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Normal concentrations of chromium in serum and urine — a TRACY project

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BRUNE D, AITIO A, NORDBERG G, VESTERBERG O, GERHARDSSON L. Normal concentrations of chromium in serum and urine — a TRACY project. *Scand J Work Environ Health* 1993; 19:39—44. The validity of "normal" concentrations for total chromium in serum and urine (S-Cr and U-Cr, respectively) in papers published mainly in the last decade were evaluated and graded by two investigators according to TRACY criteria. The results were in close agreement. Because of possible contamination during sampling from stainless steel needles, the description of the sample collection method was considered important. Documentation of analytical quality control was emphasized. The chromium concentrations were categorized according to nonoccupational conditions that could influence the levels. Eighty-seven publications reporting chromium concentrations in blood and 58 on U-Cr were evaluated, 53 dealing with S-Cr and 41 with U-Cr being found suitable for the TRACY project. In selected publications the arithmetic mean values presented for S-Cr and U-Cr in individuals with no known exposure were within the following ranges: 1—3 nmol · l^{-1} for S-Cr and 2—10 nmol · l^{-1} or 0.2—1 μmol · mol creatinine^{-1} for U-Cr.

Key terms: grading, nonoccupational, normal levels, reference values.

Published "normal" concentrations of chromium in serum (S-Cr) have apparently decreased by a factor of about 1000 during the two last decades (1), and this development has necessitated a critical review of published information before reference values can be established. The discrepancies can largely be ascribed to contamination of blood samples during sampling through the leaching of chromium from steel needles, as well as to the use of unsatisfactory analytical techniques. These errors have been eliminated over the last decade.

The present evaluation and grading of publications describing S-Cr (and also in blood cells and whole blood), as well as those describing chromium concentrations in urine (U-Cr), is part of the TRACY project for establishing reference values for heavy metals and other trace constituents in human tissues and body fluids. The project has been performed within the framework of European scientific and technological cooperation — EUREKA/EUROENVIRON. (See references 2—4.)

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Methods

Collection of publications

A literature search for data on S-Cr (blood) and U-Cr was carried out in the Medline data base. Various research groups were also requested to provide scientific information.

Evaluation and grading

The evaluation and grading of publications on the chromium concentrations in blood and urine was performed by two independent investigators (AA and DB).

The various publications were graded and categorized according to the TRACY criteria for sampling conditions and analytical treatment.

Sampling conditions. Subjects were classified in terms of the following characteristics: country, urban, rural; age, gender, and other such factors; alcohol consumption, smoking habits, and medication; health status; diet; trace-element supplementation; exercise; and iatrogenic exposure from implants (prostheses).

The experimental design included the use of steel or siliconized needles for drawing blood and the use of plastic catheters and special precautions to avoid contamination, for example, venipuncture with steel needle puncture followed by blood flow for needle rinsing before sampling (20—40 ml).

The number of subjects was given special attention (more than 40 subjects being necessary to verify the distribution).

Analytical treatment. Storage and preanalytical treatment included the addition of anticoagulants and the use of "pure" sample containers with negligible contamination caused by leaching.

The method of evaluation covered sensitivity, accuracy, and other such factors.

Tests with a reference material or analytical assessment by two or more methods should have been carried out.

The statistical distribution should have been verified, a skewness test performed, an appropriate statistical method used, and sufficient information included.

Three topics were considered of major importance in the present evaluation, namely, avoidance of contamination in sampling, documentation of analytical quality control, and verification of the statistical distribution.

Sampling

In view of serious chromium contamination of blood samples in previous studies because of the use of steel needles for venipuncture, blood collection by means of siliconized needles, plastic catheters, needle puncture followed by blood flow, and the like were considered to be of major importance in the grading. Stainless steel needles can jeopardize the analysis, since the major elements present in stainless steel, (eg, chromium, manganese, copper, cobalt and iron) can be leached from the needles and transferred to the blood during sample collection.

In addition, avoiding contamination of the urine sample during collection and storage (eg, through appropriate cleaning of the containers) was also emphasized in the evaluation.

Analytical quality control

In most of the publications evaluated atomic absorption spectrophotometry was used for the analysis. The analytical procedures were often only fragmentarily described in the various reports, and therefore the effectiveness of the analytical quality control was difficult to ascertain. In this context access to reference materials is of great importance, and the documentation of analytical conformity with an appropriate reference material has been emphasized in the grading. However, several of the reference materials used for the chromium analysis of biological matrices contain chromium at levels much higher than the normal S-Cr and U-Cr values. Certain reference materials for U-Cr and S-Cr were originally intended for use in the analysis of specimens from exposed individuals, while some reference materials have been spiked.

For several years now many reference materials have been available for use in the analysis of chromium in biological specimens. An overview of such materials has been presented by Iyengar (5). A certified chromium reference material for serum with a chromium concentration of 0.069 $ng \cdot ml^{-1}$, developed by Versieck et al (6), has been available since 1988. It is in agreement with the normal chromium mean values, which seem to be in the approximate range of 0.07—0.2 $ng \cdot ml^{-1}$. (See figure 1.) However, this reference material was not available when most of the investigations were performed.

Reference materials for U-Cr are available from, for example, Nycomed/Seronorm, Oslo, Norway. They contain a chromium concentration of 20 $\mu g \cdot l^{-1}$ (spiked in order to comply with industrial exposure conditions). Normal concentrations seem to be $< 1\ \mu g \cdot l^{-1}$.

Testing should preferably be carried out with a certified reference material of the same tissue or fluid type in the aforementioned concentration range. However, even if an ideal reference material has not been used, documentation of the use of another reference material may still be of some value in the TRACY grading procedure.

Grading

According to the preceding criteria the papers acceptable for the TRACY project were classified into one of three categories, designated by means of stars (ie, *, **, ***) corresponding to the previous TRACY unit system 3, 2, 1 (grade 1 having been the highest corresponding to ***). The star grading system was agreed upon at the TRACY expert meeting in Stockholm in May 1992.

Reference groups or individuals

In clinical chemistry, reference values are strictly defined with regard to the reference individuals meeting the health requirements specified by the International Federation of Clinical Chemistry (IFCC) and the International Committee for Standardization in Haematology (ICSH). However, a satisfactory definition of health is still lacking (7).

Reference individuals can be defined both on the basis of category (eg, population-based or subject-based) and on the basis of one (univariate) or a number (multivariate) of variables. In the present study a univariate reference value applied, for example, to the presence of an implant such as a chromium-cobalt hip prosthesis, while a multivariate reference value can be linked to parameters such as diet, chromium supplementation, and exercise.

So far, the description of reference individuals given in the publications evaluated has only partly conformed with the criteria used in clinical chemistry (IFCC) (7). In view of the lack of strictly defined reference groups, values for S-Cr and U-Cr often have to be obtained from studies devoted mainly to occupational exposure. In such cases, less attention has been given to the description of the normal or refer-

ence groups. Special attention should be paid to this question in future studies.

The reference groups in the evaluated publications were described as "healthy individuals," employees not exposed to a specific agent, "a matched reference group," volunteers, students, blood donors, and hospital employees.

Results and discussion

Evaluation and grading

Both the evaluation and grading of the various publications were performed by two independent investigators as previously mentioned. The results agreed well and did not differ by more than one grade at the most.

On the basis of the TRACY criteria, the acceptable publications were finally classified as shown in tables 1 and 2.

Altogether 53 publications describing chromium concentrations in blood were found to be suitable for the TRACY project, while 34 were found to be unsuitable. With regard to U-Cr 41 publications were found to be suitable, while 17 were unsuitable. So far, no publication has met the requirements for the highest grade (***). In order to obtain a grading higher than *, documentation of the absence of chromium contamination, performance of appropriate quality assurance, and verification of statistical distribution are required according to the TRACY system.

Publications were usually rejected because the quality assurance and procedures for avoiding contamination were poorly described. A small number of samples did not constitute grounds for exclusion. Some otherwise satisfactory reports emphasized industrial exposure conditions, while a description of normal individuals was lacking, as previously pointed out. Various reports collected during the search of the Medline data base described, for example, nutritional conditions associated with chromium deficiency, but they gave insufficient information on S-Cr or U-Cr. Such publications were also found to be unsuitable for the TRACY system.

Values for S-Cr and U-Cr were categorized according to nonoccupational conditions that could influence the levels, such as diet, trace element supplementation, exercise, and iatrogenic exposures resulting, for example, from the presence of implants (prostheses).

In most of the evaluated publications molar units were not used. Because such units are strongly advocated by the International Union of Applied and Pure Chemistry, we have used them for the range of selected mean values for S-Cr and U-Cr. However, the units in figures 1 through 3 are those used by the authors of the publications in question.

As early as 1978, Versieck et al (6) reported values for S-Cr in agreement with recent results. At that time, quality assurance could not be assessed with the same reliability as today because reference materials were only rarely available. However, Versieck and his co-workers were familiar with the serious interference effects that might occur during sampling and knew how to manage such problems. Various other groups engaged in trace element analysis also had the same skills at that time. It is therefore unreasonable to reject reports solely because they were published more than 10 years ago.

Table 1. Grading of publications on chromium concentrations in serum. (N = number of publications)

Grade	Sampling (N)	Analysis (N)
*	49	51
**	4	2
***	—	—

Table 2. Grading of publications on chromium concentrations in urine. (N = number of publications)

Grade	Sampling (N)	Analysis (N)
*	36	37
**	5	4
***	—	—

Statistical treatment

In the publications evaluated, both the values for S-Cr and U-Cr were treated mainly with the assumption of a normal or log-normal distribution. Of the two, a normal distribution was the most common. Few of the evaluated studies allowed accurate assessment of the true shape of the distribution because too few observations were made. Even if a sufficient number of observations were available in some studies, insufficient attention was often given to verifying the distribution.

However, in various studies single observations of S-Cr or U-Cr can be fitted to the normal or the log-normal distribution with about the same degree of significance. Thus Sabbioni and his co-workers (personal communication at the ISTERH symposium Huddinge, Sweden, May 25—27 1992 and reference 18) studied the distribution of S-Cr and U-Cr, and fitted single observations both to the normal and the log-normal type of distribution. The total population comprised 103 individuals divided into groups of men (N = 59) and women (N = 44). For the women, both the normal and the log-normal fittings were about equally good, since both the normal and the log-normal tests were significant at the 5% level. For the men and the men and women combined the observations fitted to the log-normal distribution were slightly better. With regard to U-Cr, the observations fitted a normal distribution best. However, the P-values indicated that neither distribution described the data very well.

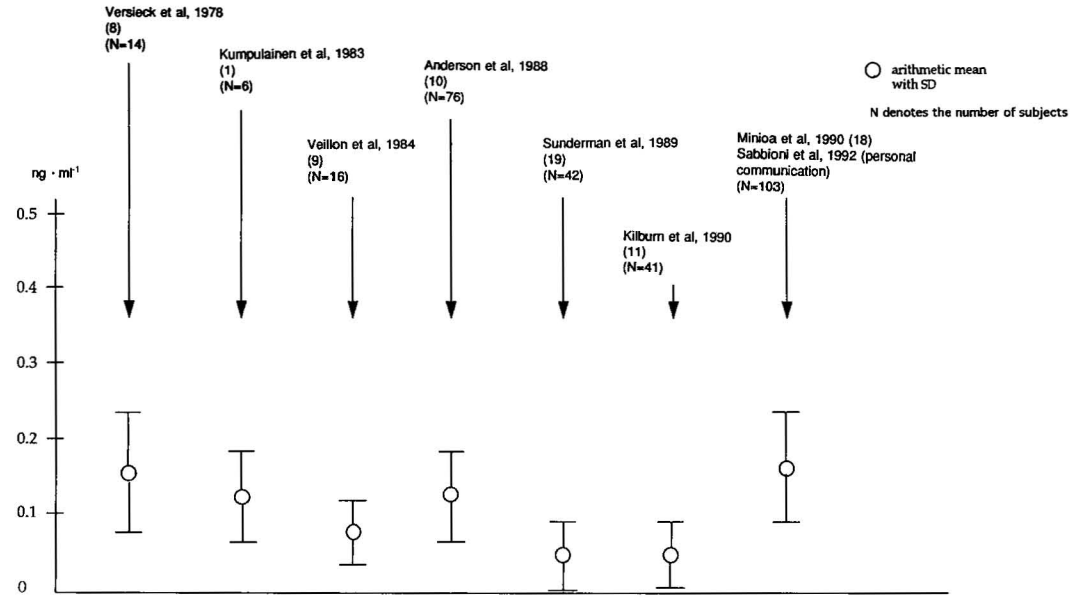

Figure 1. Concentration of chromium in serum.

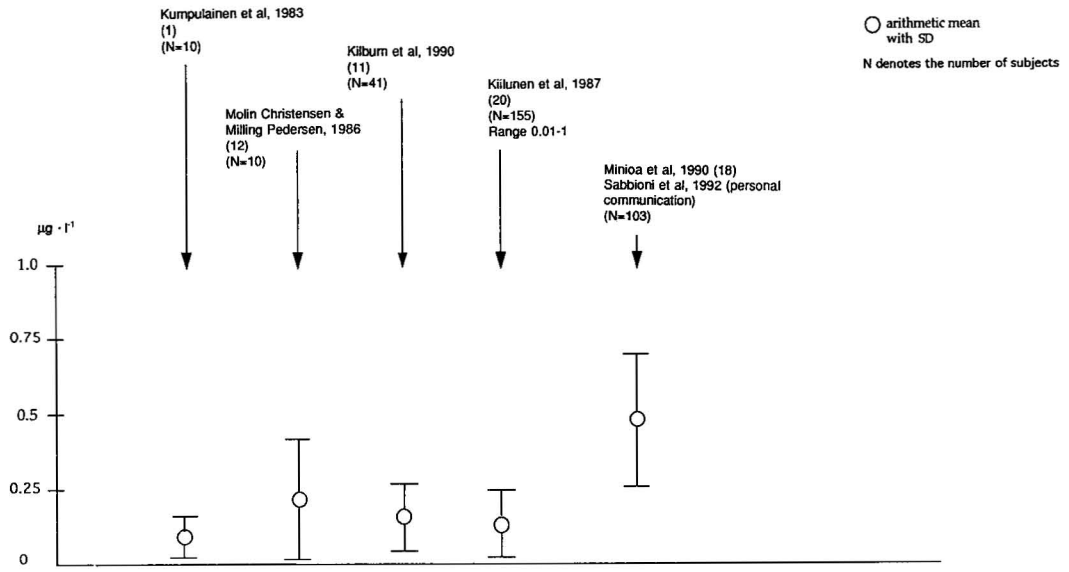

Figure 2. Concentration of chromium in urine.

Versieck et al (6) found close agreement between the arithmetic mean and the median value in a study comprising duplicate measurements of chromium in serum samples from 14 individuals [arithmetic mean 0.160 (SD 0.08), median value 0.158, range 0.038—0.351]. In a study by Sunderman et al (19) on S-Cr and U-Cr, the validity of the statistical computations based on a normal distribution was confirmed with various tests.

In the study of Kiilunen et al (20) the 155 observations of U-Cr exhibited a log-normal distribution. A log-normal distribution of U-Cr was also found by Verschoor et al (21) and Vyskocil et al (22) in studies mainly concerned with industrial exposure conditions. However, in most of the studies on U-Cr the treatment of the data was based on a normal distribution without any attempt being made to determine the true distribution.

Since difference in fit between the normal and the log-normal type of distribution of values for S-Cr and U-Cr seems slight, the choice of statistical approach does not seem to have a major effect on the provisional reference values.

In the TRACY system, the following two approaches to the statistical treatment of data from the graded publications selected are possible.

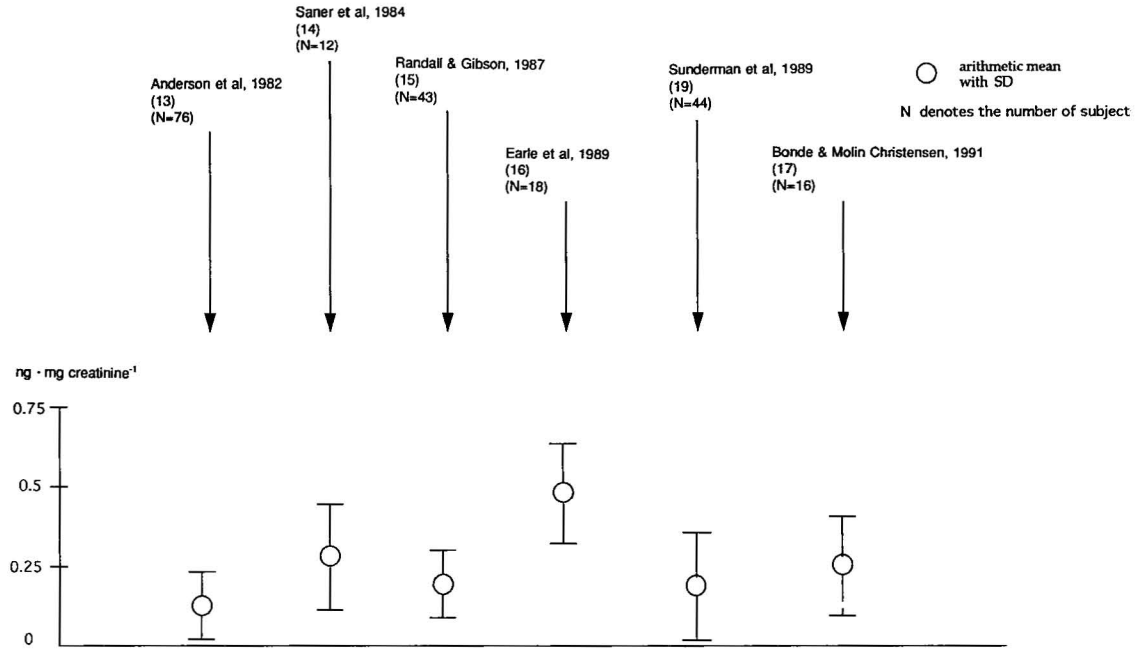

Figure 3. Concentration of chromium in urine.

Approach A: single observations (chromium concentrations for each individual available). Several statistical methods are available for data treatment following verification of the prevailing distribution. However, single observations (ie, the chromium concentrations found in individuals) are generally not available in the publications evaluated, since most journals do not usually allow the inclusion of large numbers of such values. It is difficult, therefore, to adopt this approach unless such information can be obtained from the various research groups.

Approach B: single observations not available. With approach B, the individual observations have already been treated according to parametric or nonparametric concepts, but often without verification of the prevailing statistical distribution. In a previous study dealing with mercury concentrations in blood (3) a roughly parametric method of establishing tentative reference values was used, the various selected data sets being pooled, either by weighting or by assignment of the same weight to them. This approach should be used only in cases when the distribution does not deviate greatly from the normal distribution. The approach requires that various approximations be made.

It should be emphasized that the number of observations in the various publications can vary considerably. This variation can have a major influence on the reference value when the results are pooled and weighted according to the number of observations, which can vary from about 10 to about 500 for the chromium concentration of blood or urine. Pooling, weighting, or assigning the same weight should therefore be seriously considered in order to avoid giving too much effect to the results of studies with large numbers of observations. Ideally, studies with about the same number of observations should be considered (harmonization).

Selection of data for establishing reference values

Ideally, only publications assigned the grade *** , implying the highest quality according to the present grading system, should be considered when reference values are established. However, so far, no publication has met the sampling and analytical requirements for this grade, and data sets from publications assigned the grade * (acceptable quality) or ** (good quality) were therefore provisionally used.

In the majority of the publications single observations were treated on the basis of a normal or lognormal concept. Since most of the publications suitable for the TRACY project contained data treated on the assumption of a normal distribution, only such publications have so far been taken into account.

On the basis of this approach, a tentative range of selected mean values is presented below.

Range of selected mean values for chromium in serum

Arithmetic mean values from seven publications assigned the grade * or ** were selected for inclusion

in the following range of mean values for S-Cr (see figure 1): 1—3 nmol · ml^{-1}.

Range of selected mean values for chromium in urine

Arithmetic mean values from five and six publications presenting values for U-Cr in units of μg · l^{-1} or ng · mg $creatinine^{-1}$, respectively, and assigned grades of * or ** were selected for inclusion in the following two ranges of mean values (see figures 2 and 3): 2—10 nmol · l^{-1} or 0.2—1 μmol · mol $creatinine^{-1}$.

The units ng · ml^{-1} for S-Cr and μg · l^{-1} or ng · mg $creatinine^{-1}$ for U-Cr have been used in figures 1—3 since these units were those used by the authors. In the scientific literature three units are generally used for U-Cr (in weight or molar units), namely, μg · l^{-1}, μg · 24 h^{-1} or ng · mg $creatinine^{-1}$. These are not mutually interchangeable partly due to the considerable variation in urine flow rate.

Up to the present, publications giving values for U-Cr in the unit μg · 24 h^{-1} have not been included in the selected mean value range because of the insufficient number of graded publications.

In the publication of Kiilunen et al (20), showing a log-normal distribution for U-Cr, both arithmetic and geometric mean values were presented, namely, 0.12 and 0.078 μg · l^{-1}, respectively. In figure 2 the arithmetic mean value from this study has been included. The standard deviation was estimated from the frequency distribution excluding outliers.

Conformity of selected data

In view of the considerable decrease by a factor of about 1000 in the apparently normal levels of S-Cr during the two last decades, the conformity of the data sets selected so far seems good. (See figure 1.) The conformity of the concentration levels of U-Cr indicated in figures 2—3 aids to increase the confidence in such values.

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