Biochemical composition of muscle in normal and semistarved human subjects: relevance to anthropometric measurements[1-3]

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ABSTRACT Anthropometric methods aimed at assessing muscle size in undernourished subjects assume a constant proportionality between the mass (i.e., size) and composition (specifically protein-energy content) of this tissue. This assumption was examined in three autopsy groups: controls (n = 11, sudden traumatic death), early semistarvation (n = 6, acute preterminal disease), and chronic semistarvation (n = 34, severe weight loss over time). Results of semistarved groups were expressed relative to respective control value. Early semistarvation produced no detectable change in muscle mass, protein, or total energy content (per gram wet weight), although RNA and glycogen were −50 to −70% of control value ($p < 0.05$). Chronic semistarvation caused muscle atrophy (−54.2%), but not all measured constituents were reduced to the same degree. The results were H_2O—52.9%, collagen—46%, noncollagen proteins—65.3%, total lipids—40%, DNA—54.1%, RNA—81.7%, glycogen—90.3%, and total energy—59.6%. Muscle per unit mass in chronic semistarvation thus reflects relatively more H_2O and less protein and energy when compared to normal tissue. About 85 to 95% of muscle protein-energy loss can be detected by anthropometric measurements of muscle size; the remaining 5 to 15% depletion of protein and energy is masked by muscle compositional changes. Proper interpretation of anthropometric data requires an understanding of these unmeasured but important compositional differences in normal and semistarved muscle. *Am J Clin Nutr* 1982;36:131-142.

KEY WORDS Arm muscle area, anthropometry, protein-energy malnutrition, semistarvation

Introduction

Muscle tissue comprises 40% of body weight in healthy adult man, for a total mass of about 28 to 30 kg (1). Muscle is primarily water, but protein, which constitutes most of the nonaqueous portion of muscle, has been an important focus of the nutritionist. In protein-energy starvation, muscle protein undergoes catabolism and oxidation, a process that supplies required metabolic fuel. This potential calorie reserve has, over the years, been used to judge the overall depletion of lean body mass (2) and the effectiveness of protein-energy malnutrition (PEM) treatment (3). The accessibility of skeletal muscle to anthropometric measuring techniques makes muscle unique among the components of lean body mass. However, the use in PEM of anatomic muscle mass indicators (e.g., arm muscle area) (4) is based on an unproven assumption: that in health and PEM the size of muscle bears an unknown but constant relationship to muscle composition, specifically muscle protein and energy content.

Two lines of evidence in man, histological and radiographic, suggest that muscle composition is altered in PEM. Montgomery (5), comparing microscopic sections of normal to PEM muscle, found increased connective tissue, an enlarged extracellular space, and decreased myofiber mass (5). Radiographic studies of muscle in PEM patients at our center detected a decrease in x-ray muscle

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density, which we hypothesized was a relative increase in muscle water or fat (6).

Since the a priori assumption of anthropometric muscle mass measurement is that normal and undernourished muscle have similar biochemical composition per unit of tissue, but the above cited observations appear to contradict this notion, we investigated the biochemical composition of normal and undernourished human muscle. Because of the variety of diseases included in our subject pool, and the likelihood that other nutritional deficiencies compounded inadequate protein-energy intake, we henceforth refer to undernourished study groups by the more general term, semistarvation.

Methods

Design of experiment

Anthropometry measures muscle size, or in essence, muscle mass. The experiment was designed to answer the question; is the loss in muscle mass in semistarvation accompanied by a proportionate reduction in muscle total protein, nonstructural protein [the noncollagenous protein fraction (NCP)], and energy content? This question was examined by evaluating muscle mass and composition, and then applying the following equations. The total mass of a given muscle is described by the equation:

$$\text{muscle mass} = (H_2O + DW), \text{ and} \quad (1)$$

$$DW = (\text{collagen} + NCP + TL + DNA + RNA + \text{glycogen}), \quad (2)$$

where DW is dry weight, and TL is total lipids. Total muscle protein (TP) is then equal to the sum of NCP and collagenous protein. Minerals account for about 1% of wet muscle weight and were excluded in our simplified analysis. Equations (*1*) and (*2*) can be combined to describe the major components of muscle mass or size:

$$\text{muscle mass} = (H_2O + \text{collagen} + NCP + TL + DNA + RNA + \text{glycogen}). \quad (3)$$

The energy content of wet muscle is equal to:

$$\text{muscle energy (kcal/g wet weight)} = (K_1TP) + (K_2TL) + (K_3 \text{ glycogen}) \quad (4)$$

where $K_1 = 5.65$ kcal/g, $K_2 = 9.4$ kcal/g, and $K_3 = 4.1$ kcal/g. Not all protein energy is available for metabolic processes, and utilizable protein energy can be calculated by subtracting 1.25 kcal/g protein to correct for urinary urea losses; K_1 then become 4.4 kcal/g.

Our study was aimed at estimating muscle mass from anthopometric measurements, and the biochemical composition (equation 3) and energy content of muscle (equation 4) by chemical analysis. These goals were accomplished by harvesting the psoas and biceps muscles (50 g each) from autopsy subjects at Grady Memorial Hospital from January 1979 to June 1980. Autopsies performed more than 6h postmortem were excluded, as were patients not falling into one of the three categories defined below. At the beginning of the autopsy, anthropometric measurements of the mid-upper arm were made. The muscle specimens were then quickly excised, extraneous fat trimmed, and the tissue inserted into a polyethylene container before freezing at −80°C. Biochemical composition was then analyzed as described below in "Biochemical methods."

Subjects

Of the 70 autopsies conducted during the study period, 15 were excluded because more than 6h elapsed between death and postmortem examination. Of the remaining 55 cases, 51 were selected for study who met the following criteria (**Table 1**).

The first group of cases were those subjects without a history of prior illness, who were 90 to 115% ideal body weight (%IBW) (7), and died within several seconds or minutes after traumatic injury.

The second group was composed of patients who had no history of undernutrition before hospitalization, were at or above IBW, and who were admitted for acute illnesses which terminated in death within 3 days of admission. Dextrose (5%) and water provided the only definable source of nutritional support during this period.

The third group of patients all had a history of

TABLE 1
Patient diagnoses

	Group			
	Control	Semistarvation		
		Early	Chronic	
			Cancer	Noncancer
Causes of death				
Gunshot wound	7			
Auto accident	4	1		
Malignant hypertension and CVA*		4		1
Acute myocardial infarction		1		
Malignancy				
Melanoma			2	
Pancreas			2	
Lung			4	
Pineal			1	
Leukemia			1	
Ovary			2	
Oropharynx			1	
Colon			1	
Ruptured aneurism				2
Congestive heart failure				3
				4
Chronic lung disease				1
Renal failure				
Liver disease				
Hepatitis				3
Cirrhosis				6
Total	11	6	14	20

* Cerebrovascular accident.

prehospitalization weight loss due to chronic disease. Death usually occurred after weeks of hospitalization and additional weight loss (> 2 kg). Nutrient intake (as judged from hospital records) during this period in all of these patients was far below the Recommended Dietary Allowance for protein, energy, and other essential nutrients (8). The third group was further subdivided into two types of subjects, those with and without cancer. This latter subdivision was based on earlier reports of muscle compositional changes unique to cancer patients (9–11).

We subsequently refer to these three respective subject categories as control group and early and chronically semistarved patients. It should be noted that the latter groups may also include patients with near total starvation in the immediate hours or days before death.

Clinical methods

Weight history was obtained from family and medical records on each subject. Body weight was measured at autopsy, and %IBW calculated from Metropolitan Life Insurance tables (7). Postmortem anthropometric estimates of mid-arm muscle area were made by measuring triceps skinfold thickness (TSF, in cm) with a Lange caliper, and mid-arm circumference (MAC, in cm) with a tape measure. The following equations (4) were then applied:

$$\text{arm muscle area (cm}^2\text{)} = \frac{(\text{MAC} - \pi \times \text{TSF})^2}{4\pi} - 10, \text{ for men, and} \quad (5a)$$

$$\text{for women} = \frac{(\text{MAC} - \pi \times \text{TSF})^2}{4\pi} - 6.5 \quad (5b)$$

$$\text{muscle mass (kg)} = \text{height (in cm)} [0.0125 + (0.0034 \times \text{arm muscle area})]. \quad (6)$$

Muscle mass calculated from equation 6 should be regarded as a rough approximation of total muscle mass (4); inclusion in the current report was for the purpose of estimating how changes in muscle composition per gram of tissue influenced overall body composition. If measured within 3 days of death, serum albumin was also recorded. The control group had serum albumin levels measured on postmortem intracardiac blood samples.

Biochemical methods

We measured the mass of H_2O and the six biochemical components of muscle described in equation 2. Of these six components, we found in preliminary experiments that muscle concentrations of NCP, collagen, and TL were stable in situ at 20°C up to 6 h postmortem. However, muscle glycogen, DNA, and RNA declined rapidly by about 40 to 50% in the first 60 min postmortem, then stabilized for the next 4 to 6 h. Interpretation of these three substrates therefore focuses on the relative difference between groups, rather than absolute levels within each group. Moreover, their total contribution to wet muscle mass is less than 2%. Muscle energy content was calculated from the observed glycogen, and thus will be low by about 1%.

Muscles were thawed, and the tissue was finely minced and homogenized in 25 ml of distilled water. Aliquots were then used for biochemical analysis as described below.

TP, NCP, and collagen. A 5-ml aliquot of the homogenate was mixed with 5 ml of 0.1 N NaOH and used for determination of TP, NCP, and collagen. NCP consists primarily of intracellular proteins (sarcroplasmic, contractile, and mitochondrial) (12), whereas the collagen fraction is for the most part extracellular proteins (collagen and elastin). For TP, 1 ml of the mixture was added to 9 ml of 0.2 N NaOH. The remaining 5 ml were centrifuged at 5000 × *g* for 30 min. The supernatant containing NCP was collected and diluted 10-fold with 0.2 N NaOH. The pellet was suspended in 9 ml of 0.2 N NaOH by a polytron homogenizer, and used for analysis of collagen. Total protein, NCP, and collagen were determined by the heated Biuret-Folin procedure of Dorsey et al. (13).

TL and triglyceride. The Folch (14, 15) method of purification was used to extract TL and its major subcomponent, triglyceride, from 2 ml of tissue homogenate. After the final washing in the procedure, the samples were evaporated under nitrogen at 100°C. The lipid was resuspended in 2 ml of 2:1 chloroform methanol solvent. Seventy-five microliters were used for the determination of TG by the method of Sardesai and Manning (15), and 1 ml was evaporated in a heating block at 110°C using prewashed-weighed 13 × 100 tubes. After the drying procedure, the tubes were placed in a 200°C drying oven for 1 h to remove any residual chloroform. TL was then determined by weighing these tubes.

Glycogen, RNA, and DNA. A 10-ml aliquot of the homogenate was used for sequential isolation and analysis of glycogen, RNA, and DNA according to the modified procedure of Shibko et al. (16). The aliquot was mixed with 2 ml of 70% perchloric acid (PCA) and allowed to incubate for 15 min at 0°C. The supernatant fluid containing glycogen was then removed, and the pellet washed with 5% PCA (1 × 10 ml). After a second 20 min of centrifugation at 4000 × *g*, the supernatants were pooled and assayed for glycogen according to the procedure of Carroll et al. (17). The pellet was incubated with frequent vortexing in 10 ml of 0.3 N NaOH at 37°C for 2 h. The mixture was then treated with 2 ml of 70% PCA, placed in an ice bath for 10 min, and then centrifugated at 4000 × *g* for 20 min. The supernatant contianing RNA was then separated, and the residue was washed with 5% PCA (1 × 5 ml); the pooled supernatants were than analyzed for RNA (18).

The pellet obtained in the above step was incubated in 8 ml of 1.5% PCA for 30 min at 90°C, and then mixed with 0.5 ml of 70% PCA. The samples were centrifuged at 4000 × *g* for 20 min and the supernatants collected. The pellets were washed with 1.5% PCA (1 × 5 ml) and centrifuged again. The combined supernatants were then analyzed for DNA by the Indole procedure of Hubbard et al. (19), as modified by Wiener et al. (20).

DW. Muscle H_2O and DW were determined on a 5-ml aliquot of the homogenate. The sample was placed in a small glass vial, frozen at −20°C for 24 h, and then lyopholized for 48 h. The dry residue was then weighed immediately.

Energy content. Caloric content of muscle (kilocalories) was evaluated by two methods. Method 1 determined kilocalories of dry muscle by bomb calorimetry (21) (Parr Adiabatic Bomb Calorimeter, model 1340, Moline, IL). In method 2, muscle energy was calculated from equation 4, given the measured content of muscle

protein, lipid, and glycogen. Results of both methods agreed within 2 to 3%, and we, therefore, present data for method 2 only, because this approach allowed subfractionation of total energy into each of three respective components.

Histologic methods

At the time of autopsy, a 10-mg muscle strip was removed from the belly of each muscle for histological study. After fixation in 10% formalin, the slide was prepared by hematoxylin and eosin staining, and examined by conventional light microscopy.

Results

Patient profile

The 51 subjects who met the selection criteria are described in **Table 2**. The control subjects, seven men and four women, died within minutes after traumatic injury. The average age and %IBW in this group were 30 yr and 103%, respectively. Serum albumin levels ($\bar{x} = 4.3$) were all within our laboratory normal range of 3.5 to 5.0 g/dl.

Patients with early semistarvation, three men and three women of an average age of 46 yr, died from cerebrovascular accidents (n = 4), myocardial infarction (n = 1), and auto injury (n = 1). There had been no previous history of undernutrition ($\bar{x}$ %IBW = 113%). Chronic illness, usually hypertension, had preceded the acute fatal event by an average of 8.2 yr, and hospitalization was short, lasting only 1 to 4 days ($\bar{x} = 1.6$), during which time weight loss averaged 0.4 kg. Serum albumin levels ($\bar{x} = 4.3$) were within the normal range.

The 14 cancer patients with chronic semistarvation (12 men and two women) averaging 55 yr of age, lost 16.8 kg before and 4.9 kg during hospitalization, and were at autopsy 78% IBW. The average disease duration was 3.2 yr, with a final hospitalization of 37 days. Serum albumin levels were depressed ($\bar{x} = 3.1$ g/dl). The 20 noncancer chronically semistarved patients (12 men and eight women) suffered from cardiopulmonary (n = 11), liver (n = 8), and renal (n = 1) disease. These subjects tended to be somewhat older ($\bar{x} = 61.7$ yr) and less undernourished than cancer patients. The average total weight loss was 12.1 kg over 7.2 yr, with an additional weight loss of 2.5 kg during hospitalization; the average %IBW was 88%. Serum albumin levels were low ($\bar{x} = 3.0$ g/dl).

Relation between muscle mass and composition

There were no significant differences in the composition of psoas and biceps muscles in the control group (Tables 3*A* and *B*), and therefore results for all groups are presented as "muscle" unless otherwise specified.

Control muscle mass and biochemical composition. Arm muscle area and approximate total muscle mass in controls were 48 cm^2 and 29.2 kg, respectively. The composition of this muscle tissue, expressed in kilograms and percentage (Table 3*A*) was 23.1 kg of H_2O (79.2%), 4.4 kg of NCP (15%), 1.0 kg of collagen (3.4%), 0.5 kg of TL (1.6%), 0.04 kg of DNA (0.12%), 0.1 kg of RNA (0.35%), and 0.15 kg of glycogen (0.5%) (**Fig. 1**).

Total and utilizable muscle energy content was 1.2 and 1.0 kcal/g wet weight, respectively (**Fig. 2**). There were no significant pathological or histological changes detected by light microscopy.

Muscle in early semistarvation. Compared to the control group, muscle in early semistarvation showed no detectable difference with respect to mass, composition, or histology, except for a significant reduction in muscle glycogen and RNA by about 50 to 70% ($p < 0.05$). Total and utilizable energy content of muscle was 1.32 and 1.07 kcal/g, respectively, and did not differ significantly from controls (Fig. 2). Histological examination revealed no significant abnormalities.

Muscle in chronic semistarvation. Muscle composition in cancer and noncancer patients was qualitatively similar, and pooled results are therefore presented. The mass, chemical composition, energy content, and histological appearance of chronically semistarved muscle differed from controls. Relative to the control group, arm muscle area was smaller by 54.2% (this equates to about 14.7 kg of muscle), but not all components of muscle were reduced to the same extent: the relative reductions were, respectivley, H_2O (11.3 kg or 52.9%), collagen (0.4 kg or 46%), NCP (2.7 kg or 65.3%), TL (0.1 kg or 40%), DNA (0.018 kg or 54.1%), RNA (0.021 kg or 81.7%), and glycogen (0.005 kg or 90.3%). Since each term in equation 3 changed to a different degree, the resulting composition of chronically semistarved muscle per unit mass differed from controls: H_2O ($p < 0.05$), TL ($p < 0.05$), and

TABLE 2
Duration of illness, weight loss, and nutritional indices of three groups (standard values from Reference 13)

	Age	Sex		Duration of illness			Wt loss			Serum albumin	Arm muscle area‡ (%STD)	Approximate muscle mass§
		Male	Female	Acute illness*	Chronic illness†	Hospitalization	Acute wt loss	Chronic wt loss	%IBW			
	yr			*days*	*yr*	*days*	*kg*	*kg*		*g/dl*	*cm²*	*kg*
Controls (n = 11)												
x̄	30	7	4						103	4.3	48 (98)	29.2
SD	12								8	0.2	7.2	4.4
Early semistarvation (n = 6)												
x̄	46	3	3	1.6	7.2	1.6	0.4	0.5	113	4.3	47 (96)	28.6
SD	20			0.8	8.4	0.8	0.5	1.2	18	0.7	12.4	7.5
Chronic semistarvation												
Cancer (n = 14)												
x̄	55	12	2	37	3.2	32	4.9	16.8	78	3.1	18 (37)	12.5
SD	18			23	4.3	19	1.4	11.2	19	0.7	8.5	5.9
Noncancer (n = 20)												
x̄	62	12	8	11.6	7.2	10	3.3	12	88	3.0	25 (51)	16.6
SD	11			13	5	13	1.8	9	14	0.7	11	7.3
Chronic semistarvation												
Total (n = 34)												
x̄	59	38	10	23	5.5	19	4.0	14.0	84	3.1	22 (45)	14.5
SD	14			22	5	19	1.6	10	17	0.7	9	6.1

* Acute illness and weight loss refer to immediate preterminal disease process.
† Chronic illness and weight loss refer to longstanding underlying disease process.
‡ Equation 5.
§ Equation 6.

TABLE 3
A, Biochemical analysis of hydrated muscle tissue (% wet wt ± SD)

Group	Control		Early semistarvation		Chronic semistarvation					
					Cancer		Noncancer		Cancer + noncancer	
Muscle	Biceps	Psoas	Biceps	Psoas	Biceps	Psoas	Biceps	Psoas	Biceps	Psoas
H_2O	79.2 ±2.4	77.7 ±1.6	77.3 ±2.8	78.9 ±2.4	80.6 ±21*	80.8 ±2.0*	81.9 ±2.8**	80.8 ±2.8**	81.2 ±2.5**	80.6 ±2.6**
DW	20.8 ±2.5	22.3 ±1.6	22.7 ±2.8	21.1 ±1.3	19.4 ±2*	19.2 ±2.5*	18.1 ±2.8**	19.1 ±2.8**	18.7 ±2.5**	19.3 ±2.6**
DNA	0.12 ±0.01	0.11 ±0.02	0.10 ±0.02	0.10 ±0.01	0.14 ±0.04	0.11 ±0.02	0.10 ±0.02	0.10 ±0.02	0.12 ±0.03	0.10 ±0.0
RNA	0.35 ±0.1	0.37 ±0.14	0.12 ±0.16**	0.16 ±0.12*	0.13 ±0.03**	0.15 ±0.03*	0.15 ±0.03**	0.14 ±0.06**	0.14 ±0.04***	0.15 ±0.05***
Glycogen	0.52 ±0.1	0.48 ±0.10	0.26 ±0.12**	0.22 ±0.08**	0.04 ±0.03***	0.04 ±0.03***	0.15 ±0.2**	0.19 ±0.14**	0.11 ±0.10***	0.11 ±0.08***
TP	18.4 ±3.3	19.3 ±2.4	20 ±2.4	17.9 ±5.1	15.6 ±3.13**	15.8 ±3.0**	15.3 ±3.4**	15.9 ±3.3**	15.4 ±3.2**	16.1 ±2.8**
NCP	15.0 ±2.2	15.0 ±1.6	16.1 ±3.1	15.1 ±5.6	12.0 ±2.4***	11.2 ±2.2***	11.1 ±2.9***	11.7 ±2.5***	11.4 ±2.7***	11.6 ±2.1***
Collagen	3.4 ±0.7	4.3 ±2.1	3.9 ±1.3	2.8 ±2.1	3.6 ±3.2	4.6 ±2.1	4.2 ±1.9	4.1 ±2.5	4.0 ±2.3*	4.5 ±2.2
TL	1.6 ±0.6	2.2 ±1.2	1.9 ±1.1	2.8 ±2	2.6 ±2.4**	2.7 ±1.1	2.0 ±1.7	3.4 ±3	2.1 ±1.9	3.1 ±2.7
Triglycerides	0.8 ±0.7	1.0 ±0.9	0.7 ±0.6	1.3 ±0.9	1.8 ±0.2**	1.5 ±1.7	1.2 ±1.7	1.7 ±3.0**	1.4 ±1.2**	1.6 ±2.4**

*, **, *** $p < 0.1$, 0.05, and 0.01 compared to respective control value. Analysis by Student's *t* test for nonpaired data.

TABLE 3
B, biochemical analysis of dry muscle tissue (% DW ± SD)

Group	Control		Early semistarvation		Chronic semistarvation					
					Cancer		Noncancer		Cancer + noncancer	
Muscle	Biceps	Psoas	Biceps	Psoas	Biceps	Psoas	Biceps	Psoas	Biceps	Psoas
DNA	0.56 ±0.1	0.49 ±0.1	0.44 ±0.1	0.47 ±0.1	0.72 ±0.02*	0.57 ±0.1	0.55 ±0.1	0.50 ±0.1	0.62 ±0.1	0.55 ±0.
RNA	1.7 ±0.6	1.7 ±0.6	0.5 ±0.9**	0.8 ±0.6**	0.6 ±0.2***	0.8 ±0.3**	0.8 ±0.2**	0.8 ±0.4**	0.7 ±0.2***	0.8 ±0.3**
Glycogen	2.5 ±0.4	2.2 ±0.4	1.1 ±0.3**	1.0 ±0.2**	0.2 ±0.04***	0.2 ±0.06***	0.8 ±0.02**	1.0 ±0.1**	0.6 ±0.02***	0.6 ±0.0**
TP	88.4 ±8	86 ±7	80.1 ±7	84.8 ±6	81.4 ±10**	82.3* ±8	84.3 ±11	82.8 ±10	82.4 ±11*	82.5 ±12*
NCP	72.1 ±6	67 ±8	70.9 ±6	71.6 ±2	62.9 ±13**	58.3 ±9**	61 ±10**	61.5 ±15**	61.5 ±11**	60.1 ±13**
Collagen	16.3 ±4	19 ±9	17.2 ±6	13.2 ±10	18.5 ±16	24 ±10*	23.2 ±10*	21.3 ±11*	21.9 ±12*	22.4 ±12
TL	7.7 ±3	9.7 ±5	8.4 ±3	13.2 ±15*	13.5 ±2*	14.1 ±6*	11.0 ±10	17.7 ±13*	11.2 ±8*	16.1 ±10*
Triglycerides	3.8 ±3.4	4.5 ±4	3.1 ±2.4	6.2 ±4.3**	9.3 ±1**	7.8 ±8.4*	6.6 ±9.4*	8.9 ±15**	7.9 ±6.4*	8.3 ±12**

*, **, *** $p < 0.1$, 0.05, and 0.01 compared to respective control value. Analysis by Student's *t* test for nonpaired data.

CP ($p < 0.1$) were increased, NCP ($p < 0.01$), RNA ($p < 0.01$), and glycogen ($p < 0.01$) were decreased, and DNA was unchanged. Total and utilizable muscle energy was reduced to 1.07 and 0.9 kcal/g wet weight (−10% compared to controls, $p < 0.01$) (Fig. 2), most of the reduction brought about by increased tissue H_2O; dry muscle from chronically semistarved patients was calorically equivalent to dry control muscle.

The relative change in the composition of chronically semistarved muscle leads us to focus on the primary question of the study; is the loss in muscle protein and energy content in proportion to the reduction in muscle mass in chronic undernutrition? Arm muscle area was 54.2% smaller than controls (**Table 4**), whereas TP, nonstructural protein (NCP) and

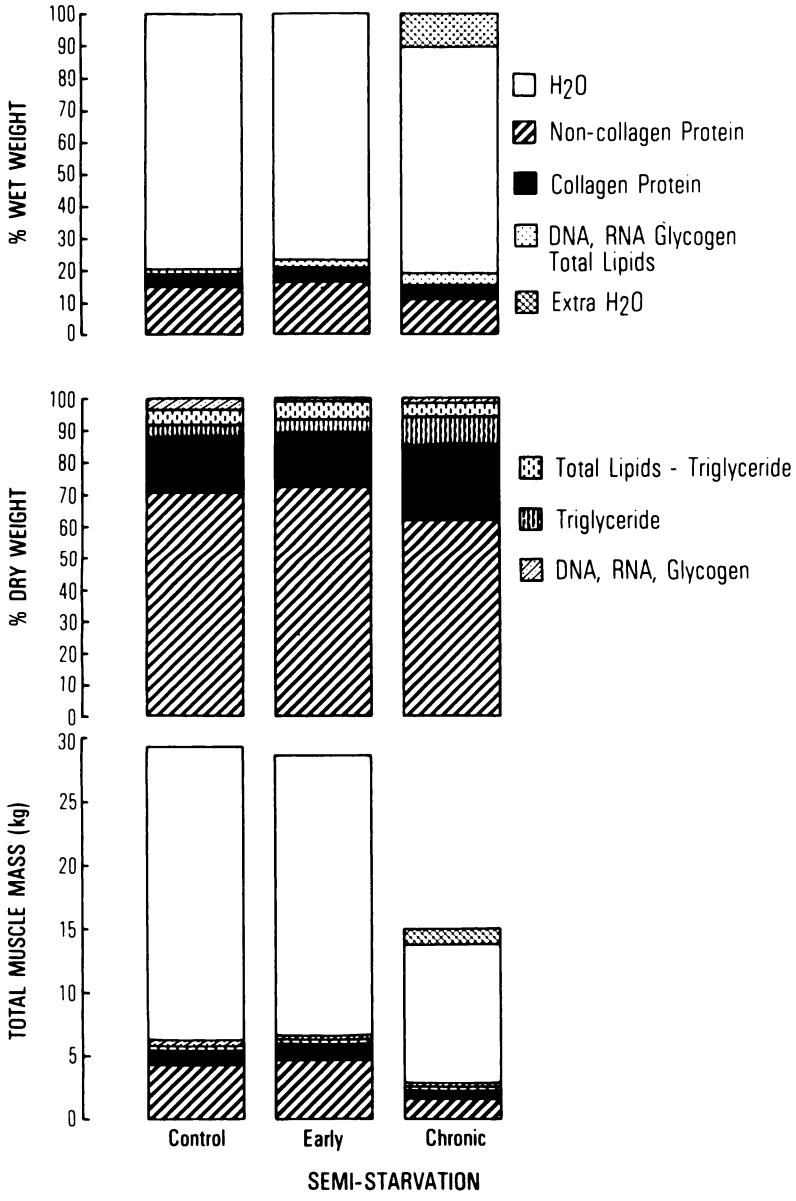

FIG. 1. Muscle tissue composition of control group, early and chronically semistarved patients. Results are expressed as percentage of wet weight (*top*), percentage of dry weight (*middle*), and as total muscle mass.

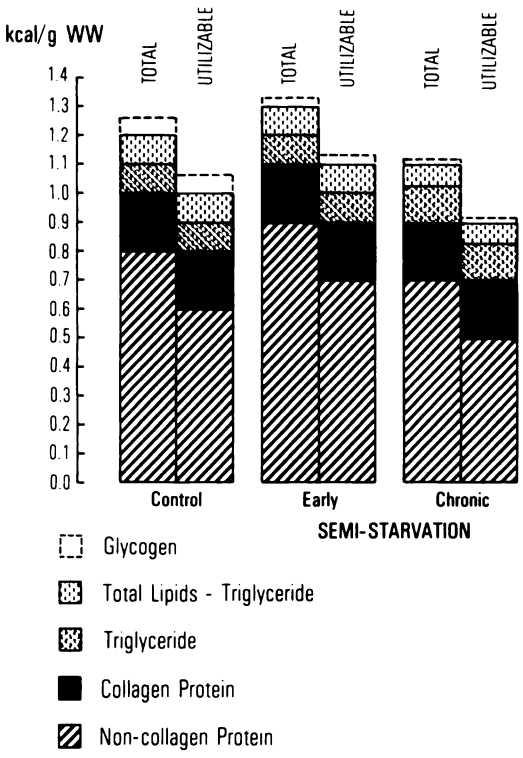

FIG. 2. Total and utilizable energy per gram of wet muscle tissue in control, early, and chronically semistarved patients. The proportion of each of the three energy yielding substrates—protein, lipid, and glycogen is shown for each group. The *broken line* depicting glycogen reflects the variable nature of this substrate, and the inability in the current study to obtain accurate in vivo measurements. The plotted values assume control glycogen is 1.5% of wet muscle weight, and adjust semistarved groups as observed value/control × 1.5%.

muscle energy were smaller by 61.6, 65.3, and 59.6%, respectively. Anthropometry alone, therefore, underestimated the relative reduction in muscle protein and energy in the chronically semistarved group by 5 to 15%.

Correlation of serum albumin and muscle composition. Serum albumin can be related to muscle composition in two respects. The first is that plasma oncotic pressure is closely related to the intravascular concentration of albumin; low levels lead to leakage of H_2O into the intercellular space, and the result is edema. The second relation is that both NCP and serum albumin are sources of metabolic fuel during semistarvation. We, therefore, correlated serum albumin with muscle DW (H_2O = muscle mass − DW), NCP and TP (**Table 5, Fig. 3**), and found significant correlations (all $p < 0.05$) for all three regression analyses.

Histological examination of chronically semistarved muscle demonstrated variable pathology, but generally there was reduction in myofiber diameter compared to controls, and in some cases there was a relative increase in connective tissue, interfibrillar edema, and increased fat.

Discussion

Evaluating an array of biochemical muscle constituents in a large number of normal and undernourished human subjects required conditions that must be discussed before interpreting our results. The first problem was the postmortem degradation of RNA, DNA, and glycogen allowed us to present only relative muscle differences of these substrates between groups; biopsy and rapid freezing techniques are required to measure accurately the tissue levels of these compounds in living man. The second problem was that the three groups differed in average age. Control subjects were youngest, while chronically diseased patients were approximately two decades older. Thus the influence of aging itself must be considered in interpreting our results, as senescence is also associated with muscle atrophy (22). An important follow-up study would, therefore, examine the relation be-

TABLE 4
Percentage change in muscle mass (arm muscle area) and composition in chronically semistarved group relative to controls

	Chronic semistarvation (% Δ)
Arm muscle area (equation 5)	−54.2
Muscle composition (equation 3)	
H_2O	−52.9
CP	−46
NCP	−65.3
TL	−40
DNA	−54.1
RNA	−81.7
Glycogen	−90.3
Muscle energy (equation 4)	
Total kcal	−59.6

TABLE 5
Correlation of serum albumin (SA) with muscle H_2O (DW) and protein*

n	Equation	SEE†	r	p
Muscle: biceps				
29	SA = 0.016 (DW) + 0.31	0.41	0.5	<0.001
32	SA = 0.019 (TP) + 0.24	0.38	0.7	<0.003
32	SA = 0.02 (NCP) + 0.77	0.36	0.7	<0.0001
Muscle: psoas				
32	SA = 0.018 (DW) − 0.29	0.39	0.5	<0.001
32	SA = 0.012 (TP) + 1.37	0.44	0.4	<0.05
28	SA = 0.019 (NCP) + 1.06	0.39	0.56	<0.001

* Units: DW, TP, and NCP mg/g wet wt; SA in g/dl.
† SE of the estimate.

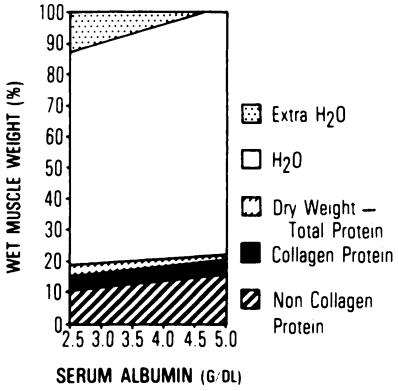

FIG. 3. Muscle composition per unit wet weight developed from regression equations in Table 5. If it is assumed that muscle is normally about 79% H_2O, then the amount (in percentage) of "excess" tissue water can be calculated as 100 − (4.76 × DW). The excess water line is included in the figure.

tween muscle mass and composition through the life span in normally nourished individuals. Two findings in the current study and a parallel rat experiment make it unlikely that aging muscle alone accounts for all of the observed differences in muscle composition between chronically semistarved and control groups. The first finding was that in early semistarvation, patients were intermediate in age ($\bar{x}$ = 47) between the control ($\bar{x}$ = 30) and chronically semistarved groups ($\bar{x}$ = 57); muscle composition, however, was nearly identical to controls, other than the expected changes in nutrition-sensitive substrates RNA and glycogen (discussed below). The second observation was that experimental chronic semistarvation in the rat produced biochemical changes in muscle nearly identical to those observed in the chronically semistarved patients (Stevens V, Heymfield S, unpublished data). Another consideration is that prolonged attempts at resuscitation might alter muscle composition in the early and chronically semistarved patient groups. Again, other than the loss of liable substrates RNA and glycogen, muscle tissue from patients with early semistarvation closely resembled that from the nonresuscitated control cases. Thus, while all of these factors must be considered in interpreting our data, it appears clear that nutritional factors can account for most of the differences in muscle composition between groups.

Muscle size and composition in semistarvation

Early changes. Early semistarvation was characterized by a 50% loss in glycogen, but no detectable change in muscle size was seen. We expect, however, that very careful measurements of muscle size in living subjects would indeed detect a reduction in arm muscle area with glycogen depletion for the following reason. For each molecule of glycogen metabolized, several molecules of associated H_2O are also removed from the muscle fiber (23). The theoretical decrease in muscle size can be calculated if we assume that *1*) normal in vivo muscle is 1.5% glycogen/g wet weight (254), and *2*) muscle remains 79% H_2O and 21% DW irregardless of glycogen content. Based on these two assumptions, a 50% loss in glycogen would result in a 3.6% reduction in muscle size, 0.75% of which is glycogen, and 2.85% H_2O. The 1:3–4 ratio of glycogen to H_2O is nearly identical to that found experimentally in living subjects (25). Similar glycogen-dependent changes in arm muscle area, but in a positive direction (**Fig. 4**), would be expected during nutritional recovery when there is an increase in muscle glycogen stores.

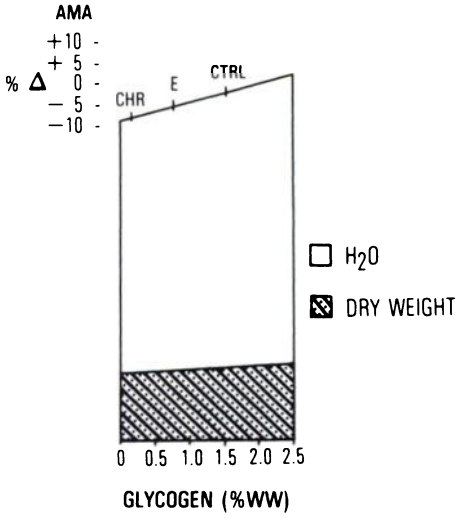

FIG. 4. The manner in which glycogen from control group (*CTRL*) (assumed to be 1.5% of wet wt) to early (*E*) and chronic semistarvation (*CHR*) influences muscle mass or arm muscle area is shown. Glycogen loss is associated with water loss, and the two together account for about a 3 to 5% theoretical decrease in arm muscle area (AMA).

Chronic semistarvation. Muscle size was reduced in patients suffering chronic semistarvation, the reduction brought about by losses of all seven measured components (equation 3): H_2O, collagen, NCP, TL, DNA, RNA, and glycogen. Each of these muscle constituents was relatively reduced to a different degree, and the final composition of the atrophied muscle differed from controls; relative losses were Δ glycogen > Δ RNA > Δ NCP > Λ muscle size = Δ DNA > Δ collagen > Δ H_2O > Δ TL. The most important conclusion from these findings is that muscle size is not an exact indicator of muscle protein and energy depletion in chronic semistarvation. The average disparity between size and composition in our study was not very large—about 5 to 15%, although in some individual cases the differences were up to 20%.

The most significant cause of size-composition dissociation was a relative increase in muscle H_2O. If we again assume normal muscle is 79% H_2O, then excess H_2O "falsely" enlarged arm muscle area by 1 to 2 cm^2, or by about 10%. This calculation is based on the following: 1 g of normal muscle = 21% DW, or 1.0/0.21 × DW (i.e., 4.76 × DW); if muscle is 19% DW, the equation becomes 5.26 × DW; "extra H_2O" for a given DW is therefore 5.26/4.76, or 10.5%. The cause of excess water accumulation (1 to 1.5 kg in 14.5 kg of chronically semistarved muscle, Fig. 1) is unknown, but at least two factors are suggested by our results. The first explanation is that the decline in serum albumin level caused a loss of intravascular oncotic pressure, and this allowed H_2O to escape into the intercellular fluid space. The strong correlation between serum albumin and muscle DW and H_2O suggests this as one possibility. An additional factor that might have increased the H_2O content of chronically semistarved muscle was that this tissue contained relatively more collagen, and this protein has a higher water binding affinity than most of the other cellular proteins (26). Other likely factors tending to expand total body (and tissue) H_2O include endocrine (27), pharmacological, and renal mechanisms.

Although muscle H_2O accumulation caused the largest discrepancy between muscle size and protein-energy content, there was also a reduction in TP, and especially NCP/g of dry muscle weight. Most of the relative decrease in TP can be accounted for by a 3 to 4% increase in TL, especially the TG (Table 3*A*) lipid faction. Fat replacement of myofibrilles (5), or an inability to adequately transport TG from muscle to other tissues (28), may account for this finding. Finally, the functional protein mass of muscle (NCP) decreased more in chronic semistarvation (65.3%) than muscle size (54.2%), energy (59.6%), and TP (61.6%). The excess H_2O and TG accounted for most of the difference between Δ size and Δ NCP, while the balance was caused by the relative excess of connective tissue protein.

Measurement of muscle mass in semistarvation: clinical perspective

Our study has demonstrated that measurements of muscle mass or size do not necessarily reflect underlying muscle composition. In vivo methods for analyzing the biochemical composition of muscle are either available (29) or under development (30). Currently, however, there is no replacement for the simple and practical bedside anthropometric techniques for quantifying muscle mass. These methods allow the practitioner to

roughly classify severity of undernutrition and establish trends over time. Although we offer no simple correction for the compositional changes observed in the current study, their appreciation should lead to a more rational interpretation and use of anthropometric data.

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