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Creatine and guanidinoacetate reference values in a French population

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ABSTRACT

Creatine and guanidinoacetate are biomarkers of creatine metabolism. Their assays in body fluids may be used for detecting patients with primary creatine deficiency disorders (PCDD), a class of inherited diseases. Their laboratory values in blood and urine may vary with age, requiring that reference normal values are given within the age range. Despite the long known role of creatine for muscle physiology, muscle signs are not necessarily the major complaint expressed by PCDD patients. These disorders drastically affect brain function inducing, in patients, intellectual disability, autistic behavior and other neurological signs (delays in speech and language, epilepsy, ataxia, dystonia and choreoathetosis), being a common feature the drop in brain creatine content. For this reason, screening of PCDD patients has been repeatedly carried out in populations with neurological signs. This report is aimed at providing reference laboratory values and related age ranges found for a large scale population of patients with neurological signs (more than 6 thousand patients) previously serving as a background population for screening French patients with PCDD. These reference laboratory values and age ranges compare rather

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favorably with literature values for healthy populations. Some differences are also observed, and female participants are discriminated from male participants as regards to urine but not blood values including creatine on creatinine ratio and guanidinoacetate on creatinine ratio values. Such gender differences were previously observed in healthy populations; they might be explained by literature differential effects of testosterone and estrogen in adolescents and adults, and by estrogen effects in prepubertal age on SLC6A8 function. Finally, though they were acquired on a population with neurological signs, the present data might reasonably serve as reference laboratory values in any future medical study exploring abnormalities of creatine metabolism and transport.

1. Introduction

The isolation of creatine is historically attributed to the French chemist Eugène Chevreul [1]. This physiological compound is supplied through body biosynthesis and the diet [2–4], and its role in muscle physiology has been largely claimed [5–8]. A creatine/creatine phosphate cycle involving creatine kinases enables the reversible exchange of the high energy phosphate bond of ATP [3]. A role of brain creatine in neurotransmission has been recently proposed ([9–12], and references therein).

Primary creatine deficiency disorders (PCDD) are inborn errors of metabolism affecting proteins which catalyze creatine biosynthesis (AGAT and GAMT) or transport (SLC6A8) [10–17]. This group of disorders has rapidly focused the attention of the physicians because, relatively unexpected, neurological dysfunctions (intellectual disability, speech delay, autistic behaviors, epilepsy) predominate sometimes over muscle signs [10–17]. Interestingly, these disorders share in common a fall in brain creatine detectable by ^{1}H-MRS [16]. This gives a basis for the neurological expression of these disorders and also explains why in many studies PCDD patients are screened by exploring populations of patients with neurological symptoms [18–26]. For these studies, reference values of a normal healthy population are often employed without knowing whether the population targeted by the screening and the normal population providing reference values differ or not in their body fluid contents in creatine and metabolites. We previously conducted a screening of primary creatine deficiencies in French patients with unexplained neurological symptoms [27]. In this cohort, laboratory data were collected in patients without PCDD (more than 6 thousand). This great number of data, here, has been used to calculate through adapted statistical tool new reference laboratory values and age intervals related to the large neurological population. Values and age intervals, obtained in this neurological population, are further compared with literature reference data given by different studies.

2. Subjects and methods

2.1. Population selection and data collection

The population was previously defined [27]. It was composed of French patients with neurological symptoms and submitted to screening for PCDD during a period of 28 months (between January 2008 and April 2010) in six major French university hospitals: Angers, Lille, Lyon and Paris (Hôpital Necker Enfants Malades, Hôpital Robert Debré and Hôpital Raymond Poincaré). The population includes 6334 persons distributing into 4411 male (age range 0–82 years) and 1923 female (age range 0–70 years) subjects. Patients diagnosed with PCDD were excluded of this study. Urine and blood creatine and guanidinoacetate values along with patient sex and age were collected for statistical analysis and generation of new reference laboratory values and age ranges.

2.2. Methods for metabolite measurements

Creatine and GAA were measured in urine and plasma by tandem mass spectrometry (LC–MS/MS) using stable isotopes as internal standards ($^{13}C_2$-GAA and $^{2}H_3$-creatine) following a method described previously [28]. Urinary creatinine was measured using LC–MS/MS using a specific internal standard ($^{2}H_3$-creatinine) except in two university hospitals of Paris (Necker Enfants Malades and Robert Debré) where this measurement was done with the colorimetric Jaffé method [29,30].

2.3. Statistics

Statistical analyses were performed with the MedCalc® software, version 11.3.1.0 (MedCalc Software, Mariakerke, Belgium). Mann–Whitney test was used to compare independent value sampling characterized by a non-Gaussian distribution (comparison of biochemical values as a function of the sex or a given age window). The studies for statistical distribution of plasma and urinary GAA and creatine levels were performed using Kolmogorov–Smirnov test. Usual values were given by 2.5 and 97.5 percentiles.

The analysis of the continuous variable «age» was performed by the non-parametric test of Kruskal–Wallis. This test has enabled us to compare adjacent age ranges, each other, for each studied parameter (urine and plasma creatine and GAA). For values of the same parameter, when no-significant difference was found between two subsequent age windows, the two age windows were fused into a larger age range towards which parameter values were expressed. This operation led, for each studied parameter, to individualize in the studied population various age ranges of interest. All statistical analyses were performed with a significance threshold of 5%.

3. Results and discussion

3.1. Reference values for creatine and metabolites in blood and urine in the population with neurological signs

For the 6334 subjects, on the basis of the creatine and guanidinoacetate laboratory values, significantly distinct sex and successive age range groups were individualized. Reference values within each of these groups were calculated and are accounted for by Table 1.

3.1.1. Comparison of GAA and creatine values as a function of the control subject gender

Median values and 95% confidence intervals plasma and urine GAA and creatine concentrations were determined in control subject sex groups, all age considered. No significant difference was found between the two female and male groups about plasma GAA ($p = 0.84$) and creatine ($p = 0.83$) values. In contrast, a significant difference was observed in the urine between the two groups for guanidinoacetate ($p < 0.0001$) and creatine ($p = 0.0003$) concentrations expressed as mmol/mol creatinine with values in female significantly higher than in male subjects.

3.1.2. Determination of sex and age range groups along with matched reference plasma and urine GAA and creatine values

Group comparisons performed by using the non-parametric test of Kruskal–Wallis have led to individualize significantly distinct age range groups for plasma and urine creatine and GAA values (Table 1).

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