The Analyst

# Occupational arsenic exposure and glycosylated haemoglobin†

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**In a group of 40 workers occupationally exposed to arsenic (As workers) biological markers for cardiovascular diseases were studied. The median arsenic concentration in urine samples from the exposed group was 22.3 nmol of As per mmol of creatinine, while the individual maximum level was 294.5 nmol of As per mmol of creatinine. That of the reference group was 12 nmol of As per mmol of creatinine and significantly below the level of the exposed group ($p < 0.001$). The arsenic concentration in urine samples from colleagues of the persons working with arsenic containing products was similar to the arsenic concentration in urine samples from the As workers. The concentration of glycosylated haemoglobin (Hgb $A_{1C}$) was increased in whole blood from the As workers. The level of the As workers was 5.4% (median), similar to that of colleagues (5.5%), while that of the reference group was 4.4%. The differences were significant ($p < 0.001$). Multiple regression analysis showed a significant connection ($p = 0.034$) between the concentration of Hgb $A_{1C}$ in whole blood and the arsenic level in urine from the As workers. The systolic blood pressure was 125 mm Hg in the As workers and 117 mm Hg in the control group. The difference was significant ($p = 0.023$). It is concluded that arsenic exposure has an influence on carbohydrate metabolism, increases the systolic blood pressure and finally may result in increased risk of development of cardiovascular diseases.**

***Keywords:*** *Arsenic; occupational exposure; glycosylated haemoglobin; blood pressure; diabetes; cardiovascular diseases*

Arsenic exposure may lead to development of cardiovascular diseases (CVD).[1] However, further data are needed to confirm this suggestion.[2] A study of biomarkers for cardiovascular diseases in a group occupationally exposed to As may support the suggestion.[3] Increased glucose level is found in whole blood from CVD patients. The increase is due to a decreased glucose tolerance.[4,5] The glucose is taken up passively by the erythrocytes; an increased level in serum results in an increased concentration of glucose in the erythrocytes. Due to a reaction with haemoglobin an increased level of glycosylated haemoglobin results.[6] As an indicator for increased glucose level in the blood Hgb $A_{1C}$ is measured.[7,8] Increased blood pressure is found in CVD patients.[9,10] Increased diastolic as well as systolic blood pressure can be used as an indicator for increased risk of CVD.

The aim of this work was: (*i*) to describe a group of occupationally exposed As workers, *i.e.*, taxidermists and persons impregnating or working with As impregnated wood; (*ii*) to evaluate the concentration of glycosylated haemoglobin (Hgb $A_{1C}$) in whole blood from As exposed workers; (*iii*) to evaluate the blood pressure in the As exposed group; and (*iv*) to discuss the results in relation to the development of cardiovascular diseases.

† Presented at the Sixth Nordic Symposium on Trace Elements in Human Health and Disease, Roskilde University, Denmark, June 29–July 3, 1997.

## Experimental

### *Chemicals*

Inorganic arsenic in the oxidation state $As^V$ and dimethylarsinic acid (DMAA) were obtained from Merck (Darmstadt, Germany) and Sigma (Poole, Dorset, UK), respectively. Pure samples of arsenite, arsenate, monomethylarsonic acid (MMAA), DMAA, arsenocholine and arsenobetaine were obtained from the Community Bureau of Reference (BCR), Brussels, Belgium.[11,12] When not stated otherwise, all chemicals were of the highest purity obtainable from Merck.

### *Groups of persons studied*

Urine samples and venous blood samples were collected from three groups of persons. Group 1: 26 persons (5 females) aged 20–60 years old without any known arsenic exposure (reference group); Group 2: 6 colleagues of workers who directly handle As containing products; and Group 3: 40 persons (4 females) working with arsenic containing products (As workers).

Group 3 was divided into following occupational categories: (*a*) taxidermists ($n = 13$), stuffing animals and birds using arsenic as a preservative; (*b*) wood workers ($n = 8$, 4 females), producing garden fences; (*c*) wood workers ($n = 6$), producing weekend cottages; (*d*) workers impregnating wood ($n = 2$), using an autoclave (length, 12 m) for impregnation; and (*e*) workers impregnating electric pylons ($n = 4$), using a hand impregnation tool for the impregnation. The occupational categories as well as the As exposure are described in detail elsewhere.[3,13]

Table 1 shows the matching of the exposed and the reference group with respect to age (mean = 37 years) and smoking habits (40% *versus* 46%). The mean age of colleagues was 29 years and the percentage of smokers was 33%.

### *Questionnaire interview*

In connection with blood and urine sampling a standardized personal interview based on a structured questionnaire was

**Table 1** Subjects in the study characterized by age and smoking habits

| Groups | Subjects, *n* | Age, mean ± *s* | Smokers, *n* | Non-smokers, *n* |
|---|---|---|---|---|
| As workers | 40 | 37.2 ± 13.2 | 16 | 24 |
| Colleagues | 6 | 29.2 ± 9.8 | 2 | 4 |
| References | 26 | 37.5 ± 10.4 | 12 | 14 |

carried out. Information obtained from the interview included name and age, type of work, duration of work with arsenic containing products, chemicals and products used, alcohol intake, cigarette smoking, physical activities, consumption of fish and fish products, consumption of vitamins and medicine as well as personal and family history of diseases including hypertension, diabetes, cardiovascular disease and cancer.[3]

### *Collection of urine samples*

Two urine samples from each participant were collected seven days apart between 12:00 pm and 6:00 pm. They were collected in the middle of the week, the initial sample on the day of blood sampling. The samples were collected in acid-washed, 250 ml polyethylene containers and were divided into smaller polyethylene containers and stored at −20 °C until arsenic and creatinine concentrations could be determined. The participants were asked not to eat fish or fish products for three days and to wash hands prior to giving the urine samples.

### *Methods*

When not stated otherwise the analyses were performed at the National Institute of Occupational Health, Copenhagen. Uncertainties and limits of detections were standard deviations ($s$) at low concentrations or blank samples and $2 \times s$, respectively. Statistical control of the methods were documented by use of control cards.

### *Determination of arsenic in urine samples*

The total amount of inorganic arsenic and metabolites (MMAA and DMAA) was determined by the direct hydride method.[12,13] A Perkin Elmer 1100 B Atomic Absorption Spectrophotometer with flow injection and autosampler AS-90, equipped with an electrodeless discharge lamp was used for detection of arsenic hydrides (Perkin Elmer, Norwalk, CT, USA). The resonance line at 197.3 nm was used.[12] Standard additions with different concentrations of As (as DMAA) was used.[12] The method had a recovery of arsenite, MMAA and DMAA in urine not significantly different from 100%, whereas a reduced sensitivity to arsenate was observed. The reduced sensitivity to arsenate has previously been considered to be acceptable since this inorganic species represents only approximately 1% of the total urinary excretion of the arsenic compounds.[12]

The results were expressed as nmol of As per mmol creatinine (1 nmol of As per mmol creatinine = 0.662 μg of As per g of creatinine). All arsenic values are the means of the two samples from each person.[13] The uncertainty was 12.9 nmol $l^{-1}$ and the limit of detection ($3 \times s$) was 38.7 nmol $l^{-1}$.

### *Determination of creatinine*

Creatinine in urine was determined by Jaffe's reaction in a Beckmann spectrophotometer.[14] The results were expressed as mmol $l^{-1}$. The uncertainty was 0.5 mmol $l^{-1}$ and the limit of detection was 1.0 mmol $l^{-1}$.

### *Collection of blood samples*

A 10 ml venous blood sample was collected from each participant. The blood was bled into venoject tubes containing EDTA. The samples were stored at −80 °C until the measurement of glycosylated haemoglobin.

### *Determination of glycosylated haemoglobin* (Hgb $A_{1C}$)

Glycosylated haemoglobin in whole blood was measured by affinity chromatography according to Little *et al.*[6] The results were reported in % of total haemoglobin. The glycosylated haemoglobin assay was performed at the Medical Laboratory, Copenhagen. The uncertainty was 0.4% and the limit of detection was 0.8%.

### *Blood pressure measurement*

Blood pressure, diastolic as well as systolic, were measured before the samplings and after the person had rested for 10 min. Initially three registrations were made; if the level was stable the registrations were used; otherwise the person rested until stable blood pressures were obtained. The blood pressures were automatically registered by use of digital blood pressure equipment, UA-751 (Takeda Medical, Japan).[3] The blood pressures used in the study were the means of three registrations.

Table 2 shows the samples, analyses and measurements involved in the study.

### *Statistical methods*

The median, mean, and standard deviation were calculated by use of the MINITAB personal computer program.[15] The differences between median values were tested by the Kruskal–Wallis non-parametric test. Multiple regression analysis was used for estimation of connection between parameters. For all tests performed, the level of statistical significance was set at 5%. Individual data points are reported in a preliminary study.[3]

## Results

In the As workers the maximum urine concentration 80 nmol of As per mmol of creatinine (median) was found in the group impregnating electric pylons, individual maximum level was 294.5 nmol of As per mmol of creatinine. The median value of all As workers was 22.3 nmol of As per mmol of creatinine. That of the reference group was 12 nmol of As per mmol of creatinine, which was significantly different from the concentrations in the group of all As workers as well as the subgroups consisting of taxidermists, garden fence makers and electric pylon impregnators, respectively ($p < 0.001$) (Table 3). The arsenic concentration in urine samples from colleagues of the persons working with arsenic containing products was at the level of occupational exposure.

The concentration of Hgb $A_{1C}$ in whole blood from the As workers was 23% higher than that in the references. The level of the As workers was 5.4% (median), similar to that of colleagues (5.5%), while that of the reference group was 4.4%. The differences were significant ($p < 0.001$) (Table 4). The Hgb $A_{1C}$ level was increased in smokers compared to non-smokers, the difference was, however, only significant for the $As^{III}$ exposed group ($p = 0.039$) (Table 5).

Multiple regression analysis showed a significant connection ($p = 0.034$) between the concentration of Hgb $A_{1C}$ in whole blood and the As level in urine from the As workers (Table 6).

**Table 2** Samples, analyses and measurements in the study

| Samples/ Analyses/ Measurements | Samples from As workers, *n* | Samples from colleagues, *n* | Samples from references, *n* |
|---|---|---|---|
| Urine samples, arsenic | 40 | 5 | 26 |
| Blood samples, Hgb $A_{1c}$ | 32 | 6 | 26 |
| Blood pressure | 34 | 5 | 25 |

Table 7 shows the systolic blood pressure in the As workers to be 125.0 mm Hg (median value). That of their colleagues was 120.0 mm Hg, while the level in the reference group was 117.0 mm Hg. The Kruskal–Wallis test showed the systolic blood pressure to be significantly increased in the As workers ($p = 0.023$). The diastolic blood pressure was also increased in the As workers, however, the difference was not significant. The levels were 77.9 *versus* 74.7 mm Hg.

In the As exposed group as well as in the reference group increased blood pressure levels were found in smokers, and increased blood pressure levels were found in As exposed non-smokers as compared to non-smoker references; however, evaluation on this limited data showed the differences not to be significant.

## Discussion

The results of this study showed an increased concentration of Hgb $A_{1C}$ in whole blood from As workers as well as from colleagues. The increase in the occupationally exposed As persons was about 25%. An increased Hgb $A_{1C}$ level is a well known marker for diabetes where the increase in Hgb $A_{1C}$ in whole blood has been estimated as 70% compared to a control level.[8] At the time of sampling none of the As exposed persons in this study suffered from diabetes mellitus. However, other studies relate As exposure to diabetes. Thus an increased frequency of diabetes mellitus has been found in As exposed persons in Taiwan as well as in Sweden.[16,17] Thus several data indicate that exposure to arsenic influences the blood glucose level.

The biochemical mechanism by which As functions is probably not due to an effect on the insulin segregation from the pancreas. Thus according to an experiment performed by Kim and Na arsenic has no effect on the serum insulin level during intoxication.[18] However, arsenic inhibits the activity of enzymes in the citric acid cycle, *i.e.*, the activity of succinic acid dehydrogenase, thus inhibiting the carbohydrate metabolism.[19] On the other hand the increased Hgb $A_{1C}$ level may also indicate an initial response against As intoxication. Thus animal experiments have shown that gluconeogenesis and interstitial glucose uptake are decreased by severe arsenic intoxication.[20,21] Treatment with glucose counteracts this toxic effect.[22]

**Table 3** Sum of inorganic arsenic, MMAA and DMAA (arsenic*) in urine samples from individuals working with arsenic containing products, from their colleagues and from reference persons

| Occupational category | $n$† | Mean | Median | $s$‡ | Range§ |
|---|---|---|---|---|---|
| Taxidermists | 13 | 30.0 | 21.0¶ | 20.9 | 12.0–84.5 |
| Garden fence makers | 8 | 33.5 | 34.9¶ | 11.4 | 14.6–48.8 |
| Weekend cottage constructors | 6 | 18.2 | 17.8 | 3.7 | 14.0–24.5 |
| Colleagues | 2 | | | | 7.5 and 25.0 |
| Wood impregnators | 2 | | | | 11.5 and 17.0 |
| Electric pylon impregnators | 4 | 127.4 | 80.0¶ | 112.5 | 55.0–294.5 |
| Colleague | 1 | | | | 61.5 |
| New house constructors | 7 | 19.1 | 15.0 | 7.3 | 13.0–29.0 |
| Colleagues | 2 | | | | 7.0 and 20.0 |
| All As workers | 40 | 35.9 | 22.3¶ | 46.2 | 11.5–294.5 |
| All colleagues | 5 | 24.2 | 20.0¶ | 22.3 | 7.0–61.5 |
| References | 26 | 14.5 | 12.0 | 8.9 | 6.0–44.0 |

* Arsenic values are reported in nmol of As per mmol of creatinine. † $n$ is the number of subjects in each category. ‡ $s$ is the standard deviation. § Minimum to maximum values. ¶ Significantly different from the reference group as estimated by the Kruskal–Wallis test.

**Table 4** Glycosylated haemoglobin (Haemoglobin $A_{1c}$)* in whole blood from individuals working with arsenic containing products, from their colleagues and from reference persons

| Occupational category | $n$ | Mean | Median | $s$ | Range |
|---|---|---|---|---|---|
| All As workers† | 32 | 5.7 | 5.4‡ | 1.1 | 4.0–10.1 |
| All colleagues | 6 | 5.8 | 5.5‡ | 1.0 | 4.7–7.5 |
| References | 26 | 4.4 | 4.4 | 0.5 | 3.2–5.1 |

* Values are reported in % of total haemoglobin. † Data from garden fence makers not available. ‡ Significantly different from the reference group as estimated by the Kruskal–Wallis test.

**Table 5** Glycosylated haemoglobin in whole blood from smokers and non-smokers

| Exposure* | $n$ | Mean | Median | $s$ | Range |
|---|---|---|---|---|---|
| $As^{III}$†, −r | 11 | 5.5 | 5.1 | 1.7 | 4.0–10.1 |
| +r§ | 6 | 6.2 | 6.2 | 0.8 | 5.3–7.5 |
| $As^{V}$‡, −r | 8 | 5.4 | 5.6 | 0.8 | 4.6–6.2 |
| +r | 7 | 5.9 | 6.0 | 0.7 | 5.0–6.9 |
| Reference, −r | 14 | 4.3 | 4.2 | 0.6 | 3.2–5.1 |
| +r | 12 | 4.5 | 4.4 | 0.4 | 4.0–5.1 |

* −r = non-smokers; +r = smokers. † Workers exposed to $As^{III}$, *i.e.*, the taxidermists and the electric pylon impregnators. ‡ Workers exposed to $As^{V}$. § Significant difference between smokers and non-smokers.

The results of the present study indicate that smoking contributes to an increased Hgb $A_{1C}$ level in whole blood. However, this increase may be due to an increased As exposure caused by the smoking.[3,13]

Smoking increases the systolic blood pressure in the reference group as well as in As workers. However, smoking habits may increase the As exposure in the As workers, which may contribute to the increase in the blood pressure in As workers who smoke. This may contribute to the finding of a significant systolic blood pressure in the As exposed workers in this study. That As exposure may increase the blood pressure is further supported by Chen *et al.*, who found increased prevalence of hypertension in long-term As exposed persons.[23] Epidemiological studies show a high prevalence of CVD in populations living in arsenic rich areas.[1] Serum levels of

**Table 6** Statistical connection between glycosylated haemoglobin and arsenic concentration in urine, years of work with arsenic containing products, and the age of the persons

| Regression equation* | $p$ |
|---|---|
| $y_1 = 4.6 + 0.0130x_1$ | 0.596 |
| $+ 0.0169z_1$ | 0.459 |
| $+ 0.0078q_1$ | 0.034 |

* $y_1$ = glycosylated haemoglobin, percentage of total haemoglobin; $x_1$ = age, years; $z_1$ = years of work with arsenic containing products; $q_1$ = arsenic concentration, nmol of As per mmol of creatinine.

**Table 7** Systolic blood pressure in persons working with arsenic containing products, colleagues and references

| Group | $n$ | Mean | Median | $s$ | Range |
|---|---|---|---|---|---|
| As exposed workers* | 34 | 127.5 | 125.0 | 14.4 | 100.0–158.0 |
| Colleagues | 5 | 122.8 | 120.0 | 7.7 | 115.0–132.0 |
| Reference | 25 | 119.9 | 117.0 | 11.9 | 105.0–153.0 |

* Significantly different from reference value.

cholesterol, triglycerides and selenium are known biomarkers for CVD.[24–27] However, the concentrations of cholesterol, triglycerides and selenium in serum from As workers have all been found to be at the reference level.[3,28] These results indicate that increased levels of Hgb $A_{1C}$(glucose) and blood pressure could be preliminary biological markers for As exposure and subsequently for increased risk of CVD.

Finally, it can be concluded that As exposure influences the carbohydrate metabolism resulting in increased blood glucose levels, identified by Hgb $A_{1C}$. Diabetes as well as increased blood pressure are known factors for development of cardiovascular diseases. As exposure by initiating increased blood pressure and diabetes subsequently may contribute to development of cardiovascular diseases. The study further supports the suggestion that As exposure can cause CVD.

The financial support of the Danish Organization for the Control of Circulatory Diseases is greatfully acknowledged. Furthermore, the authors express gratitude to Dr. I. D. Beck and Dr. A. J. L. Mürer for valuable discussions and to E. Holst, for assistance with the statistical analysis, and to A. Abildtrup for technical assistance.

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*Paper 7/05699K*
*Received August 5, 1997*
*Accepted November 3, 1997*