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DOTTORATO DI RICERCA IN

FISIOLOGIA DEI DISTRETTI CORPOREI

<u>**XX**° CICLO</u>

"Is the body cell mass a predictive of adult obesity?"

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ABSTRACT

Background: Longitudinal studies of Total Body Potassium (TBK) are rare. Assessing TBK in order to evaluate Body Cell Mass (BMC) is important in the measure of Body Composition and aging.

Objectives: The aim of this study was to assess TBK and BCM in healthy men longitudinally in order to evaluate the changes with age.

Design: Longitudinal study

Subjects: Body composition of 133 healthy Italian men, 20 to 66 years of age, was measured by whole-body counting of ^{40}K. BCM (kg) was calculated as $0.00833 \times$ TBK (mmol).

Results: There was a significant increase in overweight and obese subjects between entry into and exit from the study. There was no significant decrease in TBK in this sample. The maximum BCM score from early age (≤30 years of age) was found to be a significant predictor of BMI after age 30.

Conclusion: Subjects with high maximum BCM at early age tend to increase in BMI as they age.

Key Words: Aging, Body Cell Mass, Body Mass Index, ^{40}K, Men, Total Body Potassium, Longitudinal.

ITALIANO

Studi longitudinali di misura del potassio 40 sono rari. Effettuare tale misura è tuttavia importante nella valutazione dei cambiamenti della composizione corporea con l'età.

Lo scopo del lavoro è stato quello di misurare longitudinalmente il potassio 40 e la massa cellulare metabolicamente attiva in maschi. E' stata effettuate la misura del potassio 40, attraverso un contatore di potassio, ogni anno in 133 maschi di età compresa tra i 20 ed i 66 anni. La massa cellulare metabolicamente attiva (BCM) è stata calcolata con la seguente formula: $0.00833 \times$ TBK (mmol).

I risultati mostrano un significativo aumento di soggetti soprappeso ed obesi tra l'ingresso e l'uscita dallo studio. Non c'è stata alcun significativa diminuzione del potassio in questo campione di popolazione. Il picco massimo di BCM è stato

riscontrato al di sotto dei 30 anni di età ed era significativo del BMI dopo i 30 anni di età.

In conclusione i soggetti con una elevata BCM in età giovanile tendevano ad aumentare il BMI con il passare degli anni.

INTRODUCTION

Total body potassium (TBK) is an index of fat-free mass. TBK has therefore been the subject of intensive research during the last 50 yeasrs. However, most of the research was conducted cross-sectionally (Kehayias et al 1997, Kyle et al. 2001, De Lorenzo et al 2003, De Lorenzo et al. 2004), longitudinal studies on body composition of aging healthy human adults, and in particularly TBK, are rare (Flynn 1989).

It has been reported that loss of skeletal muscle mass occurs with advancing age in elderly men and women, even in independently living healthy subjects (Gallagher et al, 2000). Furthermore, it has been reported that men lose significantly more leg skeletal muscle mass than women (Gallagher et al, 2000). Gallagher et al. (2000) reported that skeletal muscle mass loss in men was masked by weight stability, resulting from a corresponding increase in total body fat mass. Progression of sarcopenia, particularly in men, may therefore be clinically silent and comparable to the loss of bone mineral density in osteoporosis (Gallagher et al, 2000).

A fairly new approach to assess body composition is to measure body cell mass (BCM). BCM is defined as the total mass of “oxygen-exchanging, potassium-rich, glucose-oxidizing, workperforming" cells of the body (Widdowson et al, 1964). BCM is considered the actively metabolizing portion of the body, which is known as "protoplasm" (Widdowson et al, 1964). Presently, the "gold standard" for quantifying BCM is via measurement of the naturally occurring isotope ^{40}K (Pierson et al, 1974); however, the assessment of ^{42}K, as well as the measurement of the difference between extracellular water (ECW) and total body water (TBW) using multiple isotopes are also acceptable (Forbes, 1987). Total body potassium (TBK) concentration is linearly correlated with the size of the BCM (e.g., the mass of active metabolizing tissues) (Pierson et al, 1974). An accurate measure of BCM would prove extremely useful for establishing an individual's state of health or disease over time, possibly assisting with the prevention of sarcopenia.

Whole-body counting of ^{40}K enables, by non-invasive nuclear technique, the *in vivo* determination of the naturally occurring radioactive isotope of ^{40}K in the human body (Lukaski et al, 1987). Because ^{40}K is 0.012% of natural potassium, it provides an accurate assessment of TBK. Potassium is almost exclusively an intracellular cation (95%) (Moore et al, 1963; Lukaski et al, 1987; Widdowson et al, 1964), found chiefly in muscle and viscera (hence, essentially not found in fat, bone, or extracellular water). Moore et al. (1963) have considered TBK concentrations to be linearly correlated with the size of the BCM. An accurate measure of BCM would prove extremely useful for establishing an individual's nutritional state of health or degree of malnutrition and response to nutritional measures.

The aim of this study was to assess TBK and BCM in healthy men longitudinally, in order to evaluate the changes with age.

SUBJECTS AND METHODS

The study was first approved by the Ethical Committee of the University of Rome "Tor Vergata". Each subject gave verbal and written informed consent prior to participation. Body composition of 133 healthy Italian men, 20 to 66 years of age, was measured longitudinally. These men were employees at a nuclear power plant in Italy. Whole-body counting (Figure 4) is used at this plant to monitor the ingestion of radioactivity by employees who work with radiation, when unsealed sources are used in biomedical or industrial applications. Every year, the participants were weighed, had ^{40}K by whole body counting and received a physical exam by a physician and reported changes in diet, exercise, medications, medical diagnosis and treatment, drug prescriptions and lifestyle.

Body Weight, Height, Body Mass Index

All body composition techniques were administered to each subject on the same morning. On the test day, the subjects were in a post-absorptive state (fasted for at least 10 to 12 hours) and euhydrated. Body weight, using a standard balance beam scale (Invernizzi, Rome, Italy), and height (stadiometer; Invernizzi, Rome, Italy) were measured to the nearest 1 kg and 0.5 cm, respectively. Body Mass Index (BMI) was calculated using the formula: body weight (kg)/height (m^2).

^{40}K, Total Body Potassium, Body Cell Mass

The measurement of ^{40}K was assessed using a whole-body counter, surrounded by a cell, 2.5 cm wide and 3 m high, of 10 cm thick lead bricks, with a door whose entrance was formed by a 22 cm thick iron slab (Piersson et al, 1974). The room was continuously ventilated. A single 20.3 x 10.2 cm thallium-activated sodium iodine crystal was positioned above the subject, who was measured in a sitting position for 20 minutes. During the measurements, subjects were dressed in paper pajamas. TBK was then calculated by using the formula: ^{40}K x 8.474.6 (Forbes, 1987). BCM was calculated from TBK by using the formula of Moore et al. (1963): BCM (kg) = 0.00833 × TBK (mmol).

Statistical Analyses

First, the ^{40}K values at entry and exit to the study were compared using paired t-tests, since the data was approximately normally distributed. Secondly, at each age the mean ^{40}K value, and it's 95% confidence interval, plus the maximum and minimum values were calculated. These values were graphed to visually display the trends over the age range. Mixed effects regression models were used to estimates the effect of age on ^{40}K, adjusting for other potential confounders. The relationships between BCM and BMI, and also age and BMI were similarly assessed via mixed effects models. Finally, the BCM at early age (≤30 years) was summarized for each man, using means, medians, minimum and maximums. These summaries were then evaluated as possibly predictors of BMI in later life (>30 years). All analyses were performed using SAS 8.02 for windows, and two-tailed p-values <0.05 were considered significant.

RESULTS

Entry into (exit from) the study was defined as the age at which the first (last) ^{40}K measurement was recorded. Subject characteristics for all subjects at entry and exit are listed in Table 1. As expected, there were significant differences in all variables except height. The subjects were divided by BMI into normal, overweight and obese. At entry, 54.7%; 38.7% and 6.6% were normal, overweight and obese respectively. At exit, 31,2%; 54.1% and 14.7% were normal, overweight and obese respectively. There was a significant increase in the number of overweight and obese subjects during the course of the study (p<0.001).

Figure 1 shows the mean BCM values for the age range of the study. Age was found to be significantly related to BCM in an unadjusted model (Table 2). Adjusting for BMI did not significantly affect the age effect as BMI is highly unrelated to BCM. However, height was found to be significantly related to BCM. Having adjusted for height, the age effect increased from the unadjusted model (Table 2). BCM was found to increase with age, with taller men having higher initial BCM values and increasing at a slightly faster rate than shorter men (Figure 2).

Early age was defined as ≤ 30 years of age. Sixty-five (49%) subjects in the sample had at least one BCM measurement during early age. These early age BCM measures were summarized for each subject, via minimums, means, medians, and maximums. For this sub-sample, ages ranged from 31 to 55 years, with an average age across all subjects of 40. The average BMI score was 26.2 (± 2.8). The only BCM summary measure that was significantly predictive of the BMI scores was the maximum BCM measurement. In an adjusted analysis, subjects with a low maximum BCM (< 27) at early age tend to have a decreasing BMI score as they age. Subjects with high maximum BCM at early age tend to increase in BMI as they age (Figure 3).

DISCUSSION

The use of a whole-body counter to evaluate ^{40}K, and subsequently calculate TBK and BCM, is a valuable tool that could accurately assess cellular changes in body composition with aging. The aims of this study were to assess TBK and BCM longitudinaly.

The limitation of the study is the age of the subjects because they were measused only before their retairment.

Nonetheless, the results of our study are not consistent with others who reported decreases in TBK and BCM with age (Pierson et al, 1974; Flynn et al, 1989; Forbes et al, 1970; Kehayas et al, 1997; Kyle et al, 2001; Kyle et al, 2001).

Kyle et al. (2001) reported significant declines in BCM in individuals greater than 80 years of age than those 70 to 79 years of age, our resutls could not be compared for the different age of the subjects examined. Furthermore, fat mass was shown to increase in individuals until 75 years of age (Kyle et al, 2001). Kehayias et al. (1997) stated that, "potassium depletion with age can be explained by loss of cell mass because of insufficient replacement of cells, or because of a change in inctracellular potassium concentration". Flynn et al in their longitudinal study shows that the loss of 40k increase afert 60 years of age, unfortunately our participant was younger and our findings could nont confirm this. However, our results shows that there is a significant relationship between age, maximum BCM and BMI. Subjects with a low maximum BCM (< 27) at early age tend to have a decreasing BMI score as they age. Subjects with high maximum BCM at early age then to increase in BMI as they age.

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Table 1. Descriptive Characteristics for all Subjects at ENTRY into and EXIT from the study.

Characteristic; mean ± SD	Entry n=133	Exit n=133	P-value
Age (years)	31.3 ± 7.3	52.7 ± 7.3	<0.001
Height (cm)	1.7 ± 0.07	1.7 ± 0.07	1.00
Body Weight[1] (kg)	74.1 ± 10.9	80.6 ± 11.2	<0.001
Body Mass Index[1] (kg/m^2)	25.1 ± 3.1	27.0 ± 3.2	<0.001
K40 (gr)	144.5 ± 14.8	151.7 ± 9.0	<0.001
Body Cell Mass (gr)	30.8 ± 3.1	32.3 ± 1.9	<0.001

[1] n=106

Table 2. Regression coefficients from mixed effects model for BCM.

Variable	Model 1	Model 2	Model 3	Model 4
Age (years)				
linear effect	-0.09 *	-0.08	-0.08	-0.05 *
quadratic effect	0.001 *	0.001 *	0.001 *	0.001 *
Body Mass Index (kg/m^2)		0.001		
Height (cm)			2.40 *	2.51 **
Body Weight (kg)			0.001	

* $p<0.05$, ** $p<0.01$

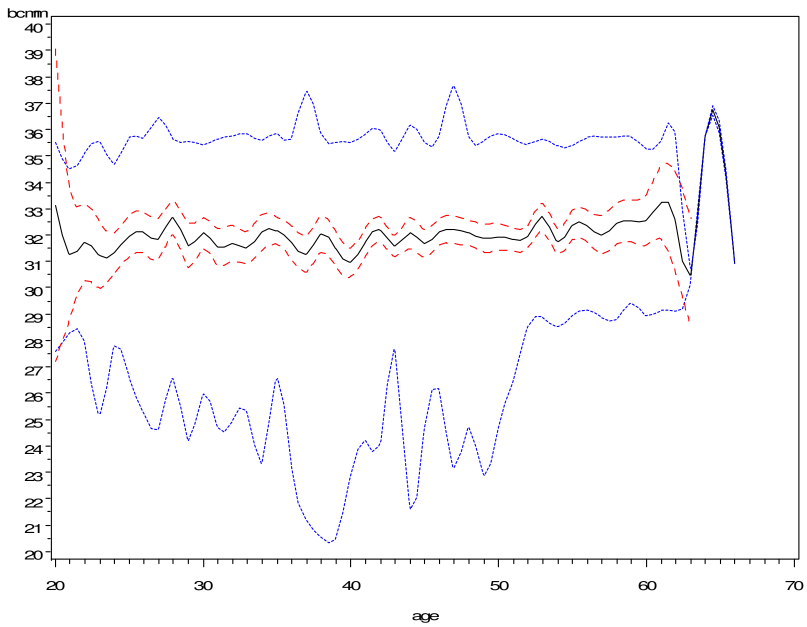

Figure 1. Body Cell Mass versus Age. Solid black line= simple mean at each age across subjects with a value at that age; dashed red lines= 95% confidence interval for the mean; dashed blue line= maximum and minimum values.

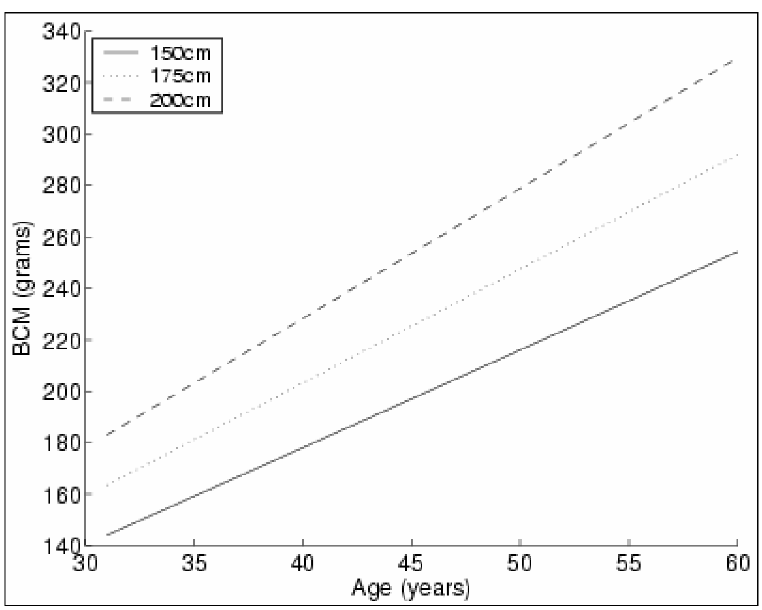

Figure 2. Predicted BCM versus Age. Predicted BCM measurements based from the mixed effects model (model 4) with quadratic age for varying height males.

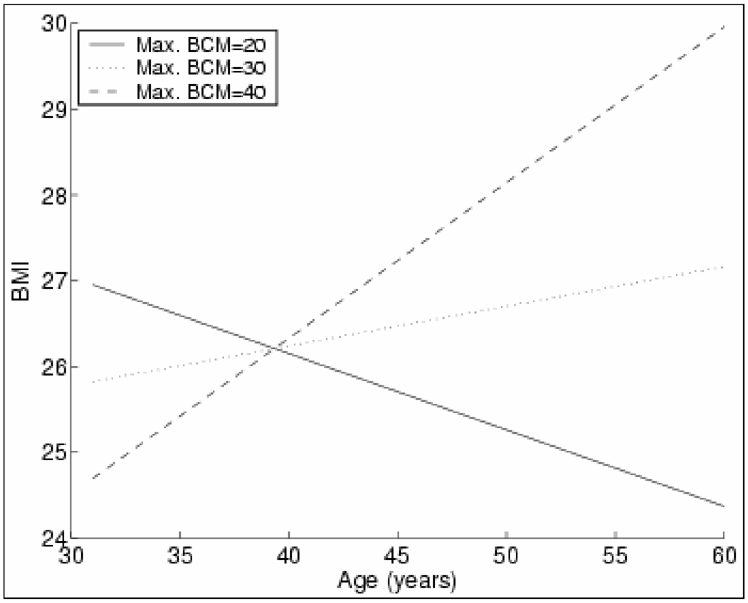

Figure 3: Predicted BMI scores for various Maximum BCM measurement at early age.

Figure 4. Whole Body Counter.