Variations in Muscle Electrolyte Composition Due to Sampling and to Aging.* (23364)

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The determination of potassium in muscle has been widely used in studies of potassium deficiency and factors relating to it. The potassium of muscle has been expressed in a variety of ways using different components of muscle as a basis of reference. These have included dry fat free muscle solids(1,2,3) and calculated intracellular water(4,5). The expression in terms of dry muscle is an effort to relate the potassium to muscle protein. This has been modified to be expressed in terms of collagen-free nitrogen(6). The expression in relation to muscle solids has the advantage of being a simply obtained analytic figure independent of calculations. It has, by now, gained widespread acceptance. On the other hand, expressing muscle potassium in terms of its concentration in intracellular water may represent a value more meaningful in physicochemical relations and does not reflect changes in muscle potassium resulting from variations in intracellular fluid volume in relation to muscle solids. This method of expression, however, is dependent on the assumption that the chloride space may be used as an index of extracellular volume.

The following report compares potassium content expressed in these 2 ways of muscle taken from the thigh to that obtained from the back in 2 groups of rats. The first group of rats consisted of normal 10-12 week old animals. The second group consisted of similar rats, allowed to grow an additional 7 weeks on a standard "chow" diet. Thus sampling from 2 different sites was compared using each method of expression in young mature rats and again in older larger rats. In addition, differences due to growth as reflected by changes noted in either sample could be compared. In this way validity of sampling and method of expression could each be explored.

Methods. Sixteen rats were fed lab chow from weaning. At approximately 10-12 weeks of age the first group of 8 were sacrificed. The second group of 8 were continued on the diet for 7 additional weeks. Group A averaged 255 grams and Group B averaged 250 g initially. Group B gained to a weight of 375 g in the ensuing 7 weeks, a gain of 125 g. The animals were killed by removing 8-12 cc blood from the abdominal aorta. Muscle from each rat was obtained separately from the thigh and from the anterior paravertebral region and was placed in separate weighing bottles. In the case of the thigh muscle, duplicate analyses on aliquots of differing weight were obtained. There was insufficient muscle for duplicate determinations on the paravertebral or back muscle. The method of analysis has been previously described(7) and was modified in only one respect. Elution of electrolyte from the muscle using nitric acid was carried out on a sample not previously fat extracted rather than on a fat extracted aliquot since slightly lower values were sometimes obtained in the latter instance. Fat extraction was done on separate aliquots of dry muscle. The method of analysis results in analytical values being directly determined in relation to muscle solids. Where duplicate analyses were done the per cent standard deviation between duplicates was as follows: Na $\pm$ 1.2%; Cl $\pm$ 1.3%; K $\pm$ .7%.

Results. Data comparing mean values for back and leg for each group are given in Table I. Differences between back and leg were observed in the case of chloride and potassium expressed in relation to fat free dry solids (FFDS) of muscle. In the young group these differences were statistically significant for chloride as well as for potassium, while in the older group the statistical significance could be demonstrated only in the case of potassium. The directions of change were the same in both groups of animals. Chloride was

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TABLE I. Data of Young and Old Groups Comparing Thigh and Back Samples.

	Expressed in terms of 100 g dry fat free solids							Per kg cell water		
	$(H_2O)_m$, g	$(Cl)_m$, mEq	$(Na)_m$, mEq	$(K)_i$, mEq	$(Na)_i$, mEq	$(H_2O)_e$, g	$(H_2O)_i$, g	$[Na]_i$	$[K]_i$	$[Na + K]_i$
								mEq		
Young—Thigh	340 ± 6	5.96 ± .41	9.51 ± .60	45.78 ± 1.16	1.54 ± .22	53.5 ± 4.5	286 ± 5	5.3 ± .7	160 ± 6	165 ± 6
Back	345 ± 15	5.22 ± .40	9.80 ± 1.37	48.83 ± .68	2.81 ± .77	47.0 ± 4.2	298 ±10	9.4 ± 2.4	164 ± 6	173 ± 5
Old—Thigh	322 ± 4	4.85 ± .26	10.62 ± .60	42.58 ± .83	4.20 ± .52	42.0 ± 2.8	280 ± 7	15.0 ± 1.9	152 ± 3	167 ± 4
Back	327 ± 10	4.54 ± .40	9.89 ± .78	44.93 ± 1.68	3.86 ± .76	39.6 ± 4.4	287 ± 5	13.5 ± 2.7	157 ± 5	170 ± 6
	Mean differences between samples from thigh and back									
Young—Δ	−5	+.74	−.29	−3.05	−1.27	+6.5	−12	−4.1	−4	−8
p*	ns	<.01	ns	<.001	<.05	<.01	ns	<.05	ns	<.05
Old—Δ	−5	+.31	+.73	−2.35	+.34	+2.4	−7	+1.5	−5	−3
p*	ns	ns	ns	<.01	ns	ns	ns	ns	ns	ns
	Mean differences between young and old using thigh and back respectively									
ΔT	−18	−1.11	+1.11	−3.20	+2.66	−11.5	−6.8	+9.7	−8	+2
p*	<.01	<.01	<.01	<.001	<.01	<.01	ns	<.01	<.01	ns
ΔB	−18	−.68	+.09	+3.90	+1.05	−7.4	−11	+4.1	−7	−3
p*	<.01	<.01	ns	<.01	<.05	<.01	<.05	<.05	<.05	ns

* Probability using Student's "T" test for significance. ns = not significant.

higher in thigh muscle than in back muscle, resulting in a larger calculated extracellular space.

Potassium, however, was lower in thigh muscle than it was in back for both young and old groups (−6.7%; −5.5% respectively). This difference was not accompanied by a parallel difference in total water (−2.4%; +1.6%), but was accompanied by a parallel difference in calculated intracellular water (−4.2%; −2.5% respectively) to potassium was observed. The difference in intracellular water did not have the same statistical significance as the difference in potassium per 100 g FFDS. However, when potassium was expressed in terms of its concentration in intracellular water, the difference in potassium noted above is no longer apparent.

In the young group only, a difference between thigh and back in the calculated values of intracellular sodium by either method of expression was also observed. This was not true in the older group, and its significance in the young group is not apparent.

The data comparing muscle composition of the young to the older group using data from either sample is given in Table I. A decrease in potassium associated with growth and aging could be demonstrated using data from thigh or from back for comparison. The decline in potassium with growth was evident by either method of expression, *i.e.*, in relation to muscle solids or expressed as concentration in intracellular water. These differences were accompanied by reciprocal increases in intracellular sodium such that the sum of sodium and potassium was not different in comparing young rats to old ones.

Discussion. The higher values of chloride and calculated extracellular volume in thigh muscle are very likely due to more connective tissue being present in thigh muscle. The samples from the thigh by simple inspection had more connective tissue. No collagen determinations were made to substantiate this.

The differences observed in the case of potassium in relation to muscle solids can be ascribed either to variations of potassium in relation to intracellular solids, to differences in the density of cell solids, or to differences in

solids from the extracellular phase to total muscle solids. The first 2 explanations imply some fundamental difference in muscle composition at the two sites. The third explanation is satisfied by postulating a difference in connective tissue in relation to muscle. If the observed differences noted in extracellular fluid between thigh and back are due to variations in connective tissue, then total muscle solids are increased in thigh muscle because of the increase in connective tissue. The lower value of potassium in relation to total muscle solids in thigh muscle might then represent an artifact due to an increase of connective tissue solids. The magnitude of connective tissue solids predicted by the difference in extracellular fluid is sufficient to account for these differences(8). The finding of a constant concentration of potassium in intracellular water lends support to this explanation.

This constancy of intracellular potassium concentration also strengthens the validity of those assumptions necessary to calculation of potassium concentration in intracellular water.

It is apparent from these data that studies relating muscle potassium values to cell solids should utilize samples from comparable sites. It is quite possible that long periods of potassium deficiency may result in greater variations in connective tissue or other muscle solids in relation to intracellular fluid as a consequence of anatomical changes in muscle from similar sites wrought by potassium deficiency. This would result in falsely low muscle potassium values when they are expressed in terms of muscle solids. Expressing potassium in terms of its concentration in intracellular water may be a more precise defintion of a real decrease in muscle potassium despite the greater number of assumptions inherent in its calculation. If these changes are due solely to changes in connective tissue solids, correcting muscle solids for collagen may serve to obviate the difficulties cited here in using muscle solids as a basis of reference.

The changes in muscle potassium associated with growth and aging are in the same direction but are even more pronounced than those reported by Lowrey and Hastings(4). It is pertinent to observe that increase in muscle sodium was equivalent to decrease in muscle potassium so that the sum of the 2 remains constant. This is in contrast to the initial decreases in potassium concentration observed early in the development of potassium deficiency(9). In the latter instance the initial decrease in muscle potassium is not accompanied by a significant increase in muscle sodium so that the sum of the 2 is substantially decreased.

Summary. 1) A difference in muscle potassium was demonstrated in samples from 2 different sites of the same animal when potassium was expressed in terms of fat free dry muscle solids. This difference did not exist when potassium was expressed in terms of its concentration in calculated intracellular water. We suggest that the discrepancy was related to differences in connective tissue or extracellular solids between the 2 samples, and that potassium expressed in terms of its concentration in intracellular water was the more meaningful expression. 2) A small but significant decrease in potassium associated with growth and aging was also noted. This difference was demonstrated expressing potassium in terms of muscle solids or intracellular water concentration. A reciprocal increase in calculated intracellular sodium was noted such that the sum of sodium and potassium remained constant.

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