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Longitudinal hair chromium profiles of elderly subjects with normal glucose tolerance and type 2 diabetes mellitus

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Abstract

Longitudinal hair chromium (H-Cr) profiles in a group of patients with type 2 diabetes mellitus (n = 59; age, 62 ± 9 years) and healthy elderly (control) subjects (n = 49; age, 59 ± 10 years) matched by age and sex were measured by solid sampling electrothermal atomic absorption spectrometry, providing data on the magnitude of variation of Cr content along the hair length. H-Cr average ($H\text{-}Cr_{av}$) and H-Cr proximal ($H\text{-}Cr_{pr}$), relating to the average Cr content of the whole hair and the proximal 3-mm hair length, respectively, were also obtained. No significant difference between the healthy and diabetic group was found in mean $H\text{-}Cr_{av}$ or $H\text{-}Cr_{pr}$ contents (248 ± 108 vs 247 ± 143 and 233 ± 98 vs 278 ± 195 ng/g, respectively. However, women in the control group had significantly lower H-Cr values ($P < .01$) compared with men, but this difference was absent in the diabetic population. The distribution of log $H\text{-}Cr_{pr}$ values in the control population displayed a Gaussian shape, in contrast to the substantially wider distribution, skewed toward lower $H\text{-}Cr_{pr}$ values, observed in the diabetic group. The magnitude of variation in H-Cr content in the patient group over an interval of approximately 2 to 3 months (time of growth of the hair sampled) was found to be a factor of more than 2 larger than that in the control population (± 58% vs ± 26%). A strong relationship ($R = 0.656$; $P < .01$) between log $H\text{-}Cr_{pr}$ and log fasting plasma Cr was observed in the diabetic group (n = 20). The mean fasting plasma Cr value of this group was 0.41 ± 0.10 μg Cr per liter. No correlation between $H\text{-}Cr_{av.}$ and duration of diabetes was observed. A strong positive association was observed in the control population between $H\text{-}Cr_{pr}$ and fasting plasma insulin (n = 22; $R = 0.6157$; $P < .01$), and $H\text{-}Cr_{pr}$ and fasting plasma glucose (n = 24; $R = 0.4118$; $P < .05$), which is indicative of the interrelation of these parameters. In the control population, $H\text{-}Cr_{av}$ showed a slight decrease with age (n = 54; $R = 0.2691$; $P < .05$), which is assumed to be the result of increased insulin resistance caused by various age-associated factors including Cr deficiency. None of the above relationships was significant in the diabetic group. Evidence is presented that justifies the assumption that the longitudinal H-Cr profile resembles the variation in Cr metabolic rate over the time span of growing hair, which is not appreciably affected by external contamination. This suggests that glucose intolerance (type 2 diabetes mellitus) is an important factor that disturbs Cr metabolism.

1. Introduction

Chromium was recognized as a biologically essential element with the discovery of Schwartz and Mertz [1] that a Cr-free diet induced impaired glucose tolerance in rats. A similar effect was observed in humans where patients on long-term total parenteral nutrition developed severe glucose intolerance, which was reversed by supplementation with Cr [2,3]. The incidence of disturbed glucose tolerance and type 2 diabetes mellitus has been increasing among the middle-aged and elderly population worldwide. This has been initially ascribed to inadequate Cr nutrition and/or higher excretion [4]. However, balance studies [5,6] on 2 different populations showed that most subjects were in equilibrium for Cr at intakes of 20 to 24 μg Cr per day, which is much lower than the lower limit of the recommended safe and adequate daily intake (50 μg Cr). Therefore, other factors should be responsible for the increase in insulin resistance and the consequent decline in glucose tolerance. It was suggested that these changes are not a primary aging effect but the result of age-associated factors such as various diseases, obesity, use of certain medications that affect glucose tolerance, and physical or mental inactivity [7,8]. Higher excretion of Cr

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was observed in most diabetic subjects [9] than in healthy people exposed to physical or mental stress [10-12]. High consumption of simple sugars also resulted in enhanced Cr losses [13]. Studies on Cr kinetics and metabolism in healthy and diabetic populations have been performed [9,14-16]. The bioavailability of various Cr compounds was measured, and the biologic role of Cr at the molecular level was investigated [17]. The effect of Cr supplementation in improving glycemic parameters in patients with type 2 diabetes mellitus was studied but the results were inconclusive [18-28]. Because impaired glucose tolerance was attributed to compromised Cr body stores, estimation of the Cr status in humans has become a most important task. Determination of Cr in blood [2,9,16,25,27,29-41], hair [2,34,36,42-55], and sweat [34,42,43,56] was carried out for that purpose. Changes in plasma Cr (P-Cr) in response to glucose challenge were measured, but the results of different research groups were inconsistent [9,15,27,29,36,57]. Very few data are available on sweat Cr [29,42,43,56], probably because of difficulties encountered in obtaining representative samples and associated contamination problems. Among various parameters measured to estimate Cr status, hair seems to be the most convenient because it is easily obtained, can be stored for a long period, and the concentration of Cr in hair is high enough to be accurately determined. The main problem in using hair as a biochemical parameter is in distinguishing between the metabolic and the anthropogenic fractions of an element in the measured part of the hair [58]. For certain elements (lead, cadmium, etc), substantial positive gradients with hair length were observed. This is clearly seen in Fig. 1 where the relative variation of hair Pb content with hair length is illustrated [59]. This means that a substantial proportion of the element was incorporated in the hair structure from outside after its formation. On the contrary, the concentration of Cr along the hair length of occupationally unexposed subjects varies randomly (see Fig. 1), which suggests that the exogenous contribution to H-Cr is likely to be insignificant.

The average content of Cr in hair (H-Cr_{av}) has been measured by several researchers in healthy and diabetic populations. Hambidge et al [60] found statistically lower H-Cr_{av} content in children with type 1 diabetes mellitus compared with healthy children. Hambidge et al [44] investigated the variation of Cr content in hair sections 1 to 10 cm in length cut at various distances from the scalp (6-30 cm). In a rather inhomogeneous group with respect to the age and sex of the healthy subjects, they found a mean variation of ± 40%. However, no significant variation (± 14%) in Cr content of the proximal 1-cm hair section sampled at different locations on the head of the same person was observed. Hambidge and Droegemueller [55] measured changes in levels of H-Cr_{av} during pregnancy. Mahalko and Bennion [61] investigated the H-Cr_{av} content of nulliparous and parous women and found considerably lower values in the latter group. Rabinowitz et al [36] performed a comprehensive study comparing the Cr status of healthy individuals and subjects displaying different categories of type 2 diabetes mellitus. Although a wide individual variation of H-Cr_{av} was observed in both populations, mean values were similar. However, the percentage of subjects with low H-Cr_{av} values was considerably higher in the diabetic groups. Aharoni et al [45] compared H-Cr_{av} contents of women with gestational diabetes and those of pregnant women displaying normal glucose tolerance. The former group showed substantially higher H-Cr_{av} contents.

This article presents the results of measurements of H-Cr longitudinal profiles of patients with type 2 diabetes mellitus and healthy elderly subjects (control population). The primary object of this research was to evaluate the significance of H-Cr data in the assessment of Cr status and, consequently, in predicting possible development of glucose intolerance. For this purpose, 3 different parameters were deduced from the results of H-Cr longitudinal profiles: the average Cr content of the whole hair length (H-Cr_{av}), the Cr content of the proximal 3-mm hair segment (H-Cr_{pr}), and the magnitude of variation of H-Cr content along the hair. The association of H-Cr values with P-Cr and glycemic parameters (plasma glucose [P-glucose] and insulin [P-insulin], glycated hemoglobin [HbA_{1c}]) and age was investigated in patients with type 2 diabetes mellitus and the control population. In addition, the influence of duration of diabetes on H-Cr_{av} and glycemic parameters was studied.

2. Subjects

Two groups were studied. The first one consisted of 59 patients with type 2 diabetes mellitus (37 men and 22 women) with an average age of 62 ± 9 years (range, 42-82 years) who were attending a clinic's outpatient department and were selected randomly. They were either on a diet or on a diet and oral hypoglycemic medication, but no one was receiving insulin therapy. The second group was composed of 49 healthy (control) individuals (30 men and 19 women) with an average age of 59 ± 10 years (range, 36-73 years). All control subjects participating in the study passed a thorough clinical examination (oral glucose tolerance test). Only those whose test results were negative were accepted. All subjects participated voluntarily, were informed

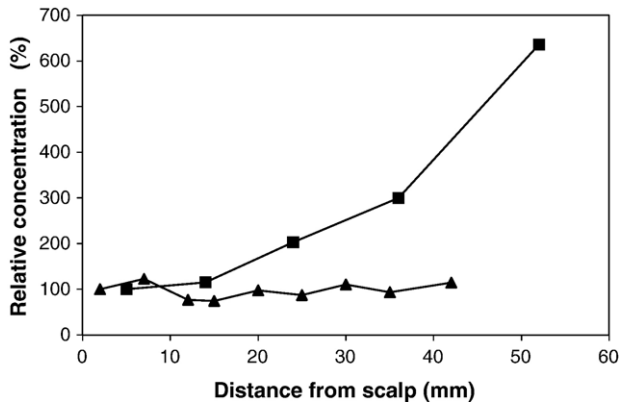

Fig. 1. Longitudinal hair Pb (■) and H-Cr (▲) profiles of a 14-year-old boy.

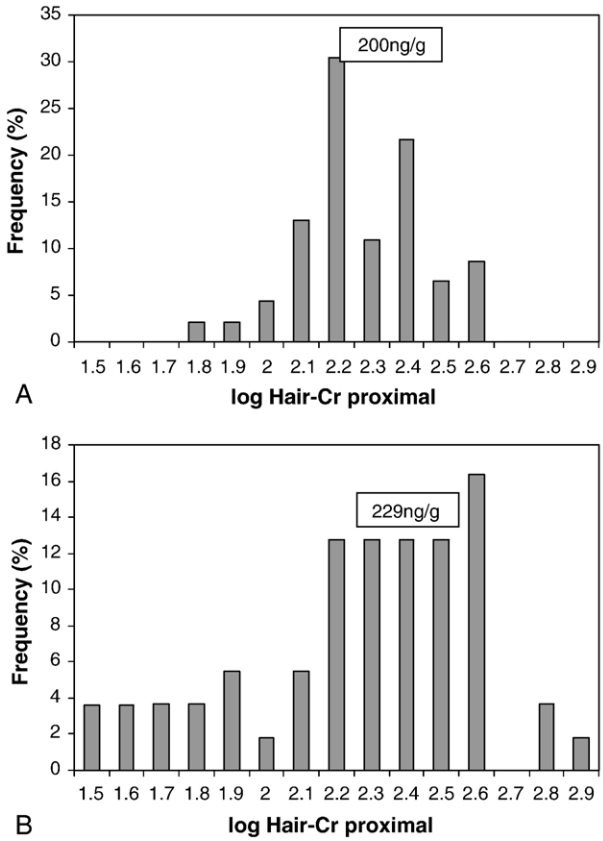

Fig. 2. Frequency distribution of log H-Cr_{pr} content of subjects with normal glucose tolerance (A, n = 47) and type 2 diabetes mellitus (B, n = 55).

about the course of the study, and gave their written consent. All participants were living permanently (more than 2 years) in the Ljubljana region (population of about 350 000). The whole region was uniformly represented in the selected population groups.

3. Methods

3.1. H-Cr longitudinal profiles

Two bundles of approximately 40 to 60 hairs were cut from the nape of the neck of each person, as close to the scalp as possible. A Teflon thread was tightened around the hairs to secure them. Hairs were washed twice in a mixture of n-hexane + ethanol (1:1) and 3 times in distilled water [52]. Hairs were dried at 80°C to constant weight (6 hours) and fixed between 2 pieces of plastic tape (Scotch MagicTape 810, USA) to make a "sandwich." Rectangular segments 3 mm wide and weighing 0.5 to 1.0 mg were cut along the hair sandwich starting from the proximal toward the distal end, and the Cr content was determined by solid sampling electrothermal atomic absorption spectrometry. A detailed description of the instrument and the experimental parameters are given elsewhere [59,62,63]. The accuracy of the results was checked by the hair reference sample (Chinese reference hair GBW09191), which was measured with each set of samples. The average value obtained for the reference sample was 4.40 ± 0.34 μg Cr per gram (certified value, 4.77 ± 0.38 μg Cr per gram). The precision of atomic absorption measurements was in the range ± 3% to ± 5%. Hair and blood samples were collected simultaneously within a 1-week interval.

3.2. Biochemical parameters

Chromium, glucose, insulin, and HbA_{1c} were measured in fasting blood samples. These were collected in carefully prepared clean sodium heparin tubes (P-Cr and P-insulin), EDTA vacutainers (HbA_{1c}), and potassium fluoride tubes (glucose). Samples for P-Cr and P-insulin were centrifuged and stored at −20°C before analysis.

P-glucose determinations were made by using an automated glucose oxidase method (Beckman, Fullerton, CA). Cationic high-performance liquid chromatography (BioRad Variant, Hercules, CA) in phosphate buffer was used for determination of HbA_{1c}. The concentration of insulin in plasma was determined by a modified two-phase radioimmunoassay technique (INSI-CTK irma, Dia Sorin, Saluggia, Italy).

Chromium in plasma was determined by electrothermal atomic absorption spectrometry using a Varian SpectrAA800, instrument with the Zeeman effect background correction (Varian, Melbourne, Australia). Calibration was performed with an aqueous solution containing 5μg Cr per liter, freshly prepared from a Cr stock standard solution of 1 g Cr per liter

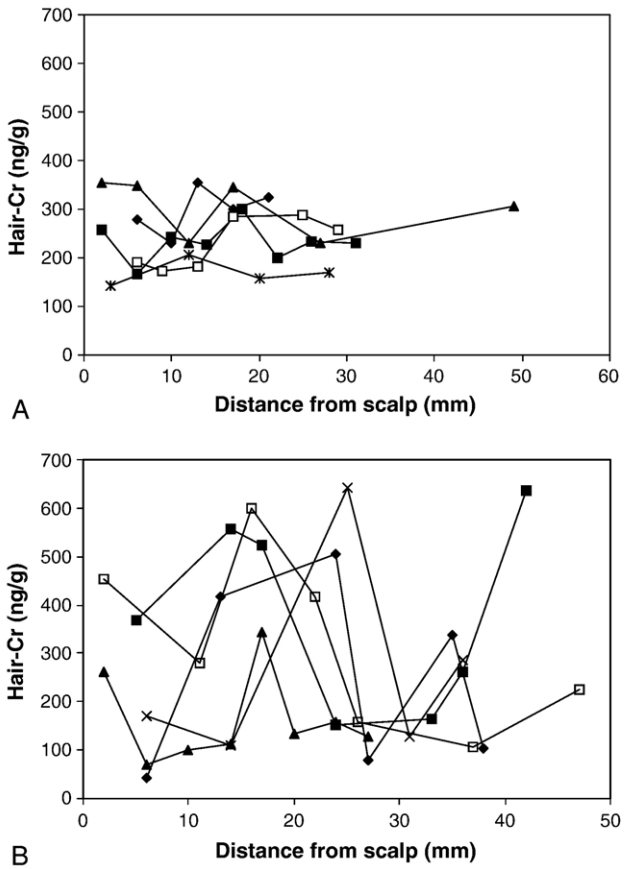

Fig. 3. Typical longitudinal H-Cr profiles of subjects (n = 5) with normal glucose tolerance (A) and type 2 diabetes mellitus (B).

(Merck Chromium Titrisol, Darmstadt, Germany). The precision of the method was ± 6% to ± 7% at a Cr concentration of 0.4 μg/L, and the calculated detection limit was 0.06 μg Cr per liter. The accuracy of the results was tested by a reference serum (Seronorm Trace Elements, Nycomed, SERO AS, Billingstad, Norway).

4. Statistical analysis

The two-tailed Student *t* test was used to determine differences in H-Cr content and longitudinal H-Cr variation between the 2 groups. The strength of association between different measured parameters was determined by Pearson correlation coefficient. In our study, the sample represented approximately 0.015% of the population, which is equal to or more than that in most other investigations where the population is in the million range. In addition, there were no differences in race in the examined population and in environmental conditions within the region.

5. Results

For each individual, at least 4 and up to 11 segments were cut from the hair bundle at various distances from the scalp and the Cr contents were determined. When results were doubtful, the second bundle was analyzed. Generally good agreement was obtained. From the Cr contents of particular hair segments the approximate H-Cr_{av}, H-Cr_{pr}, and magnitude of variation (percent) were calculated for each individual. These data are summarized in Table 2 for the 2 groups investigated.

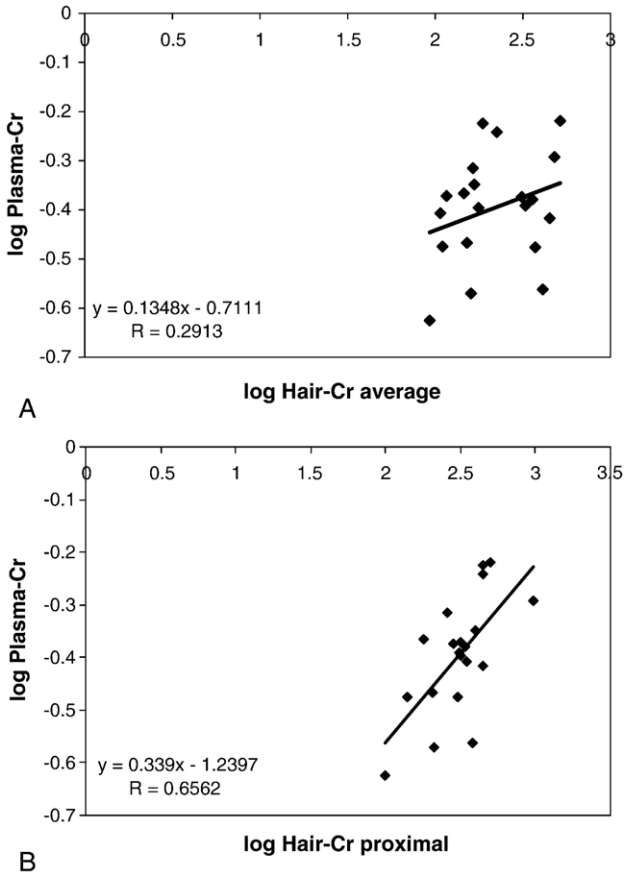

Fig. 4. Relationship between P-Cr and H-Cr in the group of diabetic subjects (n = 24). A, P-Cr and H-Cr_{av} (25-35 mm hair length). B, P-Cr and H-Cr_{pr} (3 mm hair length).

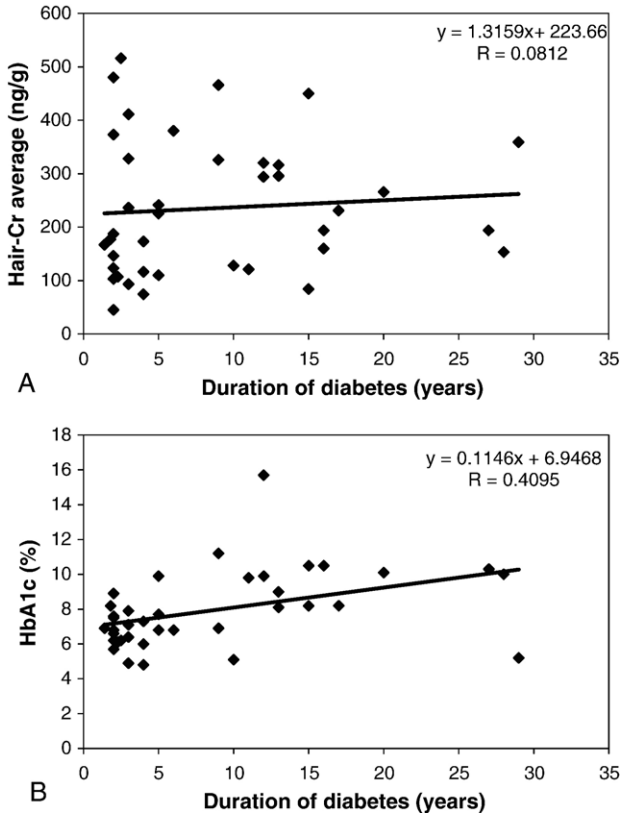

Fig. 5. H-Cr_{av} (A) and HbA_{1c} (B) as affected by the duration of diabetes mellitus in patients with type 2 diabetes mellitus (n = 39).

Fig. 2A and B shows frequency distributions of log H-Cr_{pr} values within the control (n = 47) and diabetic (n = 55) groups.

Measurement of longitudinal H-Cr profiles produced interesting results, namely, the average variation of Cr concentration along the hair length is more than twice that in the diabetic group compared with controls (see Table 2). This feature is better illustrated in Fig. 3A and B where H-Cr profiles are plotted for 5 different subjects from the control (Fig. 3A) and the diabetic (Fig. 3B) group.

Because H-Cr is less frequently used in comparison to P-Cr as a measure of Cr status or Cr metabolism, and H-Cr data are generally treated by physicians with a certain skepticism, it would be worthwhile to investigate the relationship between these parameters. The results for a number of diabetic subjects are presented in Fig. 4A and B. A highly significant association was obtained if log H-Cr_{pr} was plotted against log P-Cr, but the strength of the correlation dropped considerably when log H-Cr_{pr} was replaced by log H-Cr_{av}.

Another interesting question relates to whether the duration of diabetes, diet, and medications had any influence upon H-Cr_{av} content and HbA_{1c}. The results of this investigation are presented in Fig. 5A and B.

In addition, correlations between H-Cr_{av} and H-Cr_{pr} and 2 biochemical quantities (P-insulin and P-glucose) were investigated in the control and diabetic groups. The purpose of doing this was to identify which parameter is more sensitive to variation of H-Cr content and to verify if the effects are different in the 2 population groups. The results for the control population are illustrated in Fig. 6A and B. A statistically significant positive correlation was observed between H-Cr_{pr} and both mentioned parameters. In contrast, a complete absence of these relationships was found in the diabetic group (results not shown).

The association between H-Cr_{av} and age was investigated in the control (n = 54) and diabetic group (n = 57) separately. The results are depicted in Fig. 7A and B. A weak but statistically significant decrease of H-Cr_{av} with age was observed in the healthy population, whereas a nonsignificant opposite pattern was exhibited in diabetic patients.

6. Discussion

In addition to the interpretation of the experimental results, the major part of the discussion will address the following questions: Are P-Cr, H-Cr, and sweat Cr contents realistic measures of human Cr status? Are low or high values of these parameters characteristic of depleted body stores?

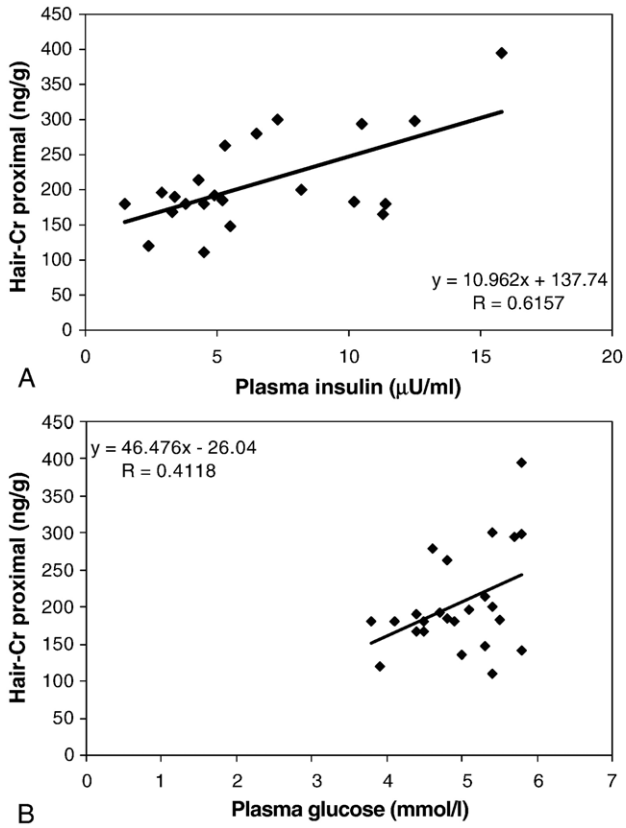

Fig. 6. Association between fasting P-insulin (A), fasting P-glucose (B), and H-Cr_{pr} in the healthy elderly population (n = 24).

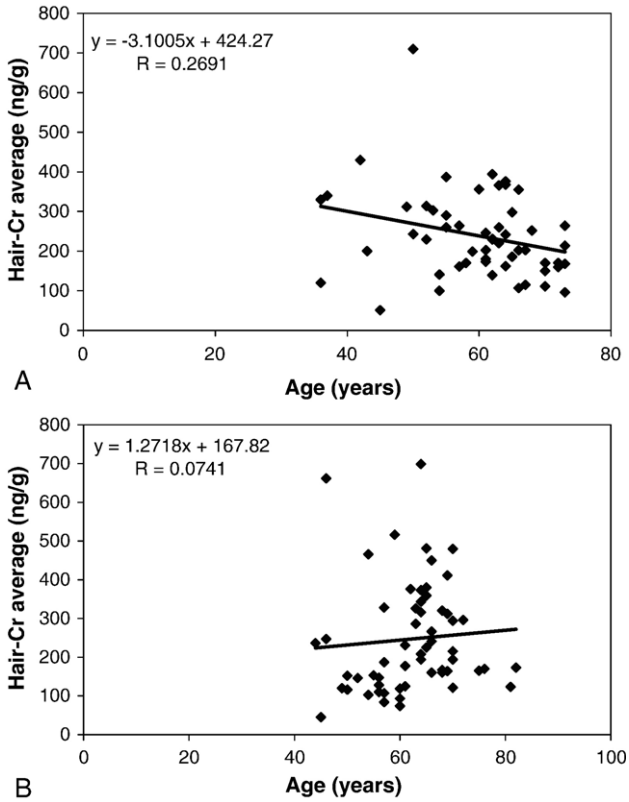

Fig. 7. Correlation between H-Cr content and age. A, Healthy control population (n = 54). B, Patients with type 2 diabetes mellitus (n = 57).

6.1. Summary of literature data on H-Cr, P-Cr, and sweat Cr

Most publications comparing P-Cr and H-Cr contents of healthy and diabetic subjects reported that low values are typical of subjects with the so-called metabolic syndrome. However, some articles, including the present one, reported no statistically significant difference was observed between these populations. Moreover, higher H-Cr contents were found in diabetic pregnant women compared with those showing normal glucose tolerance [45], raised P-Cr levels were observed in hyperglycemic subjects, and a negative Cr balance was associated with enhanced P-Cr levels [2,64]. If we consider literature data on H-Cr and P-Cr reported since 1980 (Table 1), the calculated average values ± SD of P-Cr (0.69 ± 0.62 and 0.78 ± 0.58 μg Cr per liter) and H-Cr_{av} (356 ± 228 and 381 ± 291 ng Cr per gram) for healthy and diabetic subjects, respectively, are not statistically different. The difference of 50 ppt or less in the amount of Cr at extremely low levels of P-Cr (typically 100-200 ppt) reported between these population can hardly be justified due to severe contamination problems and variability of the parameters that influence P-Cr content (diet, exposure, stressors). On the other hand, H-Cr_{av} values are less

Table 1
Summary of the published data on P-Cr, sweat Cr, and H-Cr

P-Cr (μg/L)	Sweat Cr (μg/L)	H-Cr (ng/g)[a]
0.55[b] [2]	25 [42]	154-175[b] [2]
1.67 ± 0.45; 1.89 ± 0.36[b] [29]	2.76 ± 0.66 [34]	160 [43]
0.82 ± 0.26; 0.56 ± 0.22[b] [30]	0.7; 25[c] [43]	680 ± 140 [34]
0.48 ± 0.19; 0.31 ± 0.18[b] [31]	1.0-2.9; 3.3-11[d] [56]	247-325 [44]
0.67-0.93;[e] 0.98[b] [32]		229 ± 125 [42]
0.34 ± 0.04 [9]		472 ± 61; 734 ± 155[b] [45]
0.75 ± 0.09; 0.29 ± 0.07[b] [33]		770; 505[b] [36]
0.39 ± 0.08 [34]		570 [46]
0.20 ± 0.02; 0.15 ± 0.01[b] [16]		300-360 [47]
1.09 ± 0.81; 0.75 ± 0.53[b] [35]		360-380 [48]
2.32 ± 0.39; 1.59 ± 0.48[b] [25]		200-300 [49]
0.41 ± 0.10[b] [27]		123 [50]
1.10 ± 0.2; 1.70 ± 0.2[b] [36]		100-140;[f] 240-400[g] [51]
0.73 ± 0.72; 0.28-0.41[b] [37]		151-168 [52]
0.11 [38]		201-328 [53]
0.07-0.08 [39]		430-1390 [54]
0.13 ± 0.02 [40]		160-205 [55]
0.12-0.20; 0.37-0.81[c] [41]		

[a] Average chromium content in hair up to 50 mm length.
[b] Diabetic subjects.
[c] Tannery workers.
[d] Immediately after running (1.5 hours).
[e] Obese nondiabetic subjects.
[f] Myopia.
[g] Hyperopia.

susceptible to contamination but may be influenced, in addition to the above-mentioned parameters, by hair washing and the hair length used for determination.

6.2. H-Cr_{av}, H-Cr_{pr}, and frequency distribution in the diabetic and healthy population

Analysis of the results presented in Table 2 showed that there is no statistically significant ($P \leq .05$) difference in H-Cr_{av} or H-Cr_{pr} contents between the healthy population and diabetics.

It is also evident that differences in H-Cr_{av} and H-Cr_{pr} between diabetic men and women are marginal and statistically not significant. However, in the control population, women (n = 17) have statistically ($P < .01$) lower H-Cr contents than men (n = 30) regardless of whether H-Cr_{pr} or H-Cr_{av} was used. Controversial results on sex differences in H-Cr are reported in the literature [34,47]. Whereas Davies et al [34] found female H-Cr to be statistically higher in comparison to males at all ages; the conclusion of Gordon's study [47] is the opposite and similar to ours. This can be explained, in addition to physiologic reasons, by the effect of various stressors that increase Cr metabolism and may differ in the populations examined.

It is evident from Fig. 2A and B that the homogeneity of H-Cr_{pr} values in the control group is substantially better compared with the diabetic group. A similar observation was earlier reported by Rabinowitz et al [36] for H-Cr_{av}, but no explanation has been proposed. If we consider the Gaussian distribution in the healthy group and take 1 SD of the mean H-Cr_{pr} (135-320 ng Cr per gram) as the normal range characteristic of the area, then only 39% of diabetic subjects lie in this range, in contrast to the healthy subjects (68%). Because a significantly higher percentage of diabetic subjects (22%) compared with healthy individuals (9%) exhibited H-Cr_{pr} values less than 135 ng Cr per gram, it is reasonable to assume that low H-Cr content may reflect depleted Cr body stores. However, this assumption is not consistent with the above-mentioned observations [2,64], and that some physically normal, active, healthy elderly subjects and a high proportion of young (25-35 years), well-trained long-distance runners with chronically enhanced glucose utilization showed similarly low H-Cr_{pr} contents (unpublished results). These subjects certainly have a very efficient glucose homeostatic system, and their Cr status is obviously not compromised. It has been established that this particular population exhibits substantially enhanced Cr absorption and reduced Cr excretion in comparison to the matched sedentary subjects [12,39]. Thus, persons who exercise regularly appear to develop more efficient Cr metabolism. On the other hand, it was established (Fig. 2A and B) that a large proportion of diabetic subjects (39%) showed high H-Cr_{pr} contents (>320 ng/g) compared with the controls (23%). Therefore, it may be equally correct to assume that subjects with high H-Cr_{pr} or H-Cr_{av} have depleted Cr stores or just exhibit an enhanced Cr metabolism. A possible answer to the above questions can be obtained if we assume that H-Cr_{av} and H-Cr_{pr} values in the absence of significant external contamination reflect the Cr metabolic rate integrated over the time span of growing hair

Table 2
Summary of the data on H-Cr measurements

	Healthy elderly subjects		Type 2 diabetic subjects	
H-Cr_{av} (ng/g)	All	248 ± 108; n = 47	All	247 ± 143; n = 58
	Males	275 ±74; n = 30	Males	249 ± 141; n = 37
	Females	201 ± 130; n =17	Females	244 ± 151; n = 21
H-Cr_{pr} (ng/g)	All	233 ± 98; n = 46	All	278 ± 195; n = 55
	Males	267 ± 100; n = 30	Males	255 ± 166; n = 35
	Females	170 ± 49; n = 16	Females	316 ± 236; n = 20
Longitudinal variation of H-Cr (%)	All	± 26	All	± 58
	Males	± 25	Males	± 54
	Females	± 27	Females	± 62

(several days to a few months). Reasons in support of this supposition are outlined in the following paragraph.

Chromium is present in the environment (air) in minute amounts, predominantly in a poorly soluble form (oxide). Such particles attached to the hair surface can easily be removed by washing and ultrasonic agitation. Even Cr that might be solubilized by the action of various secretions is not bound to -SH groups in the hair structure very easily, as formation of such complexes is a slow process [59]. The usual frequency of hair washing would thus effectively remove the exogenous part of H-Cr. Analysis of a number of shampoos and hair care medications showed that the contribution to H-Cr from these sources is insignificant. Similarly, the effect of sweat Cr can also be neglected, as the concentration of this element in the sweat of people not occupationally exposed to Cr is generally around 1 ng/mL [43,56]. If exogenous Cr does contribute to the total H-Cr content, H-Cr_{pr} will be significantly less affected than H-Cr_{av}.

6.3. Longitudinal Cr profiles and Cr metabolic rate

Measurements of H-Cr longitudinal profiles should thus provide an indication of the magnitude of variation of Cr metabolic rate over a definite time span, depending on the hair length measured. In addition, it has been reported [34] that H-Cr_{av} is reasonably well correlated with P-Cr, which has also been confirmed by our results (Fig. 4A and B), at least for H-Cr_{pr}. Because significant differences in the strength of association were observed when H-Cr_{pr} or H-Cr_{av} was correlated with P-Cr, it is reasonable to suppose that all 3 parameters are related to Cr metabolic rate but integrated over different time intervals (hours, days, months). It is evident from Fig. 3A and B that Cr concentration varies along the hair length (time) in a random fashion. These variations are substantially larger (± 58% on average) in subjects with impaired glucose tolerance compared with the healthy population (± 26% on average). However, it should be emphasized that part of the variation of H-Cr concentration along the hair length is a consequence of the cycling activity of hair follicles [58]. Thus, all hairs in a particular hair segment are not in the same growing phase. By using approximately 50 hairs in a bundle for analysis, this effect would be minimized. We have further assumed that other factors that may have an influence on H-Cr variation (Cr daily intake, effect of stressors) would approximately equally contribute to the H-Cr variation in both population groups. To estimate if the magnitude of long-term variation in H-Cr can be accounted for by the variation of any of the 2 glycemic parameters, the 2-month variation of fasting P-glucose was followed in one healthy subject and in a diabetic patient (27 years from the onset of diabetes). Measurements were made every second day. Coefficients of variation of ± 8% and ± 35% were calculated for the healthy and diabetic subject, respectively. These results agree well with those reported by Morris et al [16]. Thus, long term variation of H-Cr parallels that of P-glucose and P-insulin. Considering the extent and frequency of these variations, we may infer that selection of different intervals along the hair lengths (Fig. 3A and B) would result in significant variability of both H-Cr values measured. The probability of getting substantial divergence in H-Cr values, in particular H-Cr_{pr}, is much higher in diabetics. This phenomenon may, to some extent, explain the inconsistency in the results of different research groups (see Table 2). As has been mentioned before, the large divergence in P-Cr and sweat Cr contents reported is likely to be associated with analytical error.

It is assumed that H-Cr content may be an indicator of Cr body stores [34,36,42–44,46,50,53–55,60,61], which are supposed to be compromised in the diabetic state. One would then expect that diabetes would strongly influence H-Cr, which would decrease as the disease progresses. To confirm or refute this assumption, the relationship between the duration of diabetes and H-Cr_{av} was investigated. The results are presented in Fig. 5A. Surprisingly there was no relationship found, which casts doubt on the validity of using H-Cr as an indicator of Cr status. Several literature data [27,36,46] and a recent double-blind, crossover study measuring the effect of Cr supplementation (1 mg chromium picolinate per day) on improvement of glycemic parameters in patients with type 2 diabetes mellitus [27] are also consistent with the above-mentioned assumption, namely, subjects who responded positively to Cr supplementation showed no statistically significant difference in H-Cr_{pr} and H-Cr_{av} contents (394 ± 244 and 249 ± 169 ng Cr per gram, respectively) compared with those who did not respond (294 ± 102 and 254 ± 115 ng Cr per gram, respectively). It should be borne in mind that diabetic symptoms always develop as a result of severe Cr deficiency; on the other hand, diabetes as a disease of complex etiology may also appear in subjects with normal Cr body stores.

6.4. H-Cr, P-Cr, sweat Cr, and Cr status

In dealing with the question of whether low or high H-Cr (P-Cr, sweat Cr) contents are characteristic of depleted Cr stores, the mechanism of action of Cr on improving insulin sensitivity should be considered. Recent studies [17] postulated that the existence of a biologically active form of Cr, ie, Cr bound to low molecular weight protein (LMWCr), synthesized in the organism, is necessary for normal glucose utilization. It has also been reported that this form of Cr has better retention in tissues compared with inorganic Cr, which is rapidly excreted [14]. It appears that a reduced ability to convert Cr into a usable form associated with an enhanced excretion may be one of the most probable reasons for the development of glucose intolerance [65]. A genetic factor is likely to be responsible for this failure, but the inability to convert inorganic Cr to LMWCr may gradually increase as a result of the aging process. This is compatible with the enhanced Cr excretion observed in elderly subjects [5,33]. Secondly, it has been demonstrated [14] that diabetic symptoms could appear due to inhibited transport of Cr to different tissues as a consequence of

transferrin saturation (hemochromatosis). Thirdly, malnutrition, and certain diseases that influence the gastrointestinal absorption of Cr would have a similar effect [37,64]. However, signs and symptoms of diabetes in the latter 2 cases were found to develop gradually, after several years of deprived Cr supply [2,37], when slowly exchanging Cr compartments become depleted. Increased need for Cr in gestation and different kinds of chronic stress is associated with enhanced Cr utilization [10,11,13,36,45]. All these anomalies in the pathway of Cr metabolism may eventually lead to the exhaustion of Cr stores. If we consider the size and kinetics of exchange of Cr from different body pools, it becomes clear that only the blood pool and perhaps fast-exchanging compartments from other storage pools could respond to acute or chronic loads of glucose (meals, stress gestation, etc). When Cr from these sources cannot cope with demands, compensation is provided by increased absorption. As long as excretion is overcompensated by absorption, an enhanced Cr metabolic rate will be established. However, when requirements for Cr exceed that provided from absorption and transport from other compartments, the Cr metabolic rate will be reduced. These episodes of enhanced and decreased Cr metabolic rate change with time in a random fashion, as demonstrated by the shapes of the H-Cr longitudinal profiles shown in Fig. 3A and B. These changes are substantial in the diabetic population. It may be concluded from the above interpretation that H-Cr (average and proximal) is related to Cr metabolic rate rather than being an indicator of Cr status.

6.5. Relationship between duration of diabetes and glycemic parameters

In addition, it would be interesting to know how glycemic parameters (P-glucose, HbA_{1c}, P-insulin) change with the duration of diabetes in subjects treated by diet alone or in combination with hypoglycemic drugs. In view of this, a relationship between fasting P-glucose, HbA_{1c}, P-insulin, and duration of diabetes was also investigated. A statistically significant positive association (n = 39; $P < .01$; $r = 0.4660$) was found between duration of diabetes and fasting P-glucose (not shown), and glycated hemoglobin and duration of diabetes (Fig. 5B) (n = 38; $P < .02$; $R = 0.4095$). This is to be expected because of gradual diminution of the glucose homeostatic system despite treatment. In contrast, P-insulin exhibited a slight statistically insignificant (n = 20; $P > .05$; $R = 0.1453$) decrease with duration of diabetes (not shown). It should be emphasized that results from only those diabetic subjects who were registered for at least 1 year were included in the above investigation (see Fig. 5A and B).

6.6. Association between H-Cr and glycemic parameters in the diabetic and healthy populations

Morris et al [16], investigating Cr homeostasis in patients with type II diabetes mellitus (non–insulin-dependent diabetes mellitus), correlated fasting P-Cr with fasting P-glucose and fasting P-insulin. In both cases, they obtained significant negative correlations for healthy subjects (n = 33), which were absent in the group of diabetic subjects (n = 93). We investigated similar relationships between H-Cr (proximal and average) values and fasting P-glucose and P-insulin. In contrast to the findings of Morris et al [16], we found a significant positive association in the control population between $H\text{-}Cr_{pr}$ and fasting P-insulin and P-glucose (P-insulin: n = 24; $R = 0.6157$; $P < .01$; P-glucose: n = 24; $R = 0.4118$; $P < .05$). The results are presented in Fig. 6A and B. The strength of correlation was substantially reduced when $H\text{-}Cr_{av}$ was used in the calculations (P-insulin: n = 29; $R = 0.3644$; $P \sim .05$; P-glucose: n = 38; $R = 0.1711$; $P > .05$) (results not shown). This is in agreement with our observation presented earlier (see Fig. 4A and B). It is interesting to note that fasting P-insulin correlates significantly better than fasting P-glucose, which is consistent with the reported interrelationship between Cr and insulin [15]. In the group of diabetic subjects, no association was observed between these parameters (results not shown). To find a reasonable explanation for these anomalies, we considered the long-term (approximately 2 to 3 months) variation in P-glucose and P-insulin in the healthy and diabetic populations. It is generally known [9,57] that the daily oscillation of P-glucose, P-insulin, and P-Cr follows a certain cycle. In subjects with normal glucose homeostasis, these variations are marginal and increase with age because of enhanced insulin resistance and other age-related factors [7,8]. In people with various degrees of glucose intolerance, all 3 parameters (P-glucose, P-insulin, and P-Cr) are subject to significantly larger daily variations. It is generally recognized that daily variations of P-glucose and P-insulin are accompanied by simultaneous changes of P-Cr. However, the results of different research groups are contradictory. Morris et al [9,15,16,57] reported that the increase in P-glucose and P-insulin after a meal or glucose challenge is associated with a simultaneous drop of P-Cr. On the contrary, others [27,29,36,40] found that all 3 parameters oscillate in the same manner. At present, the reasons for the observed discrepancies are not clear. Although $H\text{-}Cr_{pr}$ correlates reasonably well with P-Cr, transient changes in P-Cr cannot be recorded by measurement of $H\text{-}Cr_{pr}$. On the other hand, the relationship between H-Cr and P-Cr may be different in the healthy and diabetic groups.

6.7. H-Cr and age

The association of $H\text{-}Cr_{av}$ with age was studied separately for healthy individuals and patients with type 2 diabetes mellitus. The results for the healthy and the diabetic group are illustrated in Fig. 7A and B, respectively. A weak correlation (n = 54; $R = 0.2691$; $P \sim .05$) between age and $H\text{-}Cr_{av}$ was found in the healthy population. In contrast, the absence of such correlation was found in the glucose-intolerant population (Fig. 7B). Davies et al [34] reported a highly significant negative correlation after measuring some

40 000 hair samples. Another large-scale study [47] performed on 17 000 subjects, however, found no such association. A similar observation was also reported in an earlier publication [36].

A variety of grounds could be proposed to interpret the gradual decline of H-Cr content with age. It is generally known that metabolic rates of biochemical processes in the human organism decrease in older age [8]. In addition, compromised Cr body stores resulting from an inadequate supply, reduced absorption, and inefficient transformation to a biologically active form associated with increased Cr losses should also be considered.

Two case studies are presented that might support some of the statements given in the Discussion. Two patients had remarkably higher Cr concentration than the others, and before the determination, both had serious health problems.

6.8. Case 1

One of the subjects (male, aged 66 years) had a myocardial infarction (MI) with cardiac arrest. The patient manifested typical features of the metabolic syndrome: P-glucose, 7.9 mmol/L; P-insulin, 9.8 μU/mL; cholesterol, 5.7 mmol/L; high-density lipoprotein, 0.9 mmol/L; low-density lipoprotein, 3.5 mmol/L; triglycerides, 2.9 mmol/L; body mass index, 31.0 kg/m^2; blood pressure, 140/85 mm Hg. After discharge from the hospital, he regularly took the following medications: enalapril/hydrochlorotiazide (10/12.5 mg), enalapril (10 mg), metoprolol (75 mg), acetylsalicylic acid (100 mg), and atorvastatin (20 mg). Six months later, hair was sampled for the determination of Cr longitudinal profile. The Cr contents (H-Cr_{av}, 2675 ng/g; H-Cr_{pr}, 818 ng/g) were extremely high. No source of external Cr contamination was found and he was not on a Cr-rich diet. His H-Cr content was followed for the next 2 years (5 hair samples of approximately 35 mm in length were analyzed). H-Cr_{av} exhibited a significant decrease (from 2675 to 1517 ng/g), whereas H-Cr_{pr} showed a random variation. Most interestingly, variation of Cr content along the measured hair length (35 mm) decreased in 2 years from 51% to 19.2%. His Cr excretion measured at the last hair sampling as 24-hour urine content (241 ng) was in the range reported [10,39] for the normal population and substantially lower than that of patients with type 2 diabetes mellitus (~460 ng) [9,16]. In this context it should be pointed out that significantly raised levels of plasma nickel were observed in patients after MI [66].

A significantly enhanced Cr metabolic rate coupled with a normal Cr excretion suggested that his Cr body stores were seriously depleted after MI. The repletion of stores by food as the only source of Cr was a slow process and it appears that even in 2 years equilibrium was not reached.

6.9. Case 2

The second subject (female, aged 60 years), who had partial pancreatectomy 10 years ago, was admitted to hospital for further diagnosis because of feeling unwell. High P-glucose (18.8 mmol/L) together with low P-insulin were found. Her lipid profile (cholesterol, 4.3 mmol/L; high-density lipoprotein, 1.4 mmol/L; low-density lipoprotein, 2.4 mmol/L; triglycerides, 1.0 mmol/L), body mass index (23.0 k/m^2) and blood pressure (135/80 mm Hg) were normal. She received insulin therapy and her fasting P-glucose level dropped to 10.0 mmol/L. Coincidentally, a hair sample had been collected for analysis from her scalp a month before she was admitted to the clinic. The substantially higher H-Cr_{av} (571 ng/g) compared with the mean value of the healthy elderly population in this study (233 $\pm$ 98 ng/g) suggests an enhanced Cr metabolism, presumably as a consequence of significantly increased Cr losses associated with type 1 diabetes mellitus [30].

7. Conclusions

In this article, hair analysis is critically evaluated and the potentialities of H-Cr determination in assessing Cr status in relation to the development of glucose intolerance were investigated. Conventional practice in which the average Cr content of a definite hair length is measured (H-Cr_{av}) was replaced by a novel approach in which scanning of longitudinal H-Cr profiles of a bundle of approximately 50 hairs was performed. By this means, 2 additional parameters were deduced: Cr content of the proximal 3-mm section of the hair (H-Cr_{pr}) and the magnitude of variation (in percent) of Cr content along the hair. Evidence is presented to suggest that H-Cr values resemble reasonably well the Cr metabolic rate integrated over the definite time interval given by the hair length measured. Two age- and sex-matched population groups were studied: patients with type 2 diabetes mellitus and healthy elderly subjects (controls). Mean H-Cr_{pr} and H-Cr_{av} were similar in both populations. However, substantially different patterns of the frequency distribution of log H-Cr_{pr} were observed in these populations. Characteristic of the control population was a narrow, symmetrical distribution (mean, 233 ng Cr per gram; median, 200 ng Cr per gram). In the diabetic group, the distribution was wider and skewed toward lower H-Cr_{pr} values (mean, 278 ng Cr per gram; median, 229 ng Cr per gram).

The most interesting observation relates to the magnitude of variation in H-Cr content along the hair. Large variations (on average, $\pm$ 58%) were characteristic of the glucose-intolerant population compared with the healthy subjects (on average, $\pm$ 26%). This difference was statistically significant ($P < .01$) and highly specific. Assuming a variation of H-Cr proportional to the variation of Cr metabolic rate in body tissues, this would suggest a seriously disturbed Cr metabolism in type 2 diabetes mellitus. This statement may be additionally confirmed by the observation that none of the glycemic parameters (P-glucose, P-insulin, HbA_{1c}) or age correlates with H-Cr_{pr} and H-Cr_{av} in the diabetic population, compared with the controls where a reasonably strong association was found.

H-Cr_{pr}, in contrast to H-Cr_{av}, correlates strongly with P-Cr measured in the diabetic group (n = 24; $R = 0.6562$; $P < .001$). This is compatible with the different time scale in appearance of Cr in hair and blood.

Ample evidence is presented to suggest that high and low H-Cr or P-Cr values may prove Cr deficiency or glucose intolerance.

It may be inferred from the presented experimental evidence that H-Cr and P-Cr data cannot be used as accurate predictors of Cr status unless this is confirmed by the response of the organism to Cr supplementation. The magnitude and direction of change of P-Cr or sweat Cr in response to stress may be an alternative test. This study confirmed an earlier observation [16] of considerably altered Cr metabolism in subjects displaying glucose intolerance or type 2 diabetes mellitus.

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