$), respectively, in the two groups. None of the subjects had abnormalities in renal function or serum electrolytes.

The study was approved by the Institutional Review Board as designated by the Helsinki Declaration. All the patients gave their informed consent prior to their enrollment in the present study.

Body composition

The day preceding the surgical procedure, body weight was measured to the nearest 0.1 kg by a beam scale and height to the nearest 0.5 cm using a stadiometer (Holatin, Crosswell, Wales, UK). Subjects were measured in the morning after an overnight fast, in a hospital gown, and following emptying of the bladder.

Muscle biopsy

During surgery, a rectus abdominis muscle biopsy (at least 6 g) was obtained after opening of its investing fascia. Each sample was free of any visible contamination from subcutaneous adipose depot store and was divided in three parts in order to determine chemical components. All the analyses were performed in triplicate.

Skeletal muscle water and protein analysis

SM water (∼1 g of tissue) was determined by freeze drying with continuous pumping. The difference between wet weight and dried weight was used to calculate the water content of the sample examined. The CV for SM water determination on 3 aliquots of muscle for the same biopsy (within-biopsy variability) was 2.3±0.9% (mean±SD). The dried samples were stored at −80 °C for no more than 1 month until the analyses were performed.

To measure the protein content, the dried tissue was suspended in 200 μl of a lysis buffer and homogenized by using a homogenizer (Ultra-Turrax TP 18-10; Janke and Kunkel, Ikawerk, Breisgau, Germany). The lysis buffer was composed by a 100 ml solution of TRIS-HCL 1 M, 2 ml at pH 7.4; NaCl 5 M, 2 ml; $MgCl_2$ 1 M, 0.5 ml; Tween 20 0.1%, 10 ml. The homogenate was centrifuged for 20 min at 6000 rpm at 4 °C. Protein content was determined from the clear supernatant by spectrophotometry at a wavelenght of 565 nm, by using a colorimetric test kit (Bio-rad Protein Assay; Bio-rad Laboratories, Hercules, CA).

Skeletal muscle triglyceride analysis

To measure total triglycerides, a skeletal muscle sample of about 500 mg was homogenized (Ultra-Turrax TP 18-10; Janke and Kunkel, Ikawerk, Breisgau, Germany) in a 2 ml solution of chloroform-methanol (2:1, v/v) acidified with 5−10 mg of trichloroacetic acid to precipitate proteins. Lipids were extracted twice with 8 volumes stirring the solution at 6 °C for 30 minutes.

To measure intramyocytic triglycerides, another specimen of about 500 mg was taken and immediately placed into a calcium free Hanks solution added with EDTA and bubbled with 95% O_2 and 5% CO_2. The sample was washed and then immersed in a fresh Hanks 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 PBS and centrifuged twice at 50×g for 2 minutes. The supernatant was discarded and the muscle cells were dried under a nitrogen stream. After protein precipitation with 5−10 mg of trichloroacetic acid, lipids were extracted twice with 8 volumes of chloroform-methanol (2:1, v/v) 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 System, Stone Ridge, NY, USA). The dry weight of lipid extracts was obtained by weighing the sample tube before and after drying the extracts. The low within-biopsy variability (about 5%) suggested that the TG_m measurement technique was reliable and that the between-biopsy variability was not a result of measurement error.

Skeletal muscle glycogen analysis

After a 10 minute incubation with 0.1 M NaOH at 80 °C of the fat-free material to destroy background hexose monophosphates and glucose, glycogen was degraded to glucose by amyloglucosidase and glucose was measured by a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA) as described by Bergmeyer (4). Glycogen was corrected for the highest total creatine content in the skeletal muscle specimens of a given subject.

Skeletal muscle composition model at the cellular level

Taking as a reference the model presented in (42) and the model of the fat-free body mass at the cellular body composition level, developed by Wang et al., 1999 (43), we propose the skeletal muscle model shown in Fig. 1. This model contains three components: the muscle cell mass (MCM), the extracellular space (ECS) containing fluids and the solid matrix, and the adipose tissue mass (ATM). Thus muscle mass (MM) is equal to sum of the three components

$$\mathrm{MM} = \mathrm{MCM} + \mathrm{ECS} + \mathrm{ATM}\,. \tag{1}$$

These three components consist of an aqueous and a solid compartment. Following (43), and denoting by ICW the intramyocytic water, by ECW the extracellular water and by E/I the ratio ECW/ICW, we write $\mathrm{MCM} = \mathrm{ICW}/a$ and $\mathrm{ECS} = \mathrm{ECW}/b = \mathrm{ICW} \times (E/I)/b$, where a and b are the fractions of water in the myocytes and in the extracellular space, respectively. Further, denoting by ATW (adipose tissue water) the water content of adipose tissue, we may write $\mathrm{ATM} = \mathrm{ATW}/\alpha$, α being the fraction of water in adipose tissue. The total muscle water (TMW) is thus expressed as

$$\mathrm{TMW} = \mathrm{ICW} + \mathrm{ECW} + \mathrm{ATW} \tag{2}$$

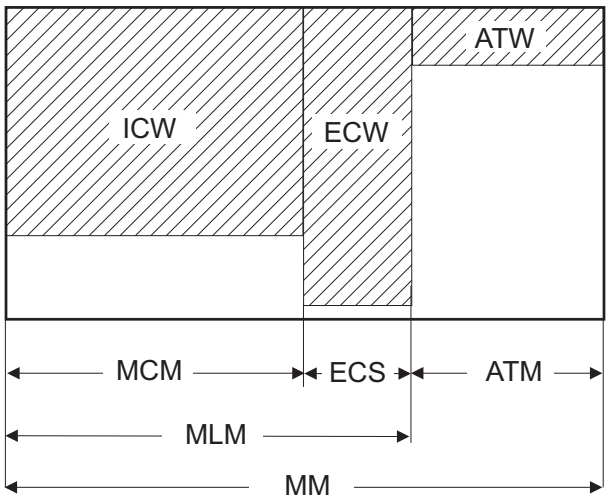

Fig. 1. Model of skeletal muscle composition at the cellular level. MM muscle mass, MLM muscle lean mass, MCM muscle cell mass, ECS extracellular space, ATM adipose tissue mass, ICW intramyocytic water, ECW extracellular water, ATW adipose tissue water. The fraction of intramuscle water is represented by the hatched area.

and we note that this amount of water was actually measured as skeletal muscle water in the SM samples examined.

We can now consider the fraction of intramuscle water, TMW/MM, as the (observed) hydration level of the *in toto* skeletal muscle mass. This ratio differs from the hydration of fat-free mass defined in (43) because it takes into account adipose tissue and the water associated with this tissue. Following the approach in (43), from Eqs. 1 and 2 we have

$$\frac{\mathrm{TMW}}{\mathrm{MM}} = \frac{\mathrm{ICW}+\mathrm{ECW}+\mathrm{ATW}}{\mathrm{MCM}+\mathrm{ECS}+\mathrm{ATM}}$$

$$= \frac{\mathrm{ICW}+\mathrm{ICW}\times(E/I)+\alpha\,\mathrm{ATM}}{\mathrm{ICW}/a+\mathrm{ICW}\times(E/I)/b+\mathrm{ATM}}\,. \tag{3}$$

To rewrite Eq. 3 in a more suitable form, we introduce as a new parameter the ratio of adipose tissue mass to muscle lean mass (MLM), ATM/MLM, with MLM =MCM+ECS (MLM includes the intramyocytic triglycerides). This parameter can be considered as an "adiposity index" of skeletal muscle. Thus we have:

$$\frac{\mathrm{TMW}}{\mathrm{MM}} = \frac{\mathrm{ICW}+\mathrm{ICW}\times(E/I)+\alpha(\mathrm{ATM}/\mathrm{MLM})\times\mathrm{MLM}}{\mathrm{ICW}/a+\mathrm{ICW}\times(E/I)/b+(\mathrm{ATM}/\mathrm{MLM})\times\mathrm{MLM}}\,.$$

Since MLM =ICW$/a$+ICW$\times(E/I)/b$, we obtain

$$\frac{\mathrm{TMW}}{\mathrm{MM}} = \frac{1+E/I+\alpha(\mathrm{ATM}/\mathrm{MLM})\times(1/a+(E/I)/b)}{(1/a+(E/I)/b)(1+\mathrm{ATM}/\mathrm{MLM})}\,. \tag{4}$$

Other important quantities that have been found to exhibit differences in SM samples from non-obese and obese patients are the fractions of intramyocytic triglycerides and of total triglycerides in skeletal muscle mass, $\mathrm{TG_m}$/MM and TT/MM respectively, where $\mathrm{TG_m}$ denotes the content of intramyocytic triglicerides and TT denotes the total content of triglycerides in the

muscle mass. We will indicate by TG_{at} the content of triglycerides in SM adipose tissue, by β the fraction (referred to myocyte mass) of triglycerides in the myocytes, and will assume that adipose tissue is essentially composed of water, proteins (5% according to (36)) and lipids (75% according to (42)). Neglecting the lipids in the ECS and following the same procedure as above, we may write

$$\begin{aligned}\frac{\mathrm{TT}}{\mathrm{MM}} &= \frac{\mathrm{TG_m}}{\mathrm{MM}} + \frac{\mathrm{TG_{at}}}{\mathrm{MM}} \\ &= \frac{\beta\,\mathrm{MCM}}{\mathrm{MCM}+\mathrm{ECS}+\mathrm{ATM}} + \frac{0.75\,\mathrm{ATM}}{\mathrm{MCM}+\mathrm{ECS}+\mathrm{ATM}} \\ &= \frac{\beta/a}{(1/a+(E/I)/b)(1+\mathrm{ATM/MLM})} + \frac{0.75(\mathrm{ATM/MLM})}{1+\mathrm{ATM/MLM}}\,. \end{aligned} \tag{5}$$

Another quantity that can be derived from the model is the fraction of proteins (P) in the SM mass. Denoting the fractions of proteins in myocytes and extracellular space as γ and γ', respectively, and since the fraction of proteins in adipose tissue has been assumed equal to 0.05, the fraction P/MM can be expressed by the following equation:

$$\frac{\mathrm{P}}{\mathrm{MM}} = \frac{\gamma/a + \gamma'(E/I)/b + 0.05\,(\mathrm{ATM/MLM})\times(1/a + (E/I)/b)}{(1/a + (E/I)/b)(1 + \mathrm{ATM/MLM})}\,. \tag{6}$$

Similarly, assuming that glycogen in the SM sample is almost exclusively present in myocytes with a fraction δ (referred to myocyte mass), an equation for the glycogen fraction in the SM sample, $\mathrm{G/MM} = (\delta/a)/(1/a+(E/I)/b)(1+\mathrm{ATM/MLM})$, can be easily obtained.

The preceding equations show that the quantites that have been measured in the SM samples: TMW/MM, TG_m/MM (given by the first term in the right hand side of Eq. 5), TT/MM, P/MM and G/MM can be expressed in terms of the following factors: a, b, E/I, α, ATM/MLM, β, γ, γ' and δ. Although these parameters will exhibit variability from sample to sample, it is not possible to estimate all their individual values on the basis of the present measurements. However, some of these parameters can be assumed to be constant in the population of samples. Parameters a and b are likely to have a reduced variability between samples since they appear to be maintained stable by regulatory mechanisms (43). On the basis of data reported in (43) for the SM cells, we assumed $a \simeq 0.72$. For parameter b, we note that the organic extracellular solids like the fibers are included in the extracellular space in the present model, whereas they were included in a compartment of extracellular solids in (43); thus, we took for b a smaller value than in (43), $b \simeq 0.97$. The value of α was set to 0.14, according to (42). Moreover, because the term representing the extracellular proteins is scarcely influent in Eq. 6, at least in the absence of atrophy or fibrosis when the content of extramyocytic proteins may become large, we gave to γ' the constant value of 0.02 (2,36). Finally, we note that almost all present data of TMW/MM in the control group are in the range 0.73–0.79. For lean subjects, where the adiposity index is likely to be small, Eq. 4 gives $\mathrm{TMW/MM} \simeq (1+E/I)/(1/a+(E/I)/b)$. Thus, for a and b as previously fixed, a reasonable estimate of E/I in the SM samples from lean subjects is around $E/I = 0.4$. Because E/I appears to be increased in obese subjects (42), we assumed $E/I = 0.4 + 0.5(\mathrm{ATM/MLM})$ which is thus substantially larger than the experimental value of 0.2 found for SM in rats and humans (6,13).

With the previous assumptions, the remaining parameters of the model: ATM/MLM (and thus E/I which is a given function of ATM/MLM), β, γ and δ were reliably determined for each sample from the experimental data of TMW/MM, TG_m/MM, TT/MM, P/MM and G/MM.

Table 1. *Chemical composition of the skeletal muscle specimens.*

	Control group (n= 16)	Obese group (n= 35)	P
H_2O (%)	76.3 ± 3.2	65.7 ± 5.8	< 0.0001
Total TG (%)	5.5 ± 2.3	19.0 ± 7.0	< 0.0001
Intramyocytic TG (%)	2.4 ± 1.1	3.1 ± 0.5	< 0.05
Proteins (%)	15.5 ± 3.7	12.6 ± 5.9	NS
Glycogen (%)	1.4 ± 0.5	1.6 ± 0.8	NS
H_2O/Proteins	5.3 ± 1.6	6.9 ± 4.3	< 0.05

The values (mean±SD) reported are referred to 100 mg of wet tissue.

Statistical analysis

Each single value represents the average of triplicate determinations. The results are presented as mean±SD, unless otherwise specified. The variability of the determinations from three samples of the same biopsy was expressed as the coefficient of variation (CV=100×SD/mean).

The Fisher's exact test was used to compare sex proportions between the two groups. Intergroup comparisons were performed using a Student's t-test for the difference of means, with significance at $P < 0.05$. The linear correlations between anthropometric data and total lipids or intramyocytic triglycerides were assessed by the correlation coefficient. In the linear regression, the sum of absolute deviations was minimized, in order to reduce the effect of outliers. The estimates of the regression parameters are given as estimate±SE (34,37).

The values of ATM/MLM, E/I, β, γ and δ were determined for each sample from the measurements of TMW/MM, TG_m/MM, TT/MM, P/MM and G/MM by least squares solution of model equations, with the other parameters set to the values specified above. The solution was found with the constraint that the sum $a+\beta+\gamma+\delta$ of water, triglycerides, proteins and glycogen in the myocyte mass, a sum that represents all the main cellular components excluding nucleic acids and miscellaneous metabolites, has to be close to 97%. The standard errors were evaluated from the inverse Hessian matrix of the least squares index at the minimum (34).

3. Results

Table 1 reports the data on tissue composition in the two groups: mean values and SD are referred to 100 mg of wet muscle tissue. The fractions of total and intramyocytic triglycerides were significantly smaller in the control group with respect to the obese group. The fraction of intramuscle water was significantly lower in the obese group. A positive correlation was found between BMI and both the fractions of intramyocytic triglycerides ($r = 0.52$, $P < 0.0001$) and of total triglycerides ($r = 0.87$, $P < 0.0001$). A negative correlation was found between the fraction of intramuscle water TMW/MM and the fraction of total triglycerides TT/MM ($r = -0.67$, $P < 0.0001$). The experimental data of TT/MM vs. TMW/MM are reported in Fig. 2, where it is noted that lean subjects are represented in the region of the plane with high fraction of intramuscle water and low lipid content. The dotted line in the figure is the regression line (slope -1.044 ± 0.032, intercept 0.874 ± 0.025).

The individual estimates of ATM/MLM, E/I, β, γ and δ were found with standard errors that were generally much smaller than 10% of the estimate. Table 2 gives the mean values and

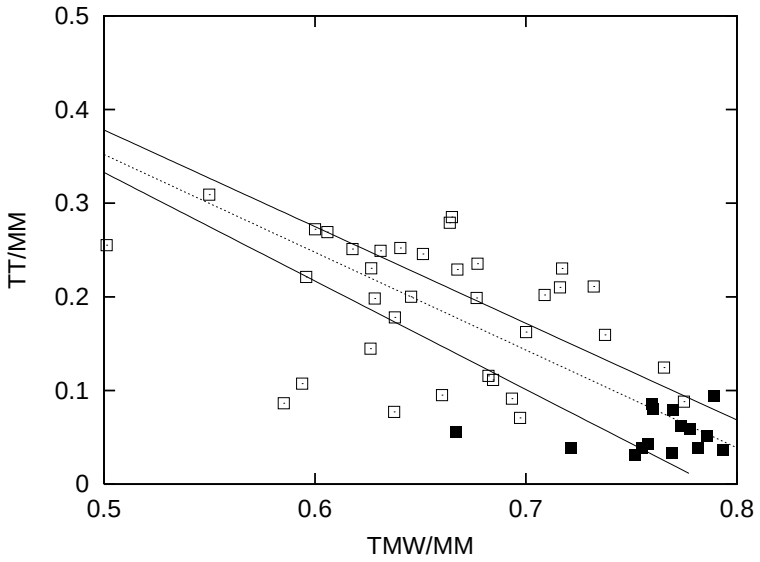

Fig. 2. Experimental data of the fraction of total intramuscle water vs. the fraction of total triglycerides in the skeletal muscle samples (controls, black squares; obese, open squares). Theoretical lines predicted by the model of skeletal muscle composition at the cellular level (Eqs. 4 and 5), with the parameter values indicated in the text (continuous lines: upper line $E/I = 0.68$ and $\beta = 9.7$; lower line $E/I = 0.40$ and $\beta = 1.5$). Regression line (dotted line).

Table 2. *Mean±SD of model parameters determined from data of chemical composition of skeletal muscle specimens.*

	Control group (n= 16)	Obese group (n= 35)	P
ATM/MLM	0.05 ± 0.03	0.28 ± 0.14	< 0.0001
E/I	0.42 ± 0.02	0.54 ± 0.07	< 0.0001
β	3.1 ± 1.3	5.6 ± 1.6	< 0.0001
γ	19.8 ± 3.6	17.4 ± 6.7	NS
δ	1.9 ± 0.8	2.7 ± 1.1	< 0.0025
H_2O/Proteins	5.14 ± 1.01	6.59 ± 2.97	< 0.01

The values of mean and SD of the estimates of β, γ and δ are given in % of myocyte mass.

the standard deviations of model parameters over the control and the obese group. As expected, the mean value of β (fraction of intramyocytic triglycerides referred to myocyte mass) is larger than the mean value of the intramyocytic triglycerides referred to the wet mass of the sample, reported in Table 1, and similarly for γ and δ compared to the fractions of proteins and glycogen in Table 1. The predicted values of the ratio H_2O/Proteins are also reported in Table 2. All the parameters of the model, except γ, are significantly different between the two groups. The values of the adiposity index, ATM/MLM, were found to range from 0.01 to 0.1 in controls and from 0.06 to 0.56 in the obese group. The fraction of SM lean mass, that can be computed from the adiposity index as MLM/MM = 1/(1+ATM/MLM), ranges thus from 0.91 to 0.99 in controls and from 0.64 to 0.94 in obese.

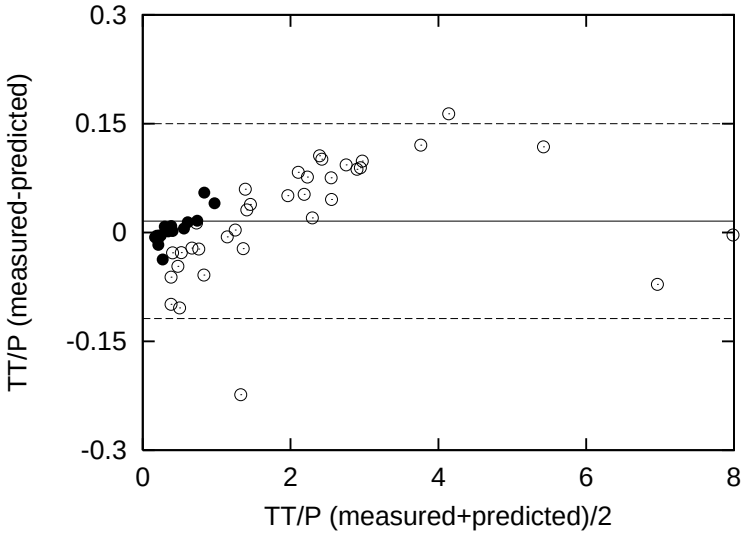

Fig. 3. Bland-Altman plot of measured values and of theoretical predictions of TT/P in the SM samples (controls, black circles; obese, open circles). The solid line indicates the mean difference and the broken lines indicate the mean difference ± 2 SDs of the difference.

The negative correlation observed between intramuscle water content and total triglyceride content can be explained on the basis of the skeletal muscle composition model at the cellular level of Fig. 1. The continuous lines in Fig. 2 were plotted by computing abscissae and ordinates by Eqs. 4 and 5, respectively, with the model parameters a, b, and α fixed at the values given in Methods, and E/I and β at the extreme values found in the SM samples (see legend of Fig. 2). The adiposity index ATM/MLM was changed from 0 to 0.9 to cover the entire range of the measured intramuscle water fractions. These lines, that are easily shown to be straight lines, are thus parametrized by the adiposity index, and the terminal point at the right corresponds to ATM/MLM = 0. If a subject would change from lean to obese, while maintaining the SM parameters a, b, α, β, and E/I constant, its representative point on the plane of Fig. 2 should move on a straight line from right to left. The two lines of Fig. 2 show the effect of the variability of three parameters of the model, the adiposity index ATM/MLM, E/I, and the fraction of intramyocytic triglycerides β.

The model in Fig. 1 shows that the hydration level of skeletal muscle can be reasonably defined in various ways. The ratio TMW/MM is the observed hydration of the *in toto* muscle mass: this quantity was seen to change in the range from 0.5 to 0.8 in the SM samples examined (see Fig. 2). We can also consider the hydration referred to the muscle lean mass, TMW/MLM, and the hydration defined as (ICW+ECW)/MLM. This latter quantity is given by Eq. 4 for ATM/MLM = 0, as seen above, and depends on the obese or non-obese status of the subject only through the parameters a, b and E/I. In contrast, the quantity TMW/MM decreases with the adiposity index because it takes into account the water contained in the adipose tissue that has a small water content. For instance, with E/I equal to 0.6, the ratio TMW/MM decreases continuously from a value of 0.797 for ATM/MLM=0 (0.797 is thus the hydration of muscle lean mass for these values of SM parameters) up to a value of 0.492 for ATM/MLM=0.9.

To assess the predictive capacity of the model, we computed the theoretical values of the ratio total triglycerides to proteins (TT/P) and of the ratio total muscle water to proteins (TMW/P), as predicted by equations 4–6 with the parameters estimated on the individual

samples as described above. A Bland-Altman plot of the experimental and theoretical values of TT/P is shown in Fig. 3. It is seen that the experimental ratios exhibit a remarkable variability from sample to sample, especially in the obese group, mainly because of the presence in this group of five samples in which a very reduced protein content (from 5.5% to 3.6%) was measured and thus a high value of the ratio was obtained. The correlation coefficient for experimental and predicted values of TT/P is equal to 0.997. For the TMW/P ratio, whose experimental values are in the range from 2 to 18, the predicted mean and SD are reported in Table 2 for the two groups, and the correlation coefficient is equal to 0.995.

4. Discussion

Only recently, the importance of maintaining the SM mass in dynamic disease conditions has stimulated new approaches to the *in vivo* assessment of this variable (12,16,17,45). A fundamental limitation to the availability of a useful technique for the measurement of SM mass is represented by the relative lack of direct information on anatomic tissue composition. In fact, data on SM mass by cadaver dissection have been reported in only 25 subjects (23,39,41,46). In a recent report (21), total body water, fat, protein, minerals and carbohydrates were measured in 2 cadavers, one underweight (BMI=19.35 kg/m^2) and the other with cachexia (BMI=10.92 kg/m^2). Indeed, the study in (21) was addressed to demonstrate the usefulness of the *in vivo* neutron activation analysis in severely wasted patients. However, the extrapolation of data obtained by cadaver dissection to the assessment of body composition, and of SM mass in particular, in living individuals with a wide range of body weights, appears at best inaccurate.

Few studies have been performed on the SM composition in humans, but without addressing the question whether the lipid content of the SM mass may influence the assessment of body composition. Landin et al. (22) found in muscle biopsies that only obese men, but not women, had a higher fat content than did lean men. Using CT of the thigh, Kelley et al. (20) reported similar findings in obese men, and these data were later confirmed in women (35). However, these investigations failed to demonstrate if fat is accumulated in the connective tissue between muscle fibers or is stored within muscle cells. Using data obtained in the "reference man" studies (36), the water content of human skeletal muscle as percentage of its total weight has been generalized for all humans to be a constant value of 81%. However, the assumption that the water content of skeletal muscle is constant throughout the population may turn to be incorrect in light of different amounts of fat mass among different subjects (44).

In the present paper, the composition of samples of skeletal muscle from subjects with a wide range of BMI was analyzed. The fractions of intramuscle water, proteins, glycogen, and both total and intramyocytic triglycerides, which represent the typical lipids stored for energy supply, were measured. Our data showed that obese subjects have a significantly larger amount of SM total triglycerides, whose fraction was found to be tripled in the obese subjects. Also the content of intramyocytic TG increased with the BMI, although to a lesser extent. The increase of SM triglycerides in obese subjects was thus mainly due to the accumulation of adipocytes between muscle fibers. As a consequence, the presence in obese subjects of larger amounts of lipids resulted in a lower fraction of intramuscle water, as confirmed by the negative correlation found. In our study the measured intramuscle water percentage for normal weight individuals was 76.3±3.3%, and thus comparable to the "Reference Man" value and to the value of 0.8034 obtained from (43), whereas the water percentage in obese subjects was much lower (65.7±5.8%), with a decrease of about 15% with respect to the reference value.

Our data were interpreted on the basis of a model of SM composition at the cellular level, that was developed starting from previously described models (42,43). The present model considers, in addition to the lean mass, the adipose tissue mass (adipocytes between muscle fibers) and is thus suitable to represent a sample of *in toto* muscle mass. The model describes the SM composition in terms of factors whose variations can account for the interindividual variability of SM composition. In particular one of these factors, denoted as adiposity index (ratio between adipose tissue mass and lean mass in the skeletal muscle), takes specifically into account the amount of triglycerides that are present in the adipocytes between muscle fibers. This factor is likely to show a wide intersubject variability (values from 0.01 to 0.56 were estimated on the present samples). A certain degree of variability, both between the control and obese groups and within the two groups, has also to be expected for the other parameters of the model, as seen in Table 2.

The model shows that the hydration level of SM (interpreted as ratio of total intramuscle water to muscle mass) decreases when the adiposity index increases while all other parameters of the model and hence the hydration of SM lean mass, are unchanged. In other words, SM hydration may still be normal in obese subjects after correcting for intramuscle lipids. However, in the transition from the lean to overweight and eventually to the obese status, all the model parameters are likely to change to some degree. A complete study of the physiological (or associated to obesity) variability of all the parameters of the model, in conjunction with the plausible effects of the experimental errors, could account for the scatter observed in the data. It has to be stressed that the measurement of other quantities in the samples, e.g. the extracellular water, would provide more accurate determinations of the unknown quantities.

The model allowed to reasonably reproduce the variability of the data, as shown by Fig. 3. We note that a subgroup of samples from obese subjects had a greatly increased value of the TT/P ratio, so a larger deviation from model predictions was found. This increased TT/P ratio is possibly associated with a specific loss of muscle proteins. Each of these subjects, in fact, in addition to morbid obesity, also presented with associated complications, namely degenerative arthritis of the hip and knee, that severely restricted their walking. For these cases, some specific modification of the model, to take into account the possible effects of the loss of proteins, should be considered.

As skeletal muscle accounts for a major portion of body cell mass, the finding that the water fraction of skeletal muscle is highly variable with BMI implies that the estimation of the fat-free mass should be revised. The assumption of a constant water fraction in skeletal muscle (equal to 0.81) should also be reconsidered. It is clear, therefore, that knowledge of a more accurate estimate of the SM water content might allow a more appropriate calculation of the TG_m amount in individuals with different anthropometric characteristics, with particular reference to lean and obese subjects. In conclusion, the classical 81% as a standard reference value for the water content in skeletal muscle is not appropriate when used across lean and obese subjects. However, the error in lean subjects is smaller compared to that found in obese individuals, in which the high lipid content of SM causes a decrease of the percentage of water.

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