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24-h Urinary Creatinine Excretion and Associated Factors: Results from Nutrition Up 65

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DEDICATION

This study is wholeheartedly dedicated to the memory of two very special people who have meant and continue to mean so much to me:

To José Dias Leitão, who has been a great source of inspiration, strength and power of mind.

To Antero Ribeiro Fonseca e Sousa, who has been my best friend all these years and has taught me so many lessons that I will carry with me always.

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ABSTRACT

Introduction: Creatinine is a by-product of muscle metabolism and its measurement in urine has multiple applications in a clinical and research context. Although urinary creatinine excretion (UCrnE) is a biochemical parameter influenced by age, data on 24-h UCrnE in older adults, described according to sex and age group, is scarce.

Aim: This studied aimed to describe 24-h UCrnE in a large sample of older Portuguese adults and evaluate individual factors (sociodemographic, clinical, anthropometric and functional) associated to it.

Methods: A cross-sectional study was conducted with a sample of 1180 individuals (56% women) ≥ 65 years from the Nutrition UP 65 study. A sample of urine was collected in a 24-h period for each participant and creatinine was measured by the Jaffe Method. The association between individuals' characteristics and UCrnE was analysed through multiple linear regression.

Results: The median for 24-h UCrnE was higher for men than women (10.38 mmol/day vs 6.56 mmol/day, $p<0.001$), even when weight and height adjusted values were considered. A decline with age, more pronounced in men, was also observed. In multivariate analysis, sex (Sβ=0.363, $p<0.001$), weight (Sβ=0.118, $p<0.001$), height (Sβ=0.161, $p<0.001$), calf circumference (Sβ=0.069, $p=0.022$), marital status (Sβ=0.085, $p<0.001$), physical activity (Sβ=0.048, $p=0.029$) and alcoholic beverages consumption (Sβ=0.11, $p<0.001$) were positively associated with 24-h UCrnE and age (Sβ=-0.178, $p<0.001$), sarcopenia (Sβ=-0.053, $p=0.015$) and smoking status (Sβ=-0.043, $p=0.045$) were negatively associated. No association was found between 24-h UCrnE and education, self-perception of health status, cognitive impairment, residence, nutritional status and waist circumference.

Conclusion: The use of the Nutrition Up 65 database provided an opportunity to present 24-h UCrnE values, described according sex and age group, in a large sample of non-hospitalized older adults. Several kinds of independent factors associated with UCrnE were found, namely: age, sex, marital status, alcoholic

beverage consumption, weight, height, calf circumference, physical activity, sarcopenia and smoking status.

Key-words: *Urinary creatinine excretion, older adults, aging, population-based study*

RESUMO

Introdução: A creatinina é o produto do metabolismo muscular e o seu doseamento na urina tem múltiplas aplicações em contexto clínico e de investigação. Apesar da excreção da creatinina urinária (EcrnU) ser um parâmetro bioquímico influenciado pela idade, são escassos os estudos que descrevem a EcrnU de 24-h, de acordo com o sexo e grupo etário, em idosos.

Objetivo: Descrever a EcrnU de 24-h numa amostra de idosos portugueses e identificar os fatores associados.

Metodologia: Estudo observacional de desenho transversal realizado numa amostra de 1180 adultos (56% mulheres) com idade ≥ 65 anos. Para cada participante foi recolhida uma amostra de urina de 24h, sendo que a análise da creatinina foi realizada através do método Jaffe. Para avaliar a associação entre a ECrnU de 24-h e as características individuais dos participantes recorreu-se a um modelo de regressão linear multivariada.

Resultados: A mediana da EcrnU de 24-h foi maior nos homens do que nas mulheres (10.38 mmol/dia vs 6.56 mmol/dia, $p<0.001$), mesmo quando os valores foram ajustados para o peso ou para a altura. Observou-se também um declínio da EcrnU com a idade em ambos os sexos. Na análise multivariada, as variáveis sexo (Sβ= 0.363, $p<0.001$), peso corporal (Sβ=0.118, $p<0.001$), estatura (Sβ=0.161, $p<0.001$), perímetro geminal (Sβ=0.069, p =0.022), estado civil (Sβ=0.085, p<0.001), atividade física (Sβ=0.048, p=0.029) e consumo de bebidas alcoólicas (Sβ=0.11 , p<0,001) foram positivamente associados a EcrnU

de 24-h enquanto uma associação negativa foi encontrada para as variáveis idade (Sβ=-0.178, *p*<0.001), sarcopenia (Sβ=-0.053, *p* = 0.015) e tabagismo (Sβ=-0.043, *p*=0.045). Não foi encontrada associação para as variáveis escolaridade, auto perceção de saúde, comprometimento cognitivo, residência, estado nutricional e perímetro da cintura.

Conclusões: O uso da base de dados do Nutrition Up 65 permitiu descrever a ECrnU de 24-h, de acordo com o sexo e faixa etária, numa amostra de idosos portugueses. As variáveis idade, sexo, estado civil, consumo de bebidas alcoólicas, atividade física, peso, estatura, perímetro geminal, sarcopenia e tabagismo foram independentemente associadas com ECrnU de 24-h.

Palavras chave: *Excreção de creatinina urinária, idosos, envelhecimento, estudo populacional*

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LIST OF ABBREVIATIONS

AGAT – Arginine: glycine amidinotransferase

BM – Body Mass Index

CC – Calf circumference

CI – Confidence Intervals

CKD – Chronic kidney disease

Cr – Creatine

Crn – Creatinine

GAA – Guanidinoacetate

GAMT – S-adenosyl-L-methionine: N-guanidinoacetate methyltransferase

GFR – Glomerular Filtration Rate

MMSE – Mini Mental State Examination

MNA – Mini-Nutritional Assessment

R^2 – Coefficient of determination

SMM – Skeletal muscle mass

UCrnE – Urinary creatinine excretion

WC – Waist circumference

WHO – World Health Organization

CHAPTER I

INTRODUCTION

Overall Introduction

The proportion of seniors within the population is increasing, particularly in the developed world[1]. This is a consequence of increased life expectancy, due to public health care and social condition improvements, and decreased fertility[1]. Similarly, increasing lifespan is also found in Portugal, as according to the last national census in 2011, 19% of the population was aged 65 or older, an increase of 3% since 2001[2].

Aging is a heterogeneous and complex process that is accompanied by functional and biological changes, with important health implications[3]. Creatinine (Crn) is a breakdown product of creatine (Cr) and it is well known as a classic marker of skeletal muscle mass (SMM) and renal function[4, 5]. Furthermore, urinary creatinine excretion (UCrnE) is frequently used in research settings to identify the completeness of 24-h urine collections[6] and to estimate average 24-h excretion rates of solutes from spot urine samples[7].

Although some evidence suggests that the relationship between SMM and adverse outcomes is not linear[8, 9], low UCrnE has consistently been associated with mortality and unfavourable health outcomes in different populations[10-20]. In a cohort of individuals with chronic kidney disease (CKD), Wilson et al. (2014)[12] found that low UCrnE rate is more strongly associated with higher mortality, independent of traditional risk factors, than fat-free-mass, evaluated by bioelectric impedance. While UCrnE is often presumed to reflect SMM, some authors speculated that UCrnE may also reflect muscle quality or improved overall metabolism[12, 21-23].

Despite 24-h UCrnE being a biochemical parameter influenced by age, data on 24-h UCrnE in older adults, described according to sex and age group, is scarce[24-27]and no Portuguese data, is available.

This thesis provides data on 24-h UCrnE in a large sample of older Portuguese adults, but first, a brief literature review on creatinine (Crn) is needed.

Creatine and creatinine metabolism – An overview

Cr is an amino acid compound that has a key role in high-energy phosphate metabolism, which is required for buffering, transport, and regulation of cellular energy[28, 29]. Cr is found mostly within SMM (>90%), for the most part in the form of creatine phosphate[30], but also in other tissues like spermatozoa, heart, and brain[31]. It is reported that from the total muscle creatine pool, amounting to approximately 120g in a 70-kg man[32], 1.5-2% is degraded into Crn in an irreversible way and excreted in the urine[33, 34]. As a result, approximately 1-2g of Cr has to be replenished daily in order to maintain body Cr stores. About half of the body Cr needs are met by a typical omnivorous diet, mainly by the ingestion of meat and seafood[35], and the remaining by endogenous synthesis[36].

Cr is endogenously synthesized from the amino acids arginine, glycine, and methionine in a two-step inter-organ process, that requires the action of two enzymes, L-Arginine: glycine amidinotransferase (AGAT) and S-adenosyl-L-methionine: N-guanidinoacetate methyltransferase (GAMT)[31].

The first step of the *de novo* synthesis of Cr occurs primarily in the kidneys, with the transamidination from arginine to glycine, to form producing guanidinoacetate (GAA) and L-ornithine[31]. This reaction is catalysed by AGAT and is thought to be the main regulated step of Cr biosynthesis[37]. The GAA is then released into circulation and is transported to the liver, where it is methylated by GAMT to Cr and S-adenosylhomocysteine. Afterward, Cr is released into the circulation, where it can be taken up by various rapid, high ATP demanding tissues, via a Na+ and Cl^- - dependent Cr transporter (SLC6A8)[28], to be phosphorylated and become part of the Cr kinase system. Most of endogenous Cr is synthesized in the liver, but evidence suggests that it can also be synthesized within the brain[38].

After both Cr and Cr phosphate are nonenzymatically converted to Crn in an irreversible manner, Crn diffuses out of the tissues into the blood and is primarily freely filtered across the glomerulus and, to a smaller extent, secreted by the proximal tubules[39]. In adults with normal renal function, the rate of secretion represents, approximately, 10-20% of Crn excreted, but it can vary substantially due to genetic and biologic factors[40]. A third renal handling of Crn, tubular reabsorption, has been documented in older adults by Musso et al. (2009)[39]. It

also hypothesized that their finding may be related to several tubular changes in the aging kidney, however more research is needed.

Extrarenal Crn clearance may also occur, as Crn may be excreted in feces and, to some degree, degraded by bacteria in the gut[41]. However, this is thought to be negligible in subjects with normal renal function[31].

Determinants of creatinine excretion

Since Crn is derived from the irreversible breakdown of Cr and as most of the latter is found in the skeletal muscle, the amount of Crn excreted daily should be proportional to the SMM[4]. Indeed, SMM is the main determinant of UCrnE, and, therefore, differs with body weight[12, 24, 42-45], height[45-47], sex[24, 42, 48, 49], race[43, 44, 49] and age[24, 45, 48-50].

Although the concept, concluded by Folin in 1905[51], that Crn excretion is relatively constant has been widely accepted[4], relatively large variations in 24-h UCrnE within individuals have been reported. According to the review performed by Heymsfield et al.(1983)[4] normal daily variation of UCrnE for healthy adults, based on metabolic ward studies, ranges from 4 to 8%. However, in less controlled studies, slightly higher intra-individual coefficients of variation are described[52-54], with a study conducted in overweight diabetics showing variations up to 15% in women and 17.4% in men[55].

This day-to-day variability cannot be attributed to collection[53] or analytical errors[56], but to physiological factors (besides SMM) that modulate UCrnE. Among these factors are: diet, health status, drug use and exercise.

1- Diet

It is well established that protein-rich foods, particularly cooked meat and seafood, can increase UCrnE[57-59]. Reductions of 10-20% in 24-h excretion occur in healthy men consuming meat-free diets for several weeks[60, 61].

Research has shown that UCrnE can be directly influenced by at least 3 dietary components: Crn, Cr, and protein itself.

The strongest acute effect on UCrnE is exerted by the ingestion of pre-formed Crn. While cooking, a variable amount of Cr is converted to Crn, which is then absorbed in the gastrointestinal tract. Since the half-life of Crn is about 4 hours[62], the substantial intake of cooked meat is reflected in an acute increase in serum Cr and UCrnE. In experimental studies with healthy adults, increases in 24-h UCrnE of 13%[63] and 23%[64] after eating an additional meal of 225g and 260g of meat, respectively, have been observed.

Additionally, dietary Cr, found mostly in meat and seafood, but also in supplements, contributes gradually for the increase in the body Cr pool, which in turn is proportional to UCrnE[32, 65]. As it is not rapidly metabolized and excreted like Crn, since the half-life of Cr pool is approximately 40 days[66], elimination of meat intake produces a slow, curvilinear decrease in the Cr muscle stores and, therefore, in UCrnE[57].

Furthermore, dietary protein is also the main source of arginine and glycine, two Cr precursors[4]. The reduction in dietary protein and these two amino acids, causes a small decrease in the activity of the first enzyme of Cr synthesis, AGAT, and in the Cr pool[58, 67].

2- Health status

In theory, any condition that leads to muscle wasting will also have an impact on the UCrnE rate[68]. However, some diseases and conditions are known to be associated with alterations in the metabolism of Crn.

Most notably, in CKD, 24-h UCrnE decreases in proportion to the magnitude of the decrease in glomerular filtration rate (GFR)[69] and it may fall to one-third of the normal level in end-stage renal disease[70]. The decrease is not necessarily related to a depletion in SMM or a reduction in protein and meat intake[71], common in this condition, but to the recycling of Crn to Cr and to extrarenal degradation of Crn[69, 70].

Also, patients with reduced liver function often have lower UCrnE. Although reduced hepatic production of Crn due to liver insufficiency is a proposed

mechanism[72], reduced renal function and low SMM, may be the main reason for the low UCrnE in these patients[52, 73].

An acute increase in UCrnE is also reported in rhabdomyolysis and other high catabolic conditions, such as states traumatic injury[74, 75] and steroid myopathy[76]. This may be related to increased protein breakdown and higher rate of release of arginine into the systemic circulation[77, 78] or the released and conversion of Cr to Crn due to acidic urine[79]. Some studies have also reported an apparent increase in Crn excretion in infection and fever[4], but the exact mechanism involved is not clear and the interference of non-Crn chromogens cannot be ruled out[42]. UCrnE also increases by 5-10% in second half of menstrual cycle[80].

The Cr deficiency syndromes (CDS), a group of rare inborn errors of Cr metabolism, can affect Cr synthesis or transport. These syndromes are characterized by Cr deficiency, particularly in the brain, and low plasma and UCrnE levels[81]. These disorders have been extensively reviewed by other authors[81-84].

Furthermore, many neuromuscular diseases, such as Duchenne muscular dystrophy and Becker muscular dystrophy are accompanied by a variety of disturbances in Cr metabolism and decreased UCrnE[31].

3- Drug use

Several drugs, such as corticosteroids, can alter Crn metabolism by reducing GFR or alter renal haemodynamic[85]. Furthermore, some drugs can also interact with different aspects of Crn physiology without decreasing GFR, as reviewed by several authors[86-88]. For example, trimethoprim[89], dronedarone[90], cimetidine and other H2-blockers[91] and some antiretroviral drugs (e.g cobicistat and rilvitpirin)[92, 93] can inhibit Crn tubular secretion. However, this interference is typically reversible with the withdrawal of these drugs.

In addition, numerous drugs, including antibiotics cefoxitin and cefazolin, acetohexamide or chemotherapeutic agents such as flucytosine, can also interfere with the Jaffe-based analytical assays[94, 95].

4- Exercise

It is well established that regular exercise increases SMM, which, therefore, increases Crn excretion. However, the impact of acute exercise sessions on Crn excretion is less clear, because decreases[96, 97], increases[4, 98] and no effects[99, 100] in UCrnE have been reported. It is possible that physical activity has an insignificant effect on UCrnE, unless it is extreme. According to Heymsfield et al. (1983)[4], an extremely strenuous exercise can increase the daily UCrnE by a magnitude of 5-10%. The precise mechanisms involved in such changes are unclear but may be related to changes in renal function and to accelerated Crn synthesis, as a consequence of Cr depletion[4, 79].

Applications in clinical and research setting

Urinary creatinine as a measure of body composition and nutritional status

For over a century ago UCrnE has been related to body composition[101]. A direct proportionality of body Cr and 24-h UCrnE has been confirmed using isotopic dilution (N^{15})[102], making the use of 24-h UCrnE a method for estimating body composition. The relation of UCrnE to body composition has been proven systematically in adults of varying ages, showing that 24-h UCrnE correlates highly with SMM[103-105], lean body mass[106, 107] and anthropometric related measurements of SMM[108].

Some studies suggest that the relation between UCrnE and SMM can be expressed by a constant ratio, based on the assumption that 24-h UCrnE is directly proportional to total body SMM. This method, usually referred to as Crn equivalence method, can be expressed mathematically as: SMM= k x Cr(g)[103].

The ideal Crn equivalence ratio (k) is not consensual, as a broad range of values between 16.2 to 23 kg of SMM/1g of UCrnE/day has been reported[103, 109-112] leading to large variability in muscle mass estimates between studies. These variations may possibly reflect the different methodologies adopted, regarding sampling, diet and method used to estimate SMM. Moreover, other studies have suggested that the relation between SMM and Crn is not constant between subjects[103, 107]. This may be related to the presence of non-SMM Cr sources[103]. The exact quantitative contribution of these non-SMM sources to UCrnE is unknown, yet it may vary as a function of SMM. As a result, alternative equations have been proposed[103, 107], but further cross validation studies are needed.

Another problem, in addition to the difficulties in getting reliable urine collections, is the day-to-day variability of UCrnE within individuals, as detailed above. For this reason, multiple collections and a meat-free-diet on the day of the collection are often recommended to improve the accuracy of the method[113], which is not very practical. The deuterated Cr dilution method, which relies on the detection of an enrichment ratio of tracer to endogenous Crn, has been recently proposed,

as an alternative method for estimating whole body Cr stores, and thus SMM, without requiring timed collections of urine[114, 115].

Overall, Crn is considered a useful biochemical marker of SMM in both young and older adults[103, 104, 106]. However, it may be of limited usefulness in individual assessment of SMM as it lacks precision compared to other methods[106]. For this reason, it is better suited for group analysis of individuals with stable renal function.

Creatinine height index

The creatinine height index (CHI) is another method of expressing Crn excretion. CHI is defined as the 24-h UCrnE compared to the values obtained from normal subjects of the same age, height and sex, expressed as a percentage[116, 117]. A decrease in the CHI is assumed to reflect in a proportionate decrease in SMM and nutrition status. Index values of 60-80% are proposed as indicative of moderate depletion; values lower than 60% are evidence of severe depletion and values below 40% suggest very severe malnutrition[116, 118].

Research has shown that (CHI) is an index of nutritional status and lean body mass[116, 117, 119] and a good predictor of successful weaning and survival in patients on prolonged mechanical ventilation[118]. In addition, CHI has the advantage that Crn is not affected by edema, obesity or acute phase reactants, like other biomarkers[42, 120].

On the other hand, CHI also has several limitations, including the lack of reference standards for older adults, which can be problematic considering the effect of age on height and on the decrease in SMM[42, 121], as well as the difficulty and unreliability of measuring height in older adults[122].

Hoeyweghen et al. (1992)[121], based on the high correlation observed between total arm length and body length, proposed an adapted index of CHI, the Crn arm index. However, the validity of this index as a nutritional marker remains to be demonstrated.

Urinary creatinine as a marker of completeness of urine collections

24-h UCrnE is commonly used to determine the completeness of urine collections, based on the assumption of the constancy of UCrnE in a given individual[64]. Several Crn-based strategies for identifying incomplete 24-h urine collections have been used by researchers[112, 123-126]. However, due to UCrnE day-to-day variability within an individual, it may only detect gross errors in urine collections[127]. In addition to Crn based methods, other available markers to check for urine completeness include duration of the collection[127, 128], volume of the collection[127, 128], self-reports assessments of completion of 24-h urine collected from participants[123, 129] and aminobenzoic acid (PABA), often considered the reference method[6, 127]. Every method has limitations and no gold standard is currently available[6].

Urinary creatinine as an estimate of average 24-h excretion rates of several analytes

24-h UCrnE is also often used to estimate average 24-h excretion rates of analytes of clinical interest, from urinary analyte/Crn ratios[7, 130, 131]. Less time-consuming spot-urine samples are much more common in large epidemiological studies than in the collection of 24-h urines[24]. One approach to estimate average 24-h analyte excretion from spot-urine samples, and to control for dilution, is multiplying the urinary analyte/Crn ratio by the predicted daily UCrnE, using 24-h reference values or regression equations[24, 105]. This method is based on the assumption that UcrnE is known to be relatively constant over 24-h[24].

Urinary creatinine as a marker of renal function

Crn is the most commonly used biochemical marker of GFR which is considered the best indicator of kidney function[132]. Crn clearance is usually measured by determining the 24-h UCrnE and sampling a single plasma Crn value[133]. This was the standard method to assess GFR and renal function for many decades[5], but in current clinical practice is not routinely used as it was largely replaced by more practical regression formulas[134].

CHAPTER II

AIMS

This research had the following aims:

1) Describe 24-h UCrnE values in a large sample of older adults and compare results to other published data.

2) Evaluate which individual factors (sociodemographic, clinical, anthropometric and functional) are strongly associated with 24-h UCrnE.

CHAPTER III

METHODS

Study design and sampling

For the present study we used data from Nutrition up 65, a cross-sectional observational study conducted in Portugal, designed with the aim of identifying and reducing nutritional inequalities in older adults (>65 years old)[135]. More information about Nutrition UP 65 project methodology is described elsewhere[135].

The sample consisted of 1500 subjects aged ≥ 65 years old. To achieve a nationally representative sample of Portuguese older adults, a quota sampling method was adopted, using data form Census 2011, regarding sex, age, educational level and regional area defined in the Nomenclature of Terriorial Units for Statistical purposes (NUTSII).

The initial sample was composed of 95% of community-dwelling and 5% of individuals institutionalized in nursing homes. These proportionalities are in accordance with the previously described for the Portuguese population[135].

Individuals willing to cooperate were eligible to participate in the study if they were Portuguese, aged ≥65 years and not presenting any condition that would impede the collection of urine (e.g., dementia or urinary incontinence).

The potential participants were contacted through the domicile, telephone, or through institutions, such as town councils and parish centres. Information about the aims of Nutrition UP 65 was provided by the interviewer, and participants were invited to participate. The face-to-face interview was conducted by trained registered nutritionists, who were also responsible for the collection of anthropometric data.

From the original sample (n=1500) we excluded individuals who reported CKD and individuals with urine sample considered incomplete. Individuals with missing values for triceps skinfold data were also excluded.

Data collection took place between December 2015 and June 2016.

Data Collection

A structured questionnaire was used to collect the following information: sociodemographic data, cognitive performance, lifestyle and physical activity.

Sociodemographic data included information about sex, date of birth, residence type, marital status and education. Marital status was dichotomized as married or in a common-law marriage and as single, divorced or widowed. Educational level was assessed by the number of completed school years. The following categories were used: no formal education, 1–4, 5–12 and >12 years of schooling. Residence type was dichotomized as home or institution (nursing home).

Cognitive performance was determined by the Portuguese version of the Mini Mental State Examination (MMSE)[136]. Cognitive impairment was dichotomized as impairment or normal, using validated cut-offs adjusted for education[136].

Lifestyle included information about tobacco use, alcoholic beverage intake and physical activity. Physical activity was assessed by the short form of the International Physical Activity Questionnaire (IPAQ)[137], which refers to the activities performed during the seven days before the interview. Low physical levels were defined as <383 kcal/week for men and < 270 kcal for women. Normal physical levels are defined as ≥ 383kcal/week for men and ≥ 270 kcal/week for women[138]. Participants were asked if they were smokers or non-smokers, and the number of alcoholic drinks they consumed per day. Alcoholic beverage consumption was dichotomized as non-drinkers and drinkers, if 1 drink/day or more was reported.

Undernutrition status was assessed by the Portuguese version of the Mini-Nutritional Assessment®-Short Form (MNA®-SF)[139, 140]. Participants with a score ≥ 12 points were classified without undernutrition risk/undernutrition. Health status was assessed by self-perceived health, categorized as: very good, good, moderate, bad and very bad.

Data concerning CKD status was collected using questions drawn from the Portuguese National Health Survey 2005-2006[141].

All anthropometric measurements were collected by trained interviewers, following standard procedure[142]. Standing height was measured with a calibrated stadiometer (SECA 213, SECA GmbH, Hamburg, Germany), with 0.1 cm resolution. For participants with visible kyphosis or when it was not possible to measure standing height due to participant's mobility, balance limitations or paralysis, height was obtained indirectly from non-dominant hand length[143], using a calibrated paquimeter (Fervi Equipment, Vignola, Italy), with 0.1 cm resolution. Body weight (in kilograms) was measured with a calibrated portable electronic scale (SECA 803, SECA GmbH, Hamburg, Germany) with 0.1 kg resolution, while participants were wearing light clothes. When it was not possible to weigh a patient, body weight was estimated from mid-upper arm and calf circumferences (CC)[144].

Calf, waist and mid-upper arm circumferences, were obtained with a metal tape measure (Lufkin W606 PM, Lufkin, Sparks, Maryland, USA), with 0.1 cm resolution. Measurement of triceps skinfold thickness was made using a Holtain Tanner/Whitehouse (Holtain, Ltd., Crosswell, United Kingdom) skinfold caliper, with 0.2 mm resolution.

Body mass index (BMI) was calculated using the standard formula (body weight (kg) /standing height^2 (m)) and categorized using World Health Organization cutoffs[145].

Handgrip strength data was obtained with a Jamar Plus® + Digital Hand Dynamometer (Sammons Preston Inc., Bolingbrook, Illinois, USA), with a resolution of 0.1 Kg. Measurements were carried out according to the procedure recommended by the American Society of Hand Therapists[146].

Gait speed was quantified over a distance of 4.6 m. Participants were asked to walk at usual pace in an unobstructed corridor and walking time in seconds was recorded by a chronometer (School electronic stopwatch, Dive049,Topgim, Portugal).

Sarcopenia was defined according to the definition of European Working Group on Sarcopenia in Older People[147]. Low muscle mass was classified as midarm muscle circumference being less than 21.1 cm or 19.2 cm in men and women, respectively[148] Low muscle strength was classified as grip strength < 20 kg in

women and < 30 kg in men, and participants with gait speed of 0.8 m/s were identified as having poorer physical performance[147]. Individuals who were unable to perform gait speed test due to mobility or balance limitations were not considered.

24-h urine collection and determination of creatinine

Participants received oral and written instructions by the study interviewers, detailing the correct method on how to collect and store the 24-h urine specimen. Participants were instructed to abstain from collecting the first urine of the day, but to record the time of the first urine, and collect all subsequent urine. The following day, participants collected their morning urine until the time they recorded the first urine the previous day. A 24-h urine container was also provided, and participants were instructed to preserve the container in the refrigerator until it was delivered for further analysis. Volume and UCrnE were analysed by a certified laboratory (Labco Portugal). UCrnE was measured by the Jaffe method. 24-h UCrnE was reported in absolute terms but also adjusted by weight and height, as all of these methods are commonly used for 24-h UCrnE in scientific literature[10, 24].

A urine sample was considered inadequate if the 24-h UCrnE was less than 3.54 mmol for women and 5.3 mmol for men or if the volume collected was below 500 mL [124].

Study population

From the original sample we excluded 166 (11.07%) with 24-h urine collection considered incomplete and 137 (9.13%) subjects that reported CKD. There were 14 (0.93%) subjects for whom weight was not possible to measure or to estimate and 3 (0.2%) subjects with triceps skinfold measurement missing who were also excluded.

As a result, a total of 1180 subjects, from the 1500 eligible, were included in the present study.

Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR) and categorical ones as frequencies. Differences in characteristics according to sex were tested using the Mann-Whitney and Chi-square tests. Differences in UCrnE medians across characteristics were tested using Mann-Whitney and Kruskal-Wallis tests.

A multivariable linear regression model was built using the stepwise method to identify independent factors associated with UCrnE (dependent variable). The following variables were used as explanatory variables: sex (categorical), age (continuous), marital status (categorical), alcoholic beverage consumption (categorical), weight (continuous), height (continuous), CC (continuous), waist circumference (continuous), physical activity (continuous), sarcopenia (categorical), education (categorical), self-perception of health status (categorical), smoking status (categorical), cognitive impairment (categorical), residence (categorical), nutrition status (categorical). Variables with more than 2 categories were dummy-coded.

Ethics statement

This research was conducted according to the guidelines established by the Declaration of Helsinki and the study protocol was approved by the Ethics Committee of the Department of Social Sciences and Health (Ciências Sociais e Saúde) from the Faculdade de Medicina da Universidade do Porto (no. PCEDCSS – FMUP 15/2015) and by the Portuguese National Commission of Data Protection (no. 9427/2015). All participants were asked to read and sign a duplicated informed consent form before their inclusion in the study.

CHAPTER IV

RESULTS

Sample characteristics

The participants´ characteristics are presented in Table 1. The age ranged from 65 to 100 with a median (IQR) of 74 (I0) for women and 72 (9) for men and there was no significant age differences between men and women (p=0.122). Within this sample, 56% was composed of women.

Most of the participants were living in their own home (96.2%) and had at least 5 years of completed education (70%). More women were unmarried than men (60.8% vs 33%, p=0.001).

In this study most of the subjects (94.7%) had normal cognitive function and only a small proportion were smokers (4.5%) and drank alcohol (3.7%).

According to the MNA-SF, the majority of the individuals were not undernourished (86.2%). According to physical activity and functional measures, women had more often low physical activity levels compared to men (79.2% vs 73.1%, p=0.016). 11.2% of the sample fulfilled criteria for sarcopenia, with similar prevalence between men and women (12.6 vs 10.1%, p=0.159).

Regarding anthropometric characteristics, the median (IQR) for weight and height was 77 (16.1) kg and 1.65 (0.093) m for men and 67.1 (16.6) kg and 1.52 (0.077) m for women. According to the WHO standards, the prevalence of obesity was higher in women compare to men (43.1% vs 30.7%, p<0.001). The prevalence of central obesity (WC > 102 in men or > 88 in women) was also higher in women compared with men (75.2% vs 50.6%, p<0.001).

Table 1. Sociodemographic, clinical, anthropometric and functional characteristics according to sex for 1180 older Portuguese ≥65 years old participating in Nutrition UP 65 study.

	Men	Women	*p*	Overall
Sociodemographic characteristics				
Age (years), median (IQR)	72 (9)	74 (10)	0.122	73 (10)
Age, n (%)				
65-69	164 (31.9)	193 (29)	0.180	357 (30.3)
70-74	139 (27)	171 (25.7)		309 (26.2)
75-79	111 (21.6)	139 (20.9)		250 (21.2)
≥80	100 (19.5)	163 (24.5)		263 (22.3)
Residence, n (%)				
Home	503 (97.9)	632 (94.9)	0.08	1135 (96.2)
Institution	11 (2.1)	34 (5.1)		45 (3.8)
Education years, n (%)				
No studies	43 (8.4)	103 (15.5)	<0.001	146 (12.4)
1-4	64 (12.5)	144 (21.6)		208 (17.6)
5-12	285 (55.4)	318 (47.7)		603 (51.1)
>12	122 (23.7)	101 (15.2)		223 (18.9)
Marital Status, n (%)				
Single. divorced or widowed	170 (33.1)	405 (60.8)	<0.001	575 (48.7)
Married or common-law Marriage	344 (66.9)	261 (39.2)		605 (51.3)
Cognition, lifestyle and nutritional status				
Cognitive Impairment (MMSE), n (%)				
Normal	493 (95.9)	624 (93.7)	0.092	1117 (94.7)
Impairment	21 (4.1)	42 (6.3)		63 (5.3)
Smoking habits, n (%)				
No	471 (91.6)	655 (98.3)	<0.001	1126 (95.4)
Yes	43 (8.4)	11 (1.7)		54 (4.6)
Alcoholic beverages consumption, n (%)				
No alcohol consumption	140 (27.2)	397 (59.6)	<0.001	537 (45.6)
≥ 1 drink a day	374 (72.8)	267 (40.1)		641 (54.5)
Self-perception of health status, n (%)				
Very good/good	211 (41.1)	179 (26.9)	<0.001	390 (33.1)
Moderate	243 (47.3)	345 (51.8)		588 (49.9)
Bad/very bad	60 (11.7)	140 (21)		200 (17)
Undernutrition status (MNA-SF), n (%)				
Not undernourished	459 (89.3)	558 (83.8)	0.006	1017 (86.2)
Risk of undernutrition/Undernutrition	55 (10.7)	108 (16.2)		163 (13.8)
Physical activity (IPAQ)¶, n (%).				
Low	407 (79.2)	487 (73.1)	0.016	894 (75.8)
Normal	107 (20.8)	179 (26.9)		286 (24.2)
Anthropometric and functional measures				

Weight, kg, median (IQR)	77 (16.1)	67.1 (16.6)	<0.001	71.8 (17.5)
Height, m, median (IQR).	1.65 (0.093)	1.52 (0.077)	<0.001	1.571 (0.14)
BMI, kg/m2	28.31 (5.16)	29.4 (6.16)	<0.001	28.8 (5.7)
BMI classification (WHO), n (%)				
Underweight/normal range	90 (17.5)	97 (14.6)	<0.001	187 (15.8)
Preobese	266 (51.8)	282 (42.3)		548 (46.4)
Obese	158 (30.7)	287 (43.1)		445 (37.7)
Waist circumference, cm	102.35 (14.3)	95.9 (16.1)	<0.001	98.91 (15.7)
Waist circumference, n (%)				
<80cm/<94	104 (20.2)	49 (7.4)	<0.001	153 (13)
80-88cm/94-102	150 (29.2)	116 (17.4)		266 (22.5)
>88/>102	260 (50.6)	501 (75.2)		761 (64.5)
Calf circumference, cm	36 (4.3)	35.5 (4.3)	0.001	35.8 (4.4)
Calf circumference. n (%)				
Normal	494 (96.1)	618 (92.8)	0.015	1112 (94.2)
Low	20 (3.9)	48 (7.2)		68 (5.8)
Sarcopenia, n (%)				
Not present	448 (87.2)	599 (89.9)	0.159	1047 (88.8)
Present	65 (12.6)	67 (10.1)		132 (11.2)

Abbreviations: IPAQ, International Physical Activity Questionnaire; MMSE, Mini Mental State Examination; MNA-SF, mini Nutritional Assessment – Short Form; BMI, Body Mass Index.
Values may not add up 100% due to rounding up.
Based on U of Mann-Whitney for continuous variables and chi-square tests for categorical variables.

Urinary creatinine excretion and associated factors

The overall median (IQR) for 24-h UCrnE was higher in men than in women (10.38 (4.42) mmol vs 6.56 (2.62) mmol, p<0.001), even after adjustments for weight (0.135 (0.052) mmol/kg vs 0.098 (0.036) mmol/kg, p<0.001) or height (6.3 (2.6) mmol/m vs 4.3 (1.6) mmol/m, p<0.001). Absolute and body-size related 24-h UCrnE excretion values according to sex and age groups are presented in Figure 1. A decrease in 24-h UCrnE with age was found, more pronounced in men. The median in ≥80 years old group was approximately 31% and 22% lower, compared to 65-69 years old group, for men and women respectively. 24-h UCrnE also varies considerably between individuals, even when considering individuals in the same age group or when 24-h UCrnE is adjusted for body weight or height.

24-h UCrnE according to individual characteristics and sex are presented in Table 2. Individuals living in institutions excreted less Crn compared to individuals living in home (men: 7.61 mmol/day vs 10.5 mmol/day, p=0.005; women: 5.6 mmol vs

6.61 mmol/day, p=0.015). Single, divorced or widowed excreted less Crn than married or in common-law marriage individuals (men: 8.95 mmol/day vs 11.14 mmol/day, $p<0.001$; women: 6.19 mmol/day vs 6.93 mmol/day, $p<0.001$). Individuals with cognitive impairment had lower UcrnE, compared with individuals with normal cognitive function (men: 8.35 mmol/day vs 10.5 mmol/day, $p=0.001$; women: 5.3 mmol/day vs 6.62 mmol/day, $p<0.001$). In addition, lower UcrnE was also observed in individuals with sarcopenia, compared with those without sarcopenia (men: 8.56 mmol/day vs 10.69 mmol/day, $p<0.001$; women 5.62 mmol/day vs 6.68 mmol/day, $p<0.001$).

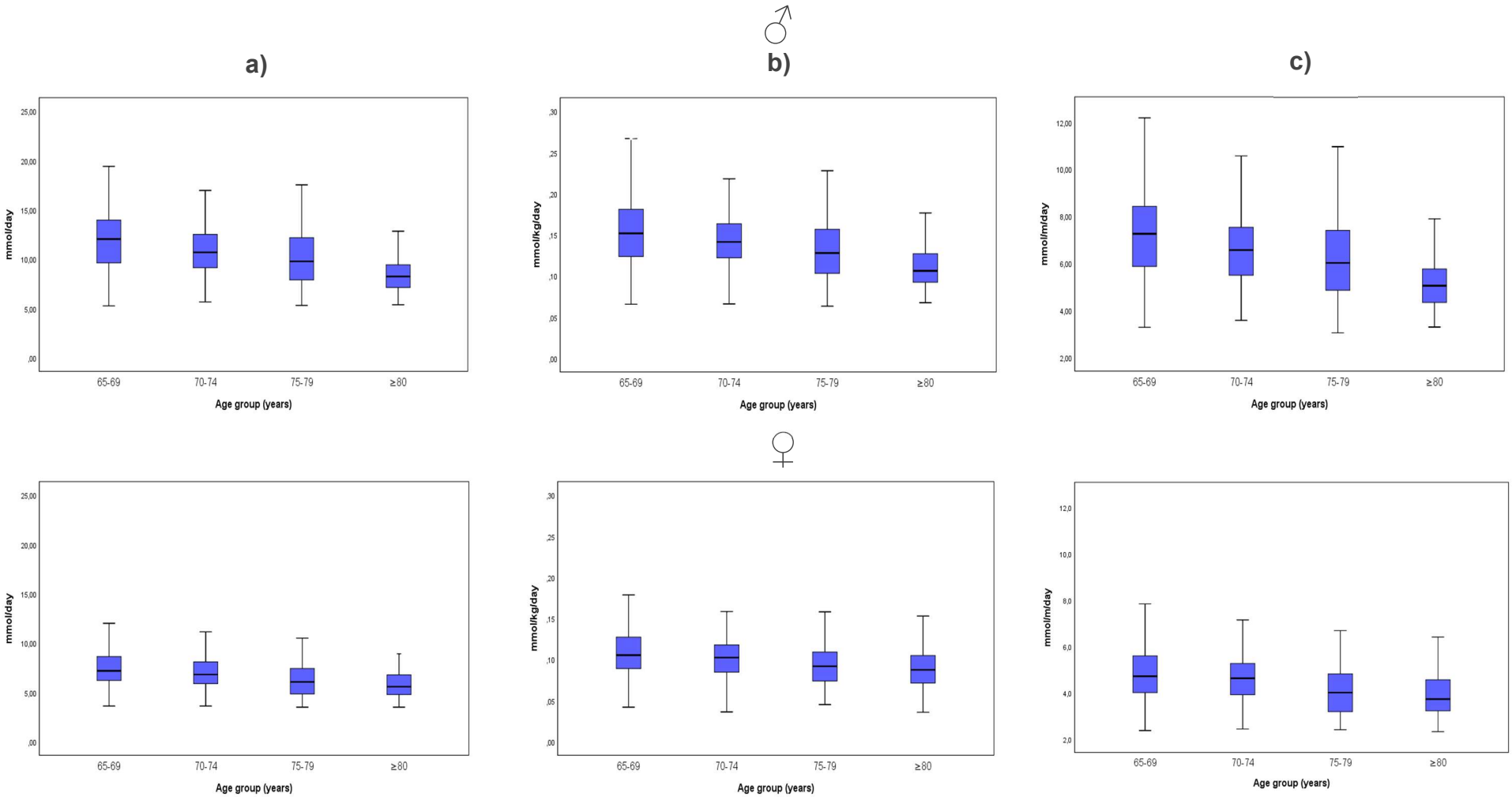

Figure 1. 24-h urinary creatinine excretion according to age and sex for 1180 older Portuguese ≥65 years old participating in Nutrition UP 65 study. a) Absolute excretion rates, b) weight-related excretion rates, c) height-related excretion rates.

Table 2. 24-h urinary creatinine excretion (mmol/day) according to individual characteristics and sex of the 1180 older Portuguese ≥65 years old participating in Nutrition UP 65 study

	Men		**Women**	
	Median (IQR)	*p*	Median (IQR)	*p*
Sociodemographic characteristics				
Age				
65-69	12.04 (4.36)	<0.001	7.16 (2.39)	<0.001
70-74	10.74 (3.44)		6.81 (2.21)	
75-79	9.78 (4.35)		6.03 (2.63)	
≥80	8.32 (2.4)		5.6 (2.06)	
Residence				
Home	10.5 (4.4)	0.005	6.61 (2.62)	0.015
Institution	7.61 (2.29)		5.6 (2.75)	
Education years				
No studies	8.17 (3.07)	<0.001	5.98 (2.71)	0.002
1-4	10.48 (4.82)		6.37 (2.76)	
5-12	10.63 (4.51)		6.72 (2.53)	
>12	10.79 (3.92)		6.74 (2.46)	
Marital Status				
Single. divorced or widowed	8.95 (3.64)	<0.001	6.19 (2.55)	<0.001
Married or common-law Marriage	11.14 (4.13)		6.93 (2.26)	
Cognition, lifestyle and nutritional status				
Cognitive Impairment (MMSE)				
Normal	10.5 (4.4)	0.001	6.62 (2.58)	<0.001
Impairment	8.35 (1.9)		5.3 (2.34)	
Smoking habits				
No	10.46 (4.41)	0.205	6.55 (2.62)	0.546
Yes	9.55 (4.29)		6.85 (2.13)	
Alcoholic beverages consumption				
No alcohol consumption	8.96 (3.71)	<0.001	6.21 (2.46)	<0.001
≥ 1 drink a day	10.98 (4.44)		7 (2.58)	
Self-perception of health status				
Very good/good	10.68 (4.35)	0.014	6.61 (2.42)	<0.001
Moderate	10.48 (4.57)		6.74 (2.66)	
Bad/very bad	9.23 (3.22)		5.99 (2.61)	
Undernutrition status (MNA-SF)				
Not undernourished	10.48 (4.37)	0.5	6.61 (2.58)	0.046
Risk of undernutrition/Undernutrition	9.95 (4.88)		6.15 (2.75)	
Physical activity (IPAQ)¶				
Low	8.94 (4.25)	<0.001	5.83 (2.39)	<0.001

Normal	10.76 (4.36)		6.79 (2.48)	
Anthropometric and functional measures				
BMI classification (WHO)				
Underweight/normal range	9.51 (4.02)	<0.001	5.8 (2.01)	<0.001
Preobese	10.37 (4.06)		6.01 (2.52)	
Obese	11.38 (5.15)		6.79 (2.78)	
Waist circumference				
<80cm/<94	9.83 (3.86)	0.113	6.01 (2.02)	0.016
80-88cm/94-102	10.25 (4)		6.51 (2.32)	
>88/>102	10.65 (4.94)		6.68 (2.73)	
Calf circumference				
Normal	10.42 (4.46)	0.165	6.67 (2.66)	<0.001
Low	9.73 (4.67)		5.63 (2.26)	
Sarcopenia				
Not present	10.69 (4.28)	<0.001	6.68 (2.55)	<0.001
Present	8.56 (3.28)		5.62 (2.36)	

Abbreviations: IPAQ, International Physical Activity Questionnaire; MMSE, Mini Mental State Examination; MNA-SF, mini Nutritional Assessment – Short Form; BMI, Body Mass Index.
Based on U of Mann-Whitney for variables with 2 categories and Kruskal-Wallis for variables with ≥3 categories.

The association between 24-h UCrnE and individual characteristics was examined using multivariable linear regression analysis. The model presented in Table 3 showed that 24-h UCrnE, after adjustment for potential confounders, was positively associated with sex (Sβ=0.363, p<0.001), body weight (Sβ=0.118, p<0.001), height (Sβ=0.161, p<0.001), CC (Sβ=0.069, p=0.027), alcoholic beverages consumption (Sβ=0.11, p<0.001), and negatively associated with age (Sβ=-0.178, p<0.001), sarcopenia (Sβ=-0.053, p=0.015) and smoking status (Sβ=-0.043, p=0.045. This model explained 52% of the total variance of the 24-h UCrnE. Education, self-perception of health status, cognitive impartment, residence, nutrition status and waist circumference were not independently associated with UCrnE.

Table 3. Multiple linear regression results for the association between 24-h urinary creatinine excretion and sociodemographic, clinical and anthropometric characteristics for the 1180 older Portuguese ≥65 years old participating in Nutrition UP 65 study.

Independent variables	Sβ (95% CI)	*p*-value
Age	-0.178 (-0.237; -0.147)	<0.001
Sex (reference: female)	0.363 (0.322; 0.447)	<0.001
Marital Status (reference: not married)	0.085 (0.04; 1.3)	<0.001
Alcoholic beverages consumption (reference: non-drinkers)	0.11 (0.065; 0.156)	<0.001
Weight	0.118 (0.051; 0.187)	<0.001
Height	0.161 (0.095; 0.229)	<0.001
Calf circumference	0.069 (0.008; 0.13)	0.027
Physical activity	0.048 (0.005; -0.093)	0.029
Sarcopenia (reference: not present)	-0.053 (-0.1; -0.009)	0.015
Smoking Status (reference: non-smoker)	-0.043 (-0.085; -0.001)	0.045

R2 = 0.52

CI, confidence interval; Sβ, standardized regression coefficient.

For the dichotomous variables, reference categories were coded as "0"

Variables excluded: Education, self-perception of health status, cognitive impairment, residence, nutrition status, waist circumference.

CHAPTER V

DISCUSSION

Main results

It is of major interest nowadays, with the global growth of the older population, to study the physiological and biochemical changes associated with aging process. To the present date, few studies have properly described UCrnE, according to sex and age groups, in adults[24-27], and in these, the older adult population were always underrepresented. Johner et al. (2015)[24] examined the 24-h UCrnE in a representative subsample of the VERA study, an observational study that took place in Germany, between 1986 and 1988, which involved individuals in community dwelling populations. This study reported mean values of 14.54 mmol/day (or 0.181 mmol/kg/day) and 10.10 mmol/day (or 0.149 mmol/kg/day) in the age group 60-69 years old, and 13.67 mmol/day (0.176 mmol/kg/day) and 9.44 mmol/day (0.142 mmol/kg/day) in the age group 70-79 years old, for men and women respectively. Similar data were reported by Kesteloot et al. (1996) in a Belgic population[27]. These data showed higher 24-h UCrnE than the present study. Body composition, health status and ethnic differences may explain those differences. In contrast, Kampmann et al. (1974)[26], in 149 Danish individuals over the age of 60, with normal serum Crn, showed lower mean 24-h UCrnE, particularly in men older than 80. Mean absolute values for men of 5.78 mmol/day (or 0.103 mmol/kg/day) and 5.41 mmol/day (or 0.083 mmol/kg/day), were observed in the age group 80-89 and 90-99 respectively. The differences observed compared to our study may be attributed to the fact that the sample include hospitalized patients while our study focused on predominantly community-dwelling adults.

The present study shows once more the impact of age and sex on 24-h UCrnE. The decrease in UCrnE observed with age is thought to be related not only to SMM atrophy[149], but also to lower meat consumption[150] and lower renal function[71]. Previous research had also suggested that the differential sex-specific rate of absolute muscle loss, the main determinant of UCrnE, can be greater in men than in women, which is in agreement with our findings[151, 152].

Taking these data into consideration, this study supports the assumption that reference ranges of UCrnE should be established not only according to sex, but also to age, as suggested recently[24, 153]. Although, some studies propose "reference values"[154-156], and also in a standard laboratory reference[157], they only give a single reference interval for each sex and do not consider variation due to age. Nevertheless, the use of these data in older adults, for e.g to determine the completeness of urine collections or to estimate average 24-h excretion rates of certain analytes, deserves some caution.

Anthropometrics, particularly weight and height, are well known determinants of UCrnE. In this study CC was also independent and positively associated with UCrnE, although not as strongly as the first two. This was an expected association as CC is an anthropometric parameter that is related to SMM[158].

Being married or in a common law-marriage, was positively associated with 24-h UCrnE. Those who are single, divorced or widowed can have worse eating habits, including less meat intake, and less SMM[159, 160]. In addition, being married is often associated with advantages in health[161] which could also impact UcnrE.

As expected, a positive association of 24-h UCrnE with physical activity was observed. Muscle disuse is a well-recognized cause of SMM atrophy[162] and physical activity decreases the likelihood of several chronic diseases[163] as such, being a good indicator of good health.

Furthermore, alcohol intake was positively associated with 24-h UCrnE, an unexpected result that was not found previously. Although alcohol abuse has a negative impact in SMM and renal function[164], the relationship between the amount of alcohol consumed and kidney function or muscle function varied from study to study[165, 166]. For instance, Steffl et al. (2016)[166], in a meta-analysis involving 13,155 participants older plus 65 years, found a protective effect of alcohol consumption in sarcopenia.

In this study, cigarette use was positively associated with 24-h UCrnE. Although this association had not been found previously, it is in agreement with some studies suggesting that smoking enhances muscle wasting and muscle dysfunction by multiple mechanisms[164].

A negative association was also found between Sarcopenia and UCrnE. Although, UCrnE has not been related to sarcopenia yet, this finding was expected as low SMM is a component of sarcopenia[147]. UCrnE is often considered a reliable index of SMM among older adults[104], however the lack of reference value for older people makes this method currently unsuitable to the study of sarcopenia[114].

Although decreased UCrnE may reflect poor nutrition, decreased SMM or reduced quality of life[21] this study does not support the use of 24-h UcrnE as a marker of undernutrition. However, increasing evidence supports the robustness of 24-h UCrnE, as a risk marker for adverse health outcomes in different populations[10-20], including in community-dwelling adults[15]. Further research is needed to evaluate if 24-h UcrnE can provide additional information of clinical interest, above and beyond traditional markers, and if so, what are the exact mechanisms involved.

Strengths and limitations

The present study has a few limitations that must be acknowledged. Firstly, renal function was not evaluated and CKD status was self-report, thus it could be under-reported[167]. This is supported by the low prevalence of CKD in the present study (9.1%), compared with the previously reported for Portuguese older adults[168]. Furthermore, the presence of other diseases and conditions, which may have explained some of our findings, were not evaluated. Additional information that might be valuable in this analysis, particularly diet (besides alcohol intake) and drugs, was also not available.

Secondly, the usefulness of 24-h UCrnE obtained from a single urine collection may be impaired by its day-to-day variation. The collection of several consecutive urine samples eliminates some of this variance and is often recommended[169] but is impractical in large epidemiological studies.

Thirdly, we cannot exclude incomplete urine collections by some participants even considering that the individuals participating in our study were carefully

instructed on the correct procedure of 24-h urine collection and potential invalid collections were excluded.

On the other hand, the use of the Nutrition Up 65 database provided an opportunity to present detailed data on 24-h UCrnE on a large sample, based in a nationally representative sample of older Portuguese adults. This is the first study that evaluated the effect of various factors (sociodemographic, clinical, anthropometric and functional) on 24-h UCrnE.

CHAPTER VI

CONCLUSION

In this study, we described 24-h UcrnE according to sex and age group, in a large sample of non-hospitalized Portuguese older adults and several independent factors associated with UCrnE were found, namely: Age, sex, marital status, alcoholic beverage consumption, weight, height, calf circumference, physical activity, sarcopenia and smoking status.

These factors reflect anthropometric, lifestyle and sociodemographic differences which may be important to improve further knowledge related to this biochemical parameter and highlight the need for additional research.

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