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ScienceDirect
Clinical Biochemistry 40 (2007) 1209–1218
CLINICAL BIOCHEMISTRY

Plasma creatinine as a determinant of plasma total homocysteine concentrations in the Hordaland Homocysteine Study: Use of statistical modeling to determine reference limits

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Received 19 March 2007; received in revised form 29 May 2007; accepted 2 July 2007
Available online 11 August 2007

Abstract

Objectives: We established population-based reference limits for plasma total homocysteine (tHcy) according to creatinine.

Design and methods: In 7042 middle-aged and elderly subjects from the Hordaland Homocysteine Study, we used statistical modeling to establish nomograms for tHcy according to creatinine in the whole population and in folate-replete and healthy subgroups.

Results: When plotted against creatinine, tHcy 97.5th percentile almost overlapped in men and women, and, in elderly, increased up to 8 μmol/L from the 2.5th to 97.5th creatinine percentiles. Folate-replete subjects had tHcy upper limits ~20% below the whole population at all creatinine levels. Healthy subjects had lower creatinine, but at a given creatinine level, tHcy was the same as in the whole population.

Conclusions: tHcy difference between men and women is mostly explained by creatinine. The tHcy-reducing effect of folate is independent of creatinine. In elderly people, creatinine should be taken into account when assessing tHcy levels.

Keywords: Homocysteine; Creatinine; Reference intervals; Nomograms; Blood; Kidney function; Folate; Gender; Age; Creatine synthesis

Introduction

Homocysteine is an amino acid intermediate of methionine metabolism that at elevated plasma levels has been implicated as an independent risk factor for cardiovascular disease, birth defects, pregnancy complications, and cognitive decline in the elderly [1]. Furthermore, plasma total homocysteine (tHcy) is increasingly used in the assessment of folate and vitamin B12 deficiency [1]. Several studies have identified renal function as a major determinant of plasma tHcy concentrations [1,2]. A slightly reduced glomerular filtration rate (GFR) increases tHcy within the reference interval [1]. Frank hyperhomocysteinemia is common among patients with chronic renal insufficiency, and almost invariably accompanies chronic renal failure [3].

The causes of hyperhomocysteinemia in renal failure are not clear. Defective urinary excretion has been ruled out as a possibility, since about 80% of plasma tHcy is bound to albumin and unavailable for filtration [4]. The remaining fraction undergoes tubular reabsorption after glomerular filtration [5], and only minute amounts of homocysteine are recovered in urine [6]. Yet tHcy levels correlate strongly with GFR [7,8]. Homocysteine uptake and metabolism by the kidney were demonstrated in rats [9], but studies in humans found no significant arteriovenous differences in tHcy across the kidney [10,11].

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doi:10.1016/j.clinbiochem.2007.07.014

Nevertheless, enzymes for homocysteine metabolism by both remethylation and transsulfuration are present in human kidney tissue [12]. It has been hypothesized that uremic toxins may depress intracellular metabolism of homocysteine in the kidney or other tissues [3], increasing its export from the cellular to the plasma pool.

Increasing age and male sex are associated with higher plasma tHcy [13,14], as well as higher creatinine levels [15]. Declining renal function may explain the age-related increase in plasma tHcy [7,13]. The gender difference, on the other hand, could partly be due to larger muscle mass in men, since muscles contain 95% of total body creatine, and creatinine is produced from creatine which is stored in muscle [16]. About 2 g of creatine is metabolized to creatinine daily in a 70 kg man, and is partly replenished from dietary sources, mainly meat, and fish [17]. The remainder is synthesized from arginine, glycine and methionine, involving a hepatic transmethylation reaction with subsequent production of homocysteine (Fig. 1) [16].

Because of the strong association between tHcy and creatinine, reference limits for tHcy are usually calculated after excluding persons with increased creatinine or impaired renal function [1], and many studies involving tHcy exclude subjects with elevated creatinine. An alternative strategy would be to establish different reference limits for different creatinine concentrations. The Hordaland Homocysteine Study [18] is a population-based cohort, with comprehensive laboratory and lifestyle data on a relatively large number of participants. Using these data allowed us to define reference limits for plasma tHcy according to serum creatinine and to establish nomograms. Using nomograms, we re-examine the association between tHcy and several factors known to influence the tHcy level.

Subjects and methods

Study population

Between April 1992 and April 1993, the National Health Screening Services, in cooperation with the University of Bergen and local health services, conducted the first Hordaland Homocysteine Study (HHS-I) in the Hordaland county of western Norway, on a total of 18,043 subjects aged 40–67 years. Details on data collection for the HHS-I have been published previously [18].

In 1997–1999, there was a follow-up study of participants living in Bergen and its surroundings (HHS-II), conducted as part of the Hordaland Health Study (HUSK). In the HHS-II, a total of 9187 subjects were invited, of whom 7074 (77%) attended. Of those, 3341 subjects belonged to the elderly group, then aged 71–73 years, while the remaining 3733 subjects belonged to the middle-aged group, aged 47–48 years [18].

Plasma concentrations of tHcy, folate, and vitamin B12 have been measured in all samples of HHS-I and HHS-II [19]. Serum and plasma creatinine, however, was only measured in the participants of the HHS-II. In the present study, we included all participants from the HHS-II study for whom creatinine and homocysteine data were available, comprising 1657 men and

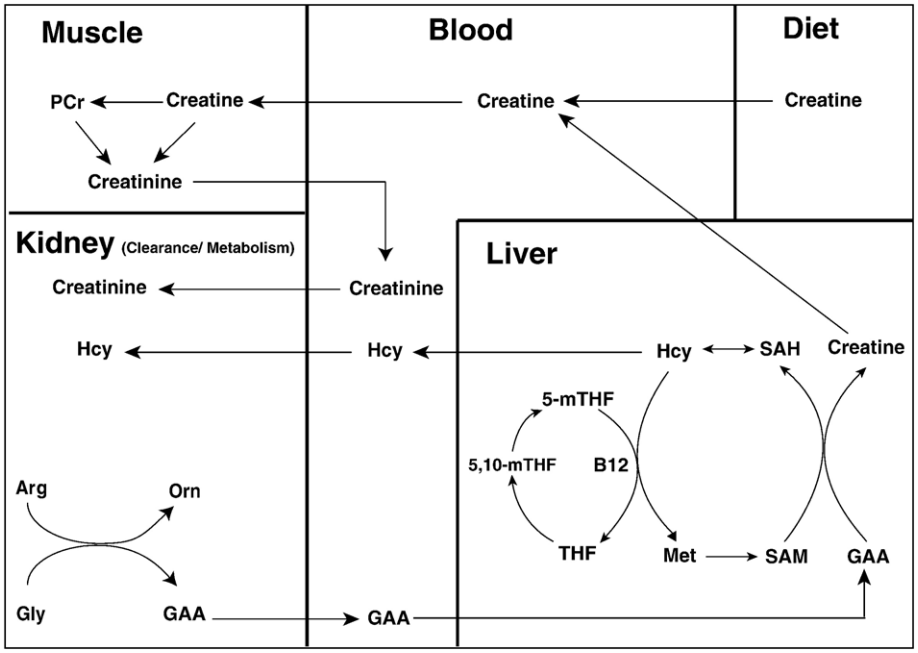

Fig. 1. Homocysteine–creatinine relationship. Creatinine is the product of intracellular non-enzymatic degradation of creatine, of which >90% is present in skeletal muscle. Muscle creatine is partly replenished by animal protein diet and the rest is synthesized, mainly by hepatic SAM-dependent methylation of GAA with subsequent production of Hcy. Remethylation of Hcy back to methionine is folate- and vitamin B12-dependent. Excess Hcy that is not metabolized intracellularly is exported to the plasma. Kidney function is critical for maintenance of normal plasma levels of both Hcy and creatinine. Arg, arginine; GAA, guanidinoacetate; Gly, glycine; Hcy, homocysteine; Met, methionine; Orn, ornithine; PCr, phosphocreatine; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; 5-mTHF, 5-methyltetrahydrofolate; 5,10-mTHF, 5,10-methylenetetrahydrofolate.

2059 women aged 47–48 years, and 1466 men and 1860 women aged 71–73 years.

The study protocols for both HHS-I and HHS-II have been approved by the Regional Ethical Committee of western Norway, whose directives are based on the Helsinki Declaration.

Data collection

Height and weight were measured in light clothing, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Blood pressure was measured three times for each individual and the average of the second and third measurements is used. Self-administered questionnaires provided information on lifestyle and medical history.

History of CVD was defined as self-reported past history of angina, myocardial infarction, and/or cerebral stroke or hemorrhage. Hypertension was defined as systolic and/or diastolic blood pressure above the upper limit for age and gender, and/or self-reported history of treatment for hypertension. Upper limit of blood pressure for age and gender was determined following the recommendations of the Norwegian College of General Practitioners by the following equations: Upper limit of systolic blood pressure = 150.3 + 0.45 * age (men) or 154 + 0.8 * age (women); Upper limit of diastolic blood pressure = 85 + 0.4 * age.

Renal insufficiency was defined following the MDRD recommendations as calculated GFR < 60 mL/min [20]. GFR was calculated using the MDRD formula: GFR (mL/min/1.73 m^2) = 186 × (serum or plasma creatinine mg/dL)$^{-1.154}$ × (age)$^{-0.203}$ × (0.742 if female) [20].

Biochemical measurements

Non-fasting plasma samples were collected in EDTA-containing tubes for tHcy, folate, and vitamin B12 analyses as previously described [21]. Plasma tHcy levels were analyzed by HPLC with fluorescence detection. Intra-assay coefficient of variation was lower than 4% [22]. Plasma folate and plasma vitamin B12 concentrations were measured by microbiological assays [23,24]. Creatinine was measured both using a conventional Jaffe assay (in serum), and by a modification of a liquid chromatography-mass spectrometry (LC-MS/MS) described previously [25] using stored plasma. The values obtained by LC-MS/MS were lower than those obtained with the Jaffe reaction regardless of age, gender, and baseline creatinine level. The correlation between the Jaffe and LC-MS/MS measurements is given by the following equation:

$$\text{Creatinine}_{\text{LC-MS/MS}} = -18.81\ \mu\text{mol/L} + 1.03^{*}\ \text{Creatinine}_{\text{Jaffe}};\quad R = 0.862$$

This difference may be related to the higher specificity of, and less interference with the LC-MS/MS method. In this report, we have used the results obtained by the LC-MS/MS method. The LC-MS/MS assay has a sensitivity of 0.1 μmol/L, within-day CV (at 75 μmol/L) of 4%, and between-day CV of 4% [26].

Reference populations

For the purpose of this study, we investigated the four age and gender groups separately, and in each age–gender group, analyses and nomograms were drawn from 3 population samples, having 2350 subjects in common:

1. The whole study population, comprising the 7042 subjects from the HHS-II study for whom tHcy and creatinine data are available.
2. A "folate-replete subgroup" comprising the 3353 subjects with a folate above the median for the whole population (6.68 nmol/L), after excluding those with severe vitamin B12 deficiency (B12 <200 pmol/L).
3. A "healthy subgroup" comprising 4930 subjects without history or evidence of major non-physiological conditions known to affect tHcy concentrations. Participants were excluded from this subgroup analysis if they had diabetes mellitus, CVD, hypertension, or renal insufficiency (defined above) [20].

Statistical methods

Distributions of tHcy, creatinine, folate, and vitamin B12 were skewed, and therefore the analyses were done either by non-parametric tests or by using $\log_{10}$ values. Geometric means are presented with their confidence intervals. All tests were two-tailed, and a p value <0.05 was considered significant.

For the tHcy reference intervals (2.5th–97.5th%) in Table 2, we calculated non-parametric bootstrap 95% confidence intervals (CIs). We replicated $B = 999$ bootstrap samples from the original data using three different methods: Basic, Percentile, and the Normal approximation methods. All three procedures gave similar results. For simplicity, we present results obtained from the percentile method (Fig. 2), indicating, in addition to the 95% CI, the mean of the replication results.

To estimate reference limits for tHcy plotted against plasma creatinine, we used the statistical model *Generalized Additive Model for Location Scale and Shape* (*GAMLSS*), within which we applied the Box-Cox t-distribution as a model for tHcy explained by creatinine. This distribution has four parameters: mean, scale, skewness, and kurtosis. Each parameter is modeled as a smooth non-parametric function of the explanatory variable, creatinine. We fitted the basic model, in which only the mean is modeled as a non-parametric function of creatinine leaving the other parameters constant, and the degrees of freedom penalty was held constant for all fits.

The advantage of the Box-Cox t-distribution is that it provides a flexible model for skewness and kurtosis. It also has an explicit formula for centiles, making it highly suitable for centile estimation, and is relatively easy to fit and interpret. However, it does not allow for hypothesis testing or estimation of CIs for the percentiles, a shortcoming which we tried to overcome by calculating bootstrap 95% CIs for the 97.5th percentile line in 4 population subgroups (Fig. 6). We fit the model using the *GAMLSS Library* developed by Rigby and Stasinopoulos [27,28] and incorporated as a package into the statistical

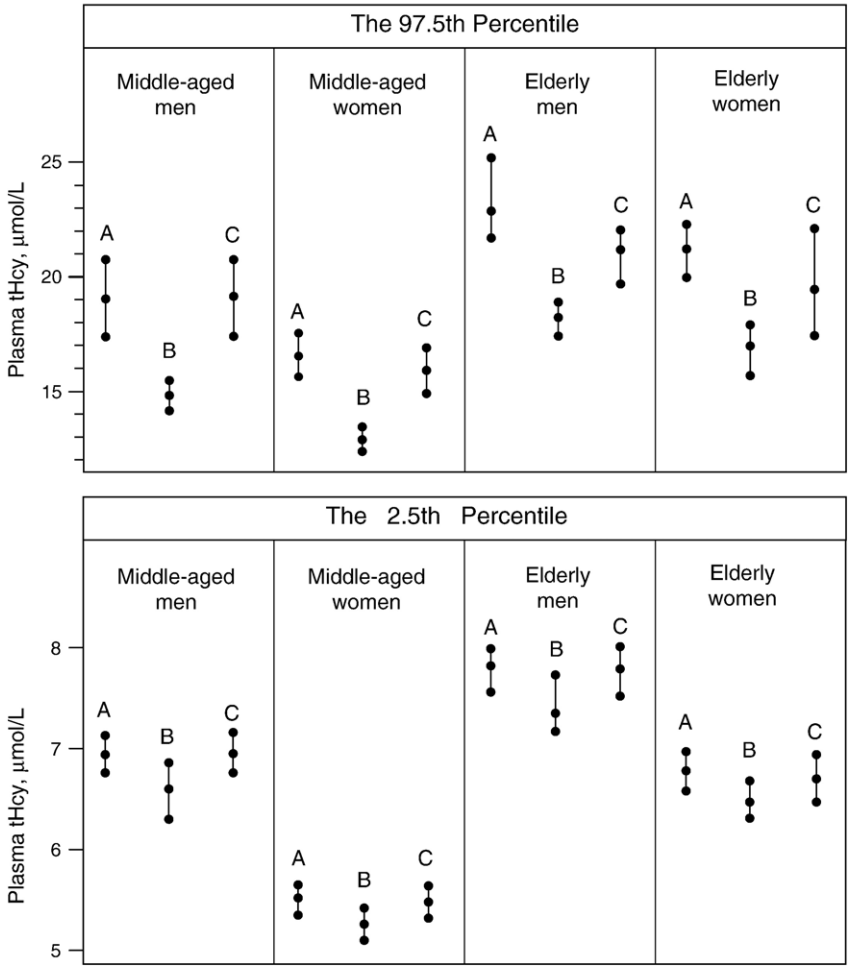

Fig. 2. Mean and 95% bootstrap confidence intervals for the 2.5th and 97.5th percentiles of total homocysteine listed in Table 2 as determined from 999 bootstrap replications. (A) Whole study population; (B) folate-replete subgroup; (C) healthy subgroup.

analysis program R [29]. To estimate the 95% CIs for the 97.5% centile curves for the 4 main groups (Fig. 6), we used a non-parametric bootstrap. We replicated, with replacement, $B=999$ samples from the original data. The model was refitted for each bootstrap sample (the smoothing degrees of freedom penalty was fixed throughout) and predictions of the tHcy 97.5% centile at fixed values of creatinine were made.

Results

Selected characteristics

Geometric means for both tHcy and creatinine differed significantly ($p<0.001$) between all 4 age–gender groups, being highest in elderly men and lowest in middle-aged women (Table 1). As expected, disease prevalence differed markedly between the two age groups, being higher at older age.

Plasma total homocysteine in three reference groups

When comparing the whole population with the folate-replete and healthy subgroups, the greatest differences observed for the tHcy levels were in the highest percentiles (Table 2, Fig. 2). Indeed, the 95% bootstrap CIs indicate a consistent difference (no overlap in CIs) between folate-replete subjects and the total population in the 97.5th but not the 2.5th percentile. The 97.5th percentile was on average 20% lower in the folate-replete subgroup than in the whole population. The greatest percent difference when comparing whole population to healthy subgroup was observed in the older population.

Plasma creatinine differed remarkably little between the whole population and folate-replete subgroup in all the percentiles, being slightly higher in the folate-replete subgroup despite the marked reduction in tHcy. Compared to the whole population, the elderly healthy subgroup had creatinine levels that did not differ greatly in the 2.5th and 50th percentiles, but were an average 24 µmol/L lower in the 97.5th percentile, probably due to exclusion of participants with renal insufficiency from the healthy subgroup.

Centiles for tHcy according to creatinine

For each of the 12 subgroups defined by age–gender and health status, Figs. 3–5 show the creatinine-tHcy association in the 2.5th to 97.5th percentile range of plasma creatinine. For tHcy, curves representing the median, 2.5th and 97.5th per-

Table 1
Selected characteristics of the study population

	Middle-aged (47–48 years)		Elderly (71–73 years)	
	Men (n=1657)	Women (n=2059)	Men (n=1466)	Women (n=1860)
Plasma variables				
Geometric mean (95% CI)				
Creatinine, μmol/L[a]	78 (78–79)	64 (63–64)	84 (83–85)	67 (67–68)
tHcy, μmol/L[a]	10.4 (10.3–10.6)	8.8 (8.7–8.9)	12.5 (12.3–12.7)	11.1 (11.0–11.3)
Vitamin B12, pmol/L[b]	353 (348–358)	358 (353–364)	340 (333–348)	359 (351–366)
Folate, nmol/L[c]	6.5 (6.4–6.7)	7.3 (7.1–7.4)	6.4 (6.2–6.6)	7.7 (7.4–7.9)
Condition				
Subjects with/without (proportion with) condition				
CVD[d]	44/1639 (2.7%)	19/2031 (0.9%)	362/1417 (25.5%)	258/1800 (14.3%)
Diabetes mellitus	26/1648 (1.6%)	11/2041 (0.5%)	105/1439 (7.3%)	117/1833 (6.3%)
Hypertension[e]	121/1657 (7.3%)	143/2059 (6.9%)	530/1466 (36.2%)	658/1860 (35.4%)
Renal insufficiency[f]	5/1657 (0.3%)	29/2059 (1.4%)	114/1466 (7.8%)	192/1860 (10.3%)
Low folate status[g]	120/1657 (7.2%)	118/2059 (5.7%)	125/1466 (8.5%)	130/1860 (7.0%)
Low vitamin B12 status[h]	40/1657 (2.4%)	79/2059 (3.8%)	105/1466 (7.2%)	111/1860 (6.0%)

tHcy, total homocysteine.
[a] $p<0.05$ between age groups within the same gender, and between genders of the same age groups.
[b] $p<0.05$ only between young and elderly men, and between elderly men and women.
[c] $p<0.05$ only between genders within the same age group, and between young and elderly women.
[d] CVD, cardiovascular disease; history of angina, infarction, or cerebral stroke.
[e] History of antihypertensive treatment and/or blood pressure>upper limit for age and gender.
[f] Estimated (by MDRD formula) glomerular filtration rate <60 mL/min/1.73 m^2.
[g] Plasma folate <3.5 nmol/L.
[h] Plasma vitamin B12 <200 pmol/L.

centiles are shown. For comparison, the nomograms are presented in three graphs where the first shows the gender effect, the second the age effect, and the third, the folate/health effect. While we describe the various trends observed in our nomograms, it should be noted that no formal statistical significance can be assigned to them since the models do not allow formal statistical testing.

Gender effect

Fig. 3 shows plasma tHcy levels plotted against plasma creatinine in panels comparing men and women of the same age group, in the three population samples according to folate and health status. Centile lines embrace the 2.5th to 97.5th creatinine percentiles. A consistent observation was that tHcy increased with increasing plasma creatinine. This increase in tHcy

Table 2
Median and reference intervals of plasma total homocysteine and creatinine in the whole population and healthy and folate-replete subgroups according to age and gender

	Whole population		Folate-replete subgroup[a]		Healthy subgroup[b]	
	Males	Females	Males	Females	Males	Females
Middle-aged						
N	1657	2059	735	1068	1473	1840
tHcy, μmol/L						
Median	10.2	8.6	9.6	8.0	10.2	8.6
2.5–97.5%	6.9–19.1	5.5–16.7	6.6–14.9	5.3–12.9	6.9–19.4	5.5–15.9
Creatinine, μmol/L						
Median	79	64	80	64	79	64
2.5–97.5%	57–106	46–87	58–107	47–87	57–105	46–83
Elderly						
N	1466	1860	591	959	674	943
tHcy, μmol/L						
Median	12.2	10.9	11.2	9.8	11.9	10.6
2.5–97.5%	7.8–22.6	6.8–21.4	7.3–18.3	6.5–17.0	7.8–21.6	6.7–19.8
Creatinine, μmol/L						
Median	83	67	84	67	81	65
2.5–97.5%	58–133	46–102	66–131	45–107	57–107	45–84

[a] Folate-replete subgroup: Subjects with folate above the median for the whole population (6.68 nmol/L) and excluding those with vitamin B12 <200 pmol/L.
[b] Healthy subgroup: Subjects without cardiovascular disease, hypertension, diabetes mellitus, or renal insufficiency (calculated GFR <60 ml/min/1.73 m^2).

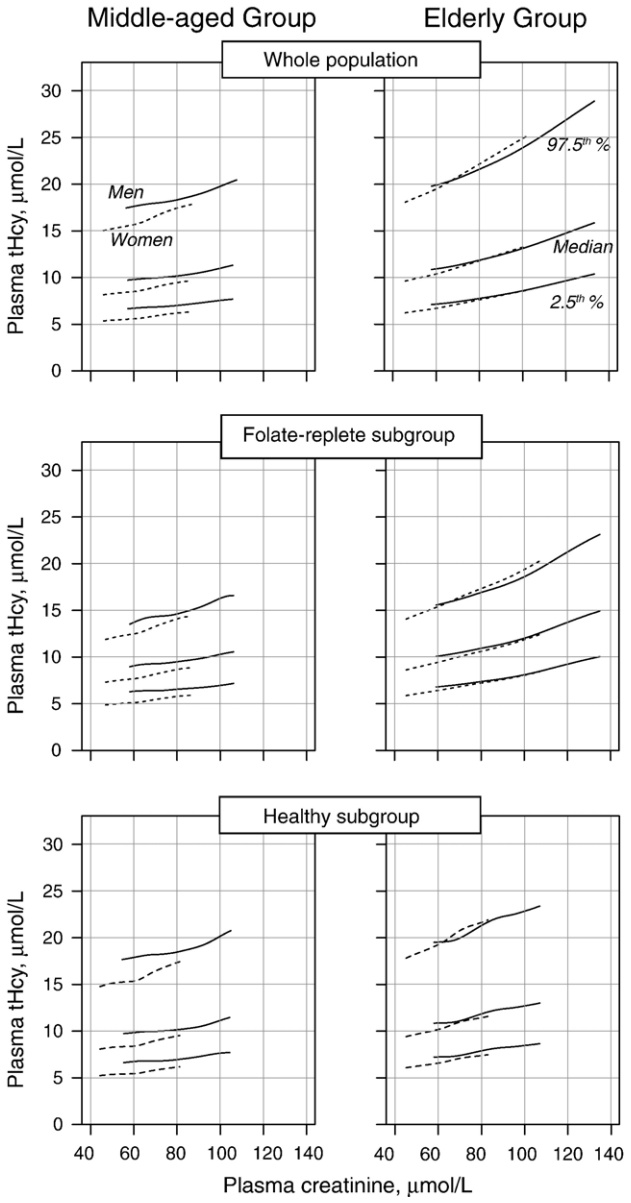

Fig. 3. Plasma total homocysteine 2.5th, 50th, and 97.5th percentiles plotted against creatinine (2.5th–97.5th percentiles): Gender effect. Groups presented are the *whole population* (all study participants), *folate-replete subgroup* (group with folate above the median [6.68 nmol/L] and excluding those with vitamin B12 <200 pmol/L), and *healthy subgroup* (subjects without cardiovascular disease, hypertension, diabetes mellitus, or renal insufficiency [calculated GFR <60 mL/min/1.73 m^2]).

was steepest in the 97.5th percentile, where it reached about 8 µmol/L tHcy across the 2.5th to 97.5th creatinine percentiles in both the whole population and in the folate-replete subgroup. It appeared as steep in men as in women, although women overall had significantly lower creatinine values. The magnitude of total increase of tHcy with creatinine in the healthy subgroup was smaller, with a maximum of 4 µmol/L increase in the 97.5th tHcy percentile. This may reflect exclusion of individuals with renal insufficiency from the healthy subgroup.

Notably, plasma tHcy at each creatinine value was higher in men than women only in middle-aged, but not in older participants. In the whole population, and folate-replete, and healthy subgroups, elderly men and women had overlapping tHcy-according-to-creatinine curves. In the middle-aged group, a convergence in tHcy curves for men and women could be observed with increasing creatinine levels.

Age effect

In Fig. 4, comparison between middle-aged and elderly groups in the same gender is presented. Creatinine 97.5th percentile increased markedly with age in the whole population and folate-replete subgroup, but not in the healthy subgroup. A constant finding in all three population samples is that the tHcy upper limit was 2–5 µmol/L higher in elderly than in middle-aged individuals at a given plasma creatinine concentration, while the difference in median and 2.5th percentile lines was 1–2 µmol/L between two age groups.

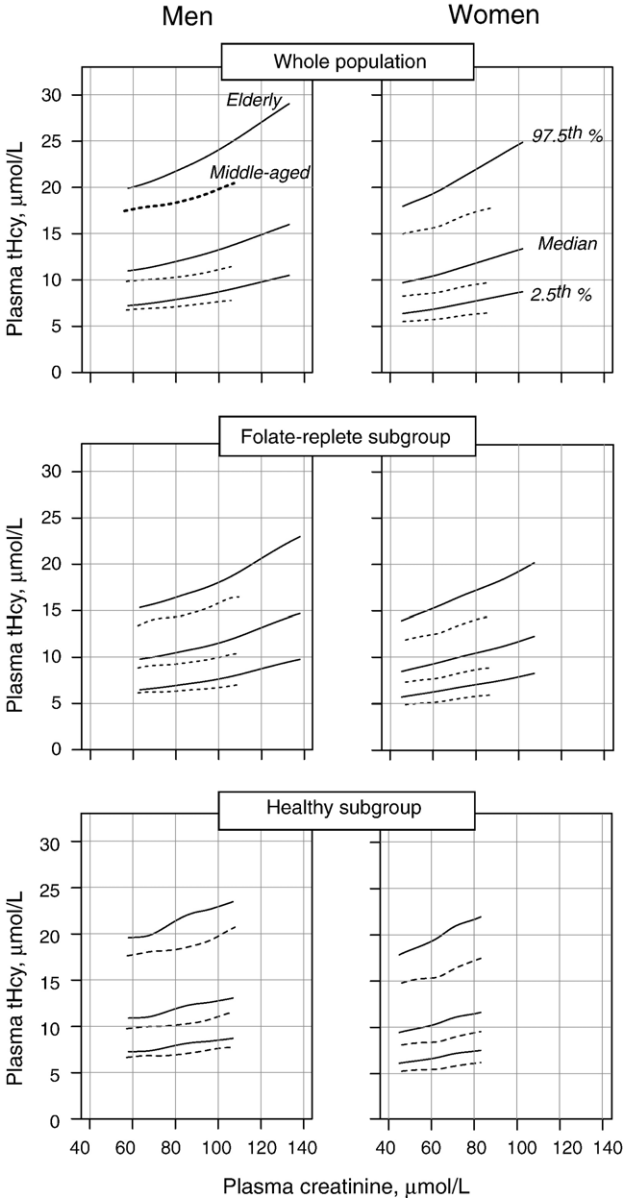

Fig. 4. Plasma total homocysteine 2.5th, 50th, and 97.5th percentiles plotted against creatinine (2.5th–97.5th percentiles): Age effect.

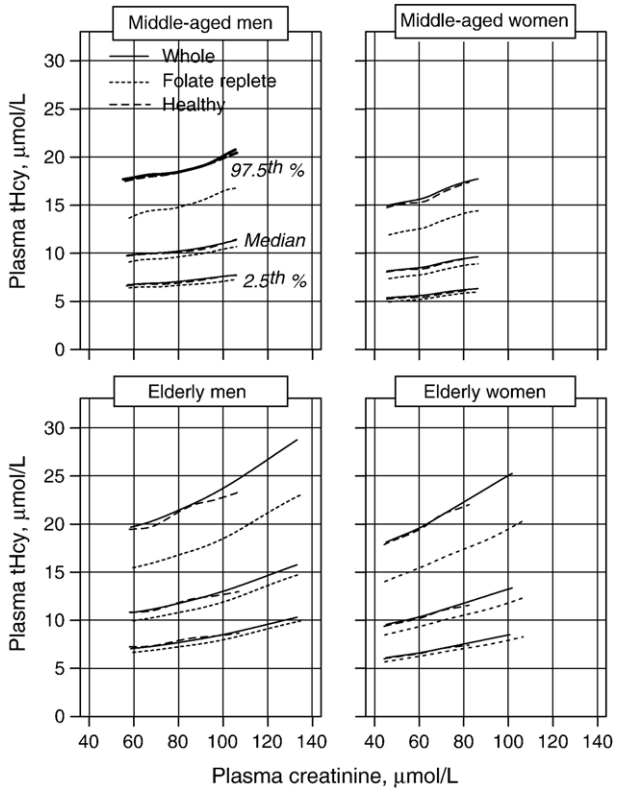

Fig. 5. Plasma total homocysteine 2.5th, 50th, and 97.5th percentiles plotted against creatinine (2.5th–97.5th percentiles): Folate and health effects.

Folate and health status effects

In Fig. 5, we compare whole population, and the folate-replete, and healthy subgroups in each age–gender group. Centiles for the healthy subgroup and whole population overlapped in all 4 age–gender groups. The folate-replete subgroup, however, had remarkably lower tHcy over the entire creatinine range. The 97.5th percentile, for example, was on the average 4 µmol/L lower in the folate-replete subgroup as compared to the whole population. Plasma tHcy appeared to be slightly more affected by folate status in older than in the middle-aged subjects.

Combined groups

The data above reveal that when tHcy is presented according to creatinine, the health and gender effects are absent or modest, while the effects of age and folate status remain strong. We therefore prepared new nomograms where we combined men and women, but had separate data for the older and middle-aged groups in the whole population vs. the folate-replete subgroup. In Fig. 6, the 97.5th percentile for each of these 4 groups is shown, together with its 95% bootstrap CIs.

Discussion

We made use of tHcy, creatinine, and other comprehensive data on over 7000 subjects to establish reference limits for tHcy according to age, gender, and creatinine levels. In doing so, the expected trend [1,2] was generally confirmed: the higher your creatinine levels, the higher your tHcy, with older age and male sex predicting higher values of both tHcy and creatinine.

The International Federation of Clinical Chemistry (IFCC) defined reference intervals as the range of values between the 2.5th and 97.5th percentiles [30]. Currently, there are two main approaches for establishing reference intervals according to co-variables, such as age and gender: the parametric model, which involves a theoretical Gaussian distribution of the data set, or non-parametric methods, which make no assumptions about the distribution [31]. In this study, we chose a partly non-parametric approach [27], given the skewed distribution of tHcy.

We investigated 3 population samples: the whole study population, which reflects a general population that has not been subjected to folate-fortification of foods, the folate-replete subgroup, which is probably quite close to that observed after folate-fortification, and the healthy subgroup. This latter subgroup is probably not appropriate as a reference group, but yields important information on the relation between creatinine and tHcy. In all age–gender groups, the tHcy-creatinine association was nearly linear within the 2.5th–97.5th creatinine percentiles, suggesting that linear models could have been used. However, it cannot be determined from this study whether this linearity continues at higher creatinine levels as observed in renal failure.

Reference ranges of tHcy and creatinine

Compared to the whole population, our folate-replete subgroup had similar creatinine reference ranges, but with reduced tHcy values especially in the upper percentiles. Compared to the whole population, tHcy in the folate-replete subgroup was 21% less in the 97.5th percentile and 8% less in the median. This difference parallels the 7% decrease in geometric mean tHcy reported for the Framingham offspring cohort after 4 years of

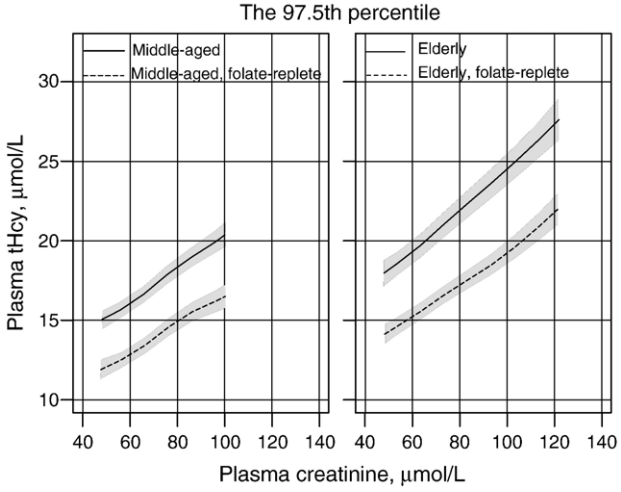

Fig. 6. Plasma tHcy 97.5th percentile plotted against creatinine (2.5th–97.5th percentile) in elderly vs. middle-aged groups in the whole population and folate-replete subgroup (subjects with folate above the median for the whole population [6.68 nmol/L] and excluding those with vitamin B12 <200 pmol/L). Central lines are the centiles and shaded areas indicate their 95% confidence intervals as obtained by bootstrapping (see Subjects and methods for details).

mandatory folic acid fortification [32] but is noticeably less than the 21% reduction of mean tHcy after 6 years of voluntary folate fortification in 468 subjects in Australia [33]. This may reflect the variations in periods and doses of fortification, and the fact that the Australian study reports arithmetic rather than geometric mean.

In our choice of a healthy subgroup, we attempted to exclude people with common non-physiological factors associated with altered tHcy levels [1]. Exclusion of these participants, particularly those with renal insufficiency, left a subgroup with remarkably lower reference ranges both for tHcy and creatinine. This is a rather "ideal" situation, in which the age effect on creatinine values disappears, indicating the well-recognized concept that the increase of plasma creatinine with age is largely a renal effect [34]. Yet, the gender difference in creatinine persists, presumably due to the different rates of creatine/creatinine production [35]. In contrast, for tHcy, both the age and gender differences are preserved in that population.

Effects of age and gender on homocysteine and creatinine

In our study population, the universally documented gender effect on tHcy levels [14,36,37] was age-dependent and more pronounced in the middle-aged than in the elderly population. Indeed, in old age, women and men with the same plasma creatinine had similar tHcy levels, indicating that the male–female difference in tHcy observed in this age group could be almost totally accounted for by the difference in plasma creatinine. Norlund et al. [13] have similarly observed that the difference in tHcy between genders disappeared when men and women were stratified by serum creatinine values.

So what accounts for the modest difference in tHcy at the same creatinine level between *middle-aged* men and women? The centile figures suggest that it does not reflect a renal or a folate effect, since it persists in the folate-replete and healthy subgroups. The fact that tHcy is compared at the same creatinine level may control for the difference in muscle mass between men and women. Previous studies point to the difference in estrogen [38] between genders, being consistent with the postmenopausal estrogen decline attenuating the tHcy difference between men and women, as observed in our elderly group. Another less extensively investigated hypothesis involves more efficient homocysteine remethylation in women [36].

When examining the age effect, the tHcy upper limit was 2–5 μmol/L higher in elderly than in middle-aged individuals *at the same plasma creatinine* in all three population samples. While this may imply that the increase of tHcy with age is not solely a renal effect, it is important to note that muscle mass decreases with age [39], and thus, creatinine would probably have decreased, had it not been for the decline in renal clearance. Therefore, decreased renal clearance may still be the strongest cause of tHcy increase with age. The age-related decline in the activity of cystathionine β-synthase that catalyses the first step of homocysteine transsulfuration [40], and intracellular functional B-vitamin deficiency [41] may also play a role.

Homocysteine as a biomarker for B-vitamin deficiency

Use of tHcy to identify preclinical folate and vitamin B12 deficiency has been recommended on the grounds that tHcy is a more sensitive marker than the levels of the B-vitamins themselves or the hematological or neurological sequelae [1,42]. But what cutoff levels should we use? Most studies use thresholds between 12 and 20 μmol/L, based on different definitions. Many exclude people with elevated serum creatinine, and use fairly low percentile cut-off. For example, Clarke et al. [43], in their population-based study on 1562 men and women above 65 years, used a tHcy cutoff of 15 μmol/L to identify functional vitamin B12 deficiency. This corresponded to the 80th percentile in their population where subjects with creatinine >100 μmol/L had been excluded. Not surprisingly, the prevalence of folate and vitamin B12 deficiency was high. In our older population, the marked variation of tHcy with creatinine means that it is crucial to take creatinine levels into account when interpreting the implications of higher tHcy values. In elderly men, for example, the upper limit of tHcy showed an increase of nearly 8 μmol/L from the 2.5th to 97.5th percentiles of creatinine. Thus the upper limit for elderly subjects was not a single value, but rather a range that depended on plasma creatinine. In our study, this range extended from 16 to 23 μmol/L in folate-replete subjects, and from 20 to 28 μmol/L in the total population. In middle-aged subjects, on the other hand, the difference in the upper limit of tHcy from the lower to upper limits of creatinine was modest (~3 μmol/L) for both men and women, suggesting that, for this age group, interpretation of high tHcy results could directly focus on B-vitamin status.

In conclusion, we have presented data on reference intervals of tHcy according to creatinine, age, gender, and health status. The nomograms revealed that the age effect on tHcy can only partly be explained by age-related increase in creatinine, while the gender effect is to a significant extent related to differences in creatinine. Compared to the whole population, healthy subjects have lower tHcy, which is mostly explained by a lower creatinine. Adequate folate status is associated with lower tHcy levels independent of the creatinine levels. Overall, our results indicate that when assessing tHcy status in older subjects, it is particularly important to consider the creatinine levels.

Acknowledgments

We would like to thank the authors of *GAMLSS*, Mikis Stasinopoulos and Bob Rigby, for their precious help in providing a code to calculate the bootstrap confidence intervals for the centile curves. The help from Mrs. Kari Juul and Elfrid Blomdal with setting up the data files and with literature support was also highly appreciated.

Grant/funding Support: Amany Elshorbagy is in receipt of an Egyptian Government Scholarship fund. This study has also received support from the Advanced Research Programme of Norway, The Norwegian Research Council, The Johan Throne Holst Foundation for Nutrition Research, University of Oslo, and the Foundation to promote research into functional B12-deficiency, Norway. None of the funding bodies was involved in

the study design; collection, analysis, or interpretation of the data; or preparation of the manuscript.

Author contributions: AE: Planning, design, statistical analysis and interpretation, and preparation of the first draft. AO: Statistical modeling of the centile figures and critical revision of the manuscript. SK: Biochemical analysis (creatinine measurement by tandem-mass spectrometry). EN: Preparation of data for analysis, critical revision of the manuscript. PMU: Planning, data collection, critical revision of the manuscript. GST: Planning, design, interpretation and critical revision of the manuscript. ON: Critical revision of the manuscript. SEV: Planning, data collection, critical revision of manuscript. HR: Planning, design, data collection, critical revision of the manuscript.

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