Introduction

In the 1970s, chromium was discovered to be an important trace element, required for maintaining normal glucose metabolism. This was discovered by chance when a patient developed elevated blood glucose after being administered long-term total parental nutrition, which did not contain chromium [1]. A small amount of chromium was administered and the blood glucose fell to within the normal range. A review of studies by Anderson in 1998 concluded that chromium supplementation improved levels of blood glucose, insulin, cholesterol and haemoglobin A1c in people with varying degrees of glucose intolerance [2]. Its physiological role appears to be that of a co-factor, potentiating the action of insulin in cellular glucose uptake. Chromium is primarily obtained from black pepper, brewer’s yeast, mushrooms, prunes, raisins, nuts, asparagus, milk, beer and wine [3].

This is the first study to analyse not only the chromium status of type 2 diabetic women compared to non-diabetics, but also to look closely at any differences in the dietary intake between the two groups.

Patients and Methods

The local ethics and research committee approved this study. The women also gave their consent to be included in the investigation. Chromium status was tested in 65 caucasian women divided into two groups. Group 1 was made up of 30 women who did not have diabetes. These women were mainly recruited from hospital and university staff and women accompanying clinic patients. Group 2 consisted of 35 women between the ages of 35 and 70 previously diagnosed with type 2 diabetes, attending the routine Diabetic Clinic over a 2-week period.

The hospital record for each diabetic woman was reviewed to assess suitability. Women who had type 1 diabetes and women whose diabetes was secondary to other pathology such as pancreatic disease and use of steroids were excluded. This study concentrated on women only, as a previous study had shown significant differences between chromium levels of the two sexes [4] and hair samples may not be consistently available from middle aged men.

Data on the use of hair care products and dietary intake was collected via a questionnaire completed during an interview with the researcher (CLM). Information regarding their diabetes was obtained from the woman’s medical records.
Analysis of Hair Specimens by Inductively Coupled Plasma Mass Spectrometer (ICPMS)

The hair sample was collected using scissors, taking a sample of the soft hair from the back of the scalp, just above the hairline. The hair sample was then placed in a plain plastic bag. The hair was weighed and placed in a Teflon Advanced Composite Vessel (ACV) with 5mL nitric acid (Romil SpA grade) and left loosely covered overnight. The ACV was then sealed and placed, with up to 11 other ACVs, in a microwave oven (CEM MDS 81D) and heated for 10 minutes at a maximum of 100 psi. After cooling, the vessels were opened and their contents diluted up to 25ml with water (Elga UHQ grade).

Fifteen samples were too small to be placed directly into the ACV and were placed with 1mL of nitric acid into 3mL and 7mL microdigestion vessels. One microdigestion vessel was then placed in an ACV with 10mL of water and a spacer. The ACV was then sealed and heated as previously described. After cooling, the contents of the microdigestion vessels were diluted with water up to 5mL. The two methods, therefore, used the same acid concentration (20%), thus making the results using the two methods comparable. The analysis of elements followed standard procedures outlined in similar studies in our laboratory [4].

The metals, including chromium, were calibrated up to 5μg/g (ppb) with standards containing 0, 1, 2, 3, 4 and 5 ppb metals, which gave linear calibrations. Analysis was performed using mass spectrometry (Thermo Elemental Plasma Quad II), with a detection limit of 0.006 μg/g for chromium.

Statistical analysis was performed by SPSS using parametric tests for numerical and non-parametric tests for ordinal data as appropriate.

Results

The average time since diagnosis of type 2 diabetes was 6.8 years. 1 (4%) woman is treated by diet only, 14 (40%) women were treated by oral medication and the remaining 20 (56%) were treated using insulin.

The diabetic women were significantly older (Table 1). The two groups were comparable with respect to their height, although the diabetic women had a greater mean weight and thus a greater mean BMI.

The two groups had very similar weekly dietary intake of most foodstuffs, apart from alcohol, chocolate, cream and milk; significantly more controls consumed these products. (Table 2 and 3).

### Table 1: General characteristics in the two groups of patients studied. Values are given as mean (SD).

| Characteristics | Control n = 30 | Diabetics n = 35 | T     | P          |
|-----------------|---------------|------------------|-------|------------|
| Age (years)     | 52.50 (6.24)  | 57.51 (8.84)     | 2.668 | .01 (CI 1.7-8.8) |
| Height (cm)     | 165.5 (8.98)  | 161.1 (8.28)     | -1.965| NS         |
| Weight (kg)     | 73.32 (15.07) | 86.6 (17.57)     | 3.114 | 0.003 (CI 4.7-21.8) |
| BMI (kg/m²)     | 26.8          | 33.4             |       |            |

### Table 2: Consumption of food and drink (Chi-square Analysis). Values are given as n (%).

| Food and Drink | Control n = 30 | Diabetics n = 35 | P    |
|----------------|---------------|------------------|------|
| Alcohol        | 23 (77)       | 6 (20)           | .000 |
| Smoking        | 3 (10)        | 6 (18)           | NS   |
| Vitamins       | 14 (47)       | 7 (20)           | .022 |
| Bread          | 30 (100)      | 35 (100)         | NS   |
| Cereals        | 28 (93)       | 29 (83)          | NS   |
| Cheese         | 28 (93)       | 28 (80)          | NS   |
| Chocolate      | 23 (77)       | 11 (31)          | .000 |
| Cream          | 10 (33)       | 4 (11)           | .032 |
| Fruit          | 28 (93)       | 35 (100)         | NS   |
| Nuts           | 8 (27)        | 4 (11)           | NS   |
| Potato         | 30 (100)      | 32 (91)          | NS   |
| Peas or beans  | 28 (93)       | 34 (97)          | NS   |
| Seafood        | 22 (63)       | 20 (58)          | NS   |
| Snacks         | 21 (70)       | 24 (69)          | NS   |
| Red meat       | 28 (93)       | 32 (91)          | NS   |
| Liver or kidney| 16 (53)       | 14 (40)          | NS   |
| Chicken or turkey| 29 (97)    | 32 (91)          | NS   |
| Milk           | 30 (100)      | 29 (83)          | .000 |
| Tea / Coffee   | 29 (97)       | 35 (100)         | NS   |
The use of most hair care products was similar for both the control and the diabetic groups (Table 4), but the use of a hair colourant was significantly greater in the control group (80% Vs 49%). If chromium levels in all women are analysed with respect to hair colouring status, there is no difference in the level of chromium in women with coloured hair compared to those without (Mann Whitney U test = 0.799 – Not Significant).

The hair sample size ranged from 0.003g to 0.1g. Chromium was found to be not significantly different in the diabetic group than the control group. (mean chromium 0.59 Vs 0.52 μg/g) (Mann Whitney U test p=NS) (table 1)

**Discussion**

Chromium levels in hair samples were analysed to assess the chronic chromium status in 2 groups of woman. Hair has previously been shown, to be an excellent material for assessing chromium [5,6,7]. Measurements of chromium status using hair samples have previously been shown to correlate well with serum levels [5]. The use of hair spray, mousse, permanent wave and bleaching

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**Table 3: Intake of food and drink in the women who consumed that food/drink (Mann Whitney U Test). Values are given as mean (SD)**

| Amount consumed/week          | Control n = 30 | Diabetics n = 35 | P    |
|-------------------------------|----------------|------------------|------|
| Alcohol (units)               | 7.0 (4.9)      | 4.3 (3.1)        | NS   |
| Smoking (cigs/day)            | 18.3 (2.9)     | 14.2 (7.4)       | NS   |
| Bread (slices)                | 19.1 (11.1)    | 23.9 (10.4)      | 0.021|
| Cereals (bowls)               | 5.8 (1.8)      | 4.4 (2.5)        | NS   |
| Cheese (helpings)             | 2.8 (1.8)      | 2.2 (1.7)        | NS   |
| Chocolate (bars)              | 1.5 (1.0)      | 1.7 (1.2)        | NS   |
| Fruit (pieces/day)            | 2.8 (1.0)      | 2.3 (1.3)        | 0.034|
| Nuts (ounces)                 | 3.2 (1.7)      | 2.5 (2.4)        | NS   |
| Peas or beans (helpings)      | 3.3 (1.8)      | 3.1 (1.7)        | NS   |
| Potato (helpings)             | 5.0 (1.9)      | 4.2 (2.0)        | NS   |
| Seafood (helpings)            | 2.0 (1.5)      | 1.7 (1.0)        | NS   |
| Snacks                        | 3.5 (2.6)      | 3.0 (3.3)        | NS   |
| Red meat (portions)           | 4.6 (2.6)      | 3.4 (1.9)        | NS   |
| Liver or kidney (portions)    | 0.8 (0.6)      | 0.8 (0.6)        | NS   |
| Chicken or turkey (portions)  | 2.6 (1.3)      | 1.9 (0.8)        | 0.011|
| Milk (pints)                  | 3.5 (2.2)      | 3.6 (2.0)        | NS   |
| Tea / Coffee (cups/day)        | 5.7 (2.0)      | 4.8 (2.0)        | NS   |
| Milk                          | 30 (100)       | 29 (83)          | .000 |
| Tea / Coffee                  | 29 (97)        | 35 (100)         | NS   |

**Table 4: General characteristics in the two groups of patients studied. Values are given as mean (SD).**

| Hair Treatment              | Control n = 30 | Diabetics n = 35 | P   |
|-----------------------------|----------------|------------------|-----|
| Hair colouring              | 24 (80)        | 17 (49)          | .009|
| Hair Spray and mousse       | 20 (67)        | 18 (51)          | NS  |
| Permanent Wave              | 5 (17)         | 11 (31)          | NS  |
| Bleaching                   | 11 (37)        | 7 (20)           | NS  |

**Table 5: Hair chromium levels (μg/g). (Mann-Whitney U test – No significant differences)**

|                | Control n = 30 | Diabetics n = 35 |
|----------------|----------------|------------------|
| Mean           | 0.524          | 0.591            |
| Median         | 0.515          | 0.586            |
| Standard Deviation | 0.361       | 0.411            |
| Minimum        | 0.050          | 0.019            |
| Maximum        | 1.565          | 1.688            |
between the two groups was not significant. The use of hair colouring products is significantly different, although this finding may not influence the results substantially, as hair was taken from the soft hair at the top of the neck, which grows rapidly and is often not reached by hair colouring products. Women with dyed hair did not differ in their chromium levels from women without dyed hair.

After comparing the general features of the two groups, it was found that the diabetic group were significantly older (52.5 years versus 57.5 years) (table 1). Chromium levels have been shown in previous studies to decrease with age [5]. It should, however, be noted that the chromium levels in the small age range of women used in this study would not decrease enough to be a possible cause of error. This is further emphasised as, if chromium levels decreased with age, then the mean level of chromium should have been correspondingly lower in the diabetics, who were older, but it was actually slightly higher.

The two groups are similar in height, but the mean weights and therefore BMI’s of the diabetics was greater (Table 1). This result therefore correlates well with previous findings that the type 2 diabetic group have a greater BMI.

Chromium is an essential trace element. Chromium deficiency and its relationship with high blood glucose levels has been documented[2]. Chromium acts by regulating or potentiating the action of insulin[7]. It is thought that chromium increases the number of insulin receptors and therefore increases insulin binding to cells. Anderson [9] suggested that the recommended daily intake of chromium for adults should be between 50 – 200 μg. Chromium, as previously mentioned, can be obtained from a wide variety of food products such as brewer’s yeast, nuts and milk. Many studies have shown that it is difficult to obtain the minimum 50 μg from diet alone [10,11]. Supplemental chromium has been shown previously to improve glucose tolerance and circulating levels of insulin and cholesterol, although other studies showed that there was no change[9]. It is thought that this may reflect the large number of factors involved in the regulation of chromium metabolism such as genetics, nutrition, stress and underlying level of glucose tolerance [9].

The dietary intakes of the two groups of women in this study were surprisingly similar. The main differences were that there were fewer diabetics who reportedly ate cereals, chocolate, cream and milk and who drank alcohol. A greater proportion of the diabetics ate fruit. This may result from the dietary advice that the diabetics receive as part of their treatment plan.

It was found, using ICPMS analytic technology, that there was no significant difference in chromium levels between the two groups (table 5). In fact, the diabetic group recorded a slightly higher mean level of chromium. There was a wide range of measured chromium levels in both groups, and the lowest chromium level, was recorded in the diabetic group (0.019 Vs 0.050 μg/g). This may be because, as previously mentioned, the dietary intake was very similar for the two groups. During the process of analysis, 2 control samples were lost due to accidental contamination, the results shown are therefore based on the results for 28 control samples.

These results are very similar to earlier findings by Rabinowitz et al in 1980 [6], who investigated chromium levels in hair samples of 46 diabetic men and compared them to levels of 20 age-matched males. The study, based in Los Angeles, found that the differences between the mean levels of chromium between the two groups were not significant. The study also found its lowest recorded chromium levels were amongst the diabetics. Kazi et al in 1999, found significantly lower levels of chromium in a group of 25 diabetics, compared to age-matched controls[7]. The study was based on a population in Pakistan. Neither of these previous studies reported use of hair care products or compared the dietary intake of the two groups. A recent study of trace elements in the blood of 53 diabetics found that diabetics had lower levels of chromium, as well as iron, manganese and selenium but had increased levels of vanadium [12]. These results may show short-term levels of the trace elements, rather then the chronic state that hair analysis reflects.

In conclusion, comparisons of chromium levels in the hair of people with type 2 diabetes and controls, in Caucasian females in a north England population, show a wide variation in levels. There was no significant difference in levels of chromium between the groups. To validate these findings it may be necessary to analyse a sample from a different population. This study also found that there were significantly less numbers of the diabetic group eating chocolate, cream, fruit, milk and alcohol. This reflects the dietary advice given to diabetic patients.

In conclusion the chromium status of type 2 diabetic women was not significantly different from a control group of women. Further work is necessary to precisely explore the relation between chromium and type 2 diabetes, if such a relationship exists.

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