Correlation between urinary nickel and testosterone plasma values in workers occupationally exposed to urban stressors


Keywords: Testosterone, urban air nickel, urinary nickel, biological monitoring
Parole chiave: Testosterone, nickel atmosferico, nickel urinario, monitoraggio biologico

Abstract

Background: The purpose of this study is to assess whether occupational exposure to low doses of nickel (Ni) present in urban air can cause alterations in the concentration of plasma testosterone in workers of the Municipal Police of a large Italian city assigned to different types of outdoor tasks.

Methods: 359 male subjects were included in the study and divided on the basis of job, age, length of service and smoking habits. The dosage of the atmospheric Ni was performed by personal dosimetries on a sample of the workers included in the study. For each worker included in the study the dosage of whole blood Ni and of the plasma testosterone was carried out. The total sample was subjected to the independent-samples T-test and the Mann-Whitney U test for variables with 2 modes (smoking cigarette) and the ANOVA test and the Kruskal Wallis test for variables with more than 2 modes (age, length of service and job function). The correlation of Pearson with p at 2 wings between the variables was evaluated in the total sample and after subdivision on the basis of smoking and on the basis of the job. After taking into account the major confounding factors the multiple linear regression was performed on the total sample and after breakdown by tasks.

Results: The correlation between the values of urinary Ni and the values of plasma testosterone on the total sample and for all classes of subdivision was found constantly negative. These results were confirmed by multiple linear regression, which indicated the Ni as the only significant variable that can contribute to the alterations of the testosterone.

Conclusions: Based on the results, the Authors suggest that occupational exposure to low doses of Ni present in the urban environment is able to influence some lines of the hypothalamic-pituitary-gonadal axis in exposed workers.

Introduction

Exposure to fine and ultra-fine particulate air pollution (PM$_{10}$ and PM$_{2.5}$), even at levels below the annual average limits established for urban air quality (50 mg/m$^3$) (1), have been associated with increased annual morbidity and mortality in several literature studies (2-13).

The PM is a complex mixture of extremely small particles that includes both liquid and solid acids, sulphates, nitrates,
metals, dust and soil particles as well as the organic compounds. The size and the composition of the PM are directly related to the negative effects that it has on human health (14-18).

The Ni present in urban air originates from natural sources (such as volcanic eruptions, forest fires and windblown dust from the rocks and soil) and from artificial sources, especially fossil combustion processes, which account for 62% of total anthropogenic emissions (19). Other anthropogenic sources are related to Ni industrial refining processes (17% of total emissions), incineration of waste (12%), the production of steel (3%), the production of other metal alloys containing Ni (2%) and the coal combustion (2%) (20). The compounds of Ni are also present as additives in unleaded gasoline (21), as catalysts in catalytic converters (22) and in paints, solvents (23) and some pesticides (24).

The Ni is an immunotoxic, neurotoxic, genotoxic, hepatotoxic and nephrotoxic metal (25). It was also recognized as an endocrine disruptor because of its adverse effects on reproduction (26-28) and disruption of steroidogenesis and spermatogenesis both in vivo and in laboratory animals (29-31). It also has the ability to bind the estrogens and the androgens receptors, together with other heavy metals (32, 33). Clinical studies on this topic associated the Ni exposure with an increased risk of prostate toxicity (34), infertility and testicular toxicity (35, 36). Studies carried out on laboratory animals and in vitro studies observed that Ni may interfere with reproductive hypothalamic hormones LH and FSH and with testosterone (37, 38). These results were also partially confirmed in studies on exposed human subjects (35, 39, 40).

A growing number of studies suggest that chemicals in the environment, such as those arising from the traffic caused and spread by human activities, can affect the male reproductive system (41-52).

Outdoor workers, such as traffic policemen, are daily exposed to a large number of pollutants arising from traffic and to various other psycho-social stressors. According to the literature, these stressors have been associated with alterations in the mean values of plasmatic androstenedione, testosterone, FSH and LH (42, 53, 54).

However studies on the effects of low occupational Ni exposure on testosterone plasma values, are deficient and, to date, not a single study has ever evaluated the relationship between occupational exposure to Ni present in urban pollutants and its effect on this hormone (55).

Based on these data, the aim of this study is to evaluate the correlation between occupational exposure to low levels of air Ni present in the urban pollution and the alterations of testosterone plasma values in workers of the municipal police of a large Italian city assigned to different types of outdoor tasks.

Materials and methods

1. Study population

The study was conducted on a sample of 359 outdoor workers, males, all enrolled in the Municipal Police of a large Italian city and assigned to different types of outdoor tasks divided as follows: traffic policemen, drivers and / or motorcycle riders, other outdoor tasks. All subjects included in the study had joined the program of health promotion in the workplace (56-58). This program is conducted in accordance with the directions of the current legislation and aims to investigate the health status of individuals occupationally exposed to urban pollutants.

The employees with the role of traffic policemen were assigned to the control of vehicular traffic in streets and areas of high and medium traffic density, monitoring and traffic control at intersections, parking lots
and limited traffic areas. The workers with the role of drivers and / or riders were assigned to traffic control and specific interventions in the event of road accidents and other activities including driving motorcycles or cars as a driver or “second patrol.” Workers with other outdoor tasks were assigned to different roles including the support to marginalized workers outdoor activities in the field of construction inspection or of the Judicial Police, Environmental Police, etc. Most of these activities were carried out in outdoor environments and only for the drivers were carried out in cars, for at least 80% of working time (8 hours a day 5 days a week). All workers were not equipped with protective equipment against dust and fumes from traffic.

The sample of 359 people was chosen from different areas of the city. We divided the city into eight areas and selected at random 42 workers from each area (25 traffic policemen, 10 drivers, 7 employed in other outdoor tasks); in the busiest central area we selected 65 employees (35 traffic policemen, 20 drivers, 10 employed in other outdoor tasks).

Each worker was monitored once during the morning shift (7:00 to 14:00 hours) on a working day between September 2010 and April 2011. A reasonable period of time of 8 months, in order with the purpose of this study and tests performed (environmental monitoring, biological monitoring and medical examinations), was then used. Each worker completed a clinical case history on the same day of the sampling. The questionnaire included information on age, area of residence in the last five years, physiological anamnesis (especially focused on diet, consumption of water from the water supply and / or mineral water and exposure to cigarette smoking), near and remote medical history. Following the recommendations of the World Health Organization (WHO), we classified as smokers all subjects who reported having smoked at least 100 cigarettes in their life or having stopped smoking less than six months before or being a smoker (59, 60).

The questionnaire also included items relating to impairment of fertility collected using a binary method (yes / no). For the acquisition of these items each subject was asked the following three direct questions: “Have you ever been diagnosed with fertility problems?” “Have your partner ever tried to get pregnant for at least 6 months without success?” , “If so, as a result of this failure, have you carried out investigations on fertility and what were the results of these investigations?”.

The drug history was collected in order to verify the assumption of drugs and the related therapeutic interventions for the treatment of this type of diseases.

To avoid the influence of confounding factors we excluded from the study those workers who reported being exposed to solvents, paints and pesticides during their leisure activities (61), subjects using illicit drugs and habitual drinkers of alcohol (alcohol consumption exceeding 2 units of alcohol per day for men, where 1 unit of alcohol corresponds to about 12 grams of ethanol) (62). We also excluded subjects older than 50, subjects working on shift work and / or night shifts (63), those practicing competitive sports activities (64) or performing outdoor tasks until less than 1 year before. Those with levels of urinary Ni below the lower limit of detection (LOD) of the method used were also excluded because their values were not representative and not statistically analyzable. So, the final number of workers included in the study went down from 359 to 274.

For the purposes of statistical evaluation we considered the following factors: job positions (traffic policemen, drivers, motorcyclists and outdoor workers with other tasks), age, seniority and smoking habit. We stratified the sample basing on age and on seniority in service into three groups.
(Age: Group A: 20-35 years; Group B: 36-45 years; Group C: > 45 years - seniority in service: Group A: < 10 years; Group B: 10-20 years; Group C: 21-40 years) in order to better evaluate the influence of these variables on the parameters under study.

All subjects agreed to make their personal information available and were aware that such data would be classified as “sensitive information”; they also agreed that data would be treated in an anonymous and collective form, and examined with scientific methods and analyzed for scientific purposes in accordance with the principles of the Declaration of Helsinki.

2. Environmental monitoring of the Ni: personal dosimetry

The characterization of the exposure to atmospheric Ni was evaluated through the execution of the personal dosimetry. In total we performed \( n = 8 \) personal dosimetries on traffic policemen selected from 8 different work areas considered to be most representative of the city’s air quality in this study, as well as \( n = 4 \) personal dosimetries on police drivers of cars with at least \( n \geq 2 \) policemen for each shift. So even if only one worker was wearing the dosimeter, the results were representative of the colleagues who were in the car with him. The air, blood and urine samples were taken in the same day, to avoid the influence of weather conditions on the Ni in the air. All subjects were asked not to smoke during the sampling. The personal air samples were collected using Dorr-Oliver cyclones of the type with a cut-point for the 5 micron diameter particles. Each cyclone was attached to a pump for personal sampling of air; the pump was calibrated to a flow rate of 1.7 L air/min, following the directions of the NIOSH. Each cyclone was fitted with a cassette holding a membrane filter of 37 mm in polyvinyl chloride (PVC). The cyclone and the cassettes were attached to the worker’s collar in the breathing area. The pump was placed in a padded envelope. After sampling, the cyclones were carefully removed. The filter membranes containing the collected particulates were analyzed to collect the Ni according to the method indicated by the NIOSH 7521 (65). The “digested” particulate samples were analyzed by atomic absorption spectrometry in graphite furnace (Perkin Elmer, model HGA-2100). Each subject wore the air sampler for the entire shift (7 hours).

For each sample of air the level of personal TWA exposure to Ni for 7 hours was calculated. The American Conference of Governmental Industrial Hygienists (66) proposed a limit value (TLV-TWA) of 1.5 mg/m\(^3\) for subjects occupationally exposed to Ni.

3. Ni urinary and plasma testosterone

The measurement of urinary Ni represents the most appropriate test for the evaluation of an occupational exposure to this metal, as there is a good correlation between the concentrations of Ni in the air and those present in the urine of exposed subjects (67).

The assay of urinary Ni and the examination of plasma testosterone were made for each worker, after 5 continuous working days, at the end of their shift. Each worker was asked to abstain from the consumption of food containing cocoa, soybeans, oatmeal, walnuts and almonds, fresh and dried legumes during the four days prior to examination (68).

For the urinary Ni, urine samples were transferred to the lab in an appropriate thermal bag at the temperature of +4 °, and then were stored in a refrigerator at -20° until the analytical determinations of Ni and urinary creatinine were performed. The determination of urinary Ni was performed by the complexation with ammonium pyrrolidinedithiocarbamate (APDC) and the atomic absorption analysis in graphite furnace. The LOD was 1.0 mg/g of urinary creatinine.
The determination of urinary creatinine to adjust the values of the biological indicators was carried out by the Jaffe method (69).

In order to take into account the dilution of the concentration of Ni in different urine samples, we divided the Ni (g/L) for the urinary creatinine (g/L) and expressed the urinary concentration of Ni in terms of g/g urinary creatinine.

For the plasma testosterone, a venous blood sample of 10 mL from each worker was collected. The blood samples were stored at the place of work in a refrigerator at +4° until the moment in which they were transferred (in a suitable container and at the same temperature) to the laboratory where they were centrifuged and then stored at -20° until they were analyzed (within 3 days). The immunoassay method (EIA) was used to analyze the plasma testosterone. Normal levels of plasma testosterone were those routinely considered by the laboratory for male subjects clinical analysis: 1-11 ng/mL.

4. Statistical Analysis

The normal distribution of variables was assessed using the Kolmogorov–Smirnov test, which was statistically significant for the urinary Ni and for testosterone, so these parameters were converted into logarithmic form for the analysis of the index of correlation and multiple linear regression.

The results for the values of atmospheric Ni measured at the individual dosimeter, for the values of urine Ni, of the plasma testosterone levels and for all confounding factors were expressed in terms of mean, standard deviation (SD), median, geometric mean and range (min-max).

The comparison between means was performed using the T test for independent samples and the Mann-Whitney U test for variables in 2 modes (smoking cigarette) and using the ANOVA test and the Kruskal Wallis test for the variables with more than 2 modes (age, length of service and job function).

The Pearson correlation coefficient was applied after the logarithmic transformation of the data, to evaluate the correlation between Ni and urinary testosterone.

Multiple linear regression analysis was performed after the logarithmic transformation of the data, considering the plasma testosterone as a dependent variable and considering the urinary Ni, the age, the length of service and smoking cigarette as independent variables.

Also the multiple linear regression analysis was repeated using the urinary Ni as dependent variable and the atmospheric Ni, the age, the length of service and the smoking cigarette as independent variables. The results were considered significant when the p values were less than 0.05.

Statistical analysis was performed using the software SPSS ® 10.0 Advanced StatisticalTM.

Results

1. Characteristics of the study population

The total sample of 274 male subjects was composed as follows: 191 nonsmokers and 83 smokers, 170 subjects were traffic policemen; 59 subjects were drivers and/or 2° patrol; 15 subjects were motorcycle riders; 30 subjects performed other outdoor tasks. These characteristics of are shown in Table 1.

The average values of Ni urinary and plasma testosterone levels were 4.78 (SD = 3.68) mg/100 mL in smokers and 4.62 (SD = 4.22) mg/100 mL in nonsmokers.

No statistically significant differences between the values of urinary Ni (test variable), and the habit of cigarette smoking (grouping variable) were found at T test for independent samples and at the Mann-Whitney U test. No statistically significant differences between the values of urinary Ni (dependent variable) and the age and the length of service (independent variables)
Table 1 - Characteristics of the study population divided for task

<table>
<thead>
<tr>
<th>Variables</th>
<th>Traffic policemen</th>
<th>Police drivers</th>
<th>Police motorcyclists</th>
<th>Policemen with other outdoor activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>(n. 170)</td>
<td>(n. 59)</td>
<td>(n. 15)</td>
<td>(n. 30)</td>
</tr>
<tr>
<td>Smoking habit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n° (%)</td>
<td>43 (25,2)</td>
<td>23 (38,9)</td>
<td>2 (13,3)</td>
<td>15 (50)</td>
</tr>
<tr>
<td>Age (ys)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>45,6 (7,8)</td>
<td>46,1 (7,8)</td>
<td>41,9 (9,2)</td>
<td>49,3 (6,7)</td>
</tr>
<tr>
<td>Geometric Mean</td>
<td>45</td>
<td>45,5</td>
<td>40,9</td>
<td>48,9</td>
</tr>
<tr>
<td>Min-Max</td>
<td>29 – 64</td>
<td>28 – 63</td>
<td>28 – 57</td>
<td>36 – 60</td>
</tr>
<tr>
<td>Median</td>
<td>44</td>
<td>46</td>
<td>42</td>
<td>49</td>
</tr>
<tr>
<td>Working life (ys)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>14,3 (8,4)</td>
<td>15,9 (7,3)</td>
<td>14,06 (7,7)</td>
<td>19,2 (8)</td>
</tr>
<tr>
<td>Geometric Mean</td>
<td>12</td>
<td>7,36</td>
<td>12,1</td>
<td>16,97</td>
</tr>
<tr>
<td>Min-Max</td>
<td>1 – 36</td>
<td>5 – 35</td>
<td>5 – 31</td>
<td>4 – 34</td>
</tr>
<tr>
<td>Median</td>
<td>14</td>
<td>16</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Nickel (μg/g creat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>4,79 (3,68)</td>
<td>4,62 (4,22)</td>
<td>4,67 (3,77)</td>
<td>4,76 (4,68)</td>
</tr>
<tr>
<td>Geometric Mean</td>
<td>3,56</td>
<td>3,26</td>
<td>3,42</td>
<td>3,23</td>
</tr>
<tr>
<td>Min-Max</td>
<td>1-17,2</td>
<td>1-24,9</td>
<td>1 - 19,4</td>
<td>1 – 24,9</td>
</tr>
<tr>
<td>Median</td>
<td>3,6</td>
<td>3,1</td>
<td>3,55</td>
<td>2,8</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3,99 (2,29)</td>
<td>4,2 (2,37)</td>
<td>4,36 (2,39)</td>
<td>4,01 (2,29)</td>
</tr>
<tr>
<td>Geometric Mean</td>
<td>2,88</td>
<td>2,95</td>
<td>3,14</td>
<td>2,86</td>
</tr>
<tr>
<td>Min-Max</td>
<td>1,5-10,7</td>
<td>1,2 -11</td>
<td>1,2 – 10,6</td>
<td>1,4 – 9,4</td>
</tr>
<tr>
<td>Median</td>
<td>4,41</td>
<td>4,48</td>
<td>4,69</td>
<td>4,41</td>
</tr>
<tr>
<td>Air Ni (ng/m³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of subjects</td>
<td>8</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>178.4 (142.3)</td>
<td>113.2 (123.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric Mean</td>
<td>104,52</td>
<td>85,18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min-Max</td>
<td>11.6-378.3</td>
<td>30.2-538.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>96,3</td>
<td>78</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD = Standard Deviation; ys = year

were found at the univariate ANOVA test and at Kruskal Wallis test. The results of the t test for independent samples, the Mann-Whitney U test, the ANOVA test and the Kruskal Wallis test are reported in Table 2. There were no statistically significant difference among the different outdoor tasks (traffic policemen, drivers, motorcyclists and subjects with other tasks) in relation to the average values and the distribution by age, length of service and smoking habit (smoking, nonsmokers). In the sample studied no subjects reported being diagnosed or treated for fertility disorders, either past or present.

2. Environmental monitoring of the Ni: individual dosimetry

The values of individual exposure to atmospheric Ni are shown in Table 1. All subjects reported they had not smoked during the sampling. No sample exceeded the limit value of 1.5 mg/m³ ACGIH proposes for occupationally exposed subjects.

At the multiple linear regression analysis a significant correlation (p <0.01) between the atmospheric Ni and the urinary Ni in both the total sample and after subdivision on the basis of the job (traffic policemen and drivers, Table 3) was found.
### Table 2 - Independent sample T test and ANOVA univariate test between dependent variables (Plasmatic Testosterone and Urinary Ni) and independent variables (smoking habit, age, working life and kind of task).

<table>
<thead>
<tr>
<th>Statistical analysis with dependent variable: Plasmatic Testosterone</th>
<th>Statistical analysis with dependent variable: Urinary Nickel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indipendent sample T test (p)</td>
<td>Univariate Anova test (p)</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>0.48</td>
</tr>
<tr>
<td>Age</td>
<td>0.93</td>
</tr>
<tr>
<td>Working life</td>
<td>0.45</td>
</tr>
<tr>
<td>Kind of task</td>
<td>0.97</td>
</tr>
</tbody>
</table>

### Table 3 - Multiple linear regression analysis, in the group of subjects who carried out the personal air samplings, between the Log urinary nickel values (dependent variable) and Log air Ni with the main confounding factors (independent variables).

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Total sample</th>
<th>Traffic policemen</th>
<th>Police drivers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t (beta)</td>
<td>P</td>
<td>t (beta)</td>
</tr>
<tr>
<td>Log nickel in air</td>
<td>23,431 (0.924)</td>
<td>0.000</td>
<td>23,581 (0.924)</td>
</tr>
<tr>
<td>Age (ys)</td>
<td>-0,261 (-0.014)</td>
<td>0.795</td>
<td>0,369 (-0.020)</td>
</tr>
<tr>
<td>Working life (ys)</td>
<td>-0,430 (0.024)</td>
<td>0.668</td>
<td>0.583 (0.032)</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>0,505 (-0.020)</td>
<td>0.615</td>
<td>-0,498 (0.020)</td>
</tr>
<tr>
<td>Model</td>
<td>F (R² Ad.)</td>
<td>P</td>
<td>F (R² Ad.)</td>
</tr>
<tr>
<td></td>
<td>142,339 (0.850)</td>
<td>0.000 *</td>
<td>142,606 (0.850)</td>
</tr>
</tbody>
</table>

ys = years  
R² Ad.= R² Adjusted  
*= Statistically signific  

3. **Ni urinary and plasma testosterone**

All 274 workers had been living and working in the same urban area for at least 5 years.

All of them reported that they had not eaten food containing cocoa, soybeans, oatmeal, walnuts and almonds, fresh and dry vegetables, during the 4 days prior to collection of blood for the determination of Ni.

Dietary habits and the consumption of water from the water supply and / or mineral water were similar in all subjects studied.
All values of the urinary creatinine analyzed were within the normal range (0.3-3.0 g/L) recommended by the World Health Organization (70).

The values of the concentrations of urinary Ni and plasma testosterone were expressed in terms of mean, standard deviation (SD), geometric mean, median and range (min-max) and are shown in Table 1.

The analysis of the ANOVA test and the Kruskal Wallis test showed no statistically significant differences in the comparison between the different tasks (independent variable) and the urinary Ni (dependent variable) and between the different tasks and plasma testosterone, as shown in Table 2.

In the total sample and in all subgroups evaluated, stratified on the basis of smoking habit and job position, the Pearson’s correlation analysis showed the values of urinary Ni negatively but significantly correlated, (p with 2 tails) with the values of plasma testosterone, which is why the concentration of plasma testosterone is reduced in a statistically significant manner when the urinary Ni increases (Table 4).

The multiple linear regression analysis confirmed the significance of the negative correlation between plasma testosterone and urinary Ni (R = -0.240, p = 0.027), compared to other confounding factors (age, length of service, cigarette smoking), both in the total sample and after subdivision on the basis of the work task (Table 5).

No worker had plasma levels of testosterone outside the normal range of the laboratory for males (1-11 ng/mL).

Discussion and conclusions

Endocrine disruptors are defined as “exogenous agents that interfere with the production, release, transport, metabolism, binding, action or elimination of the hormones naturally occurring in the body and responsible for the maintenance of the homeostasis and regulation of developmental processes” (42, 43, 46, 49-52).

It has been shown in literature that various environmental toxicants contained in the PM are endocrine disruptors that

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### Table 4 - Pearson correlation coefficient (R) between log peripheral blood count values and log total blood Ni in the total sample and after subdivision on the basis of sex, cigarette smoking and kind of task

<table>
<thead>
<tr>
<th>Variables</th>
<th>Biological indicator (log transformed)</th>
<th>Testosterone (log transformed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sample (n. 274)</td>
<td>Blood Nickel</td>
<td>r: -0.468</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p: 0.000 a</td>
</tr>
<tr>
<td>Non smoker subjects (n. 191)</td>
<td>Blood Nickel</td>
<td>r: -0.437</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p: 0.000 a</td>
</tr>
<tr>
<td>Smoker subjects (n. 83)</td>
<td>Blood Nickel</td>
<td>r: -0.548</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p: 0.000 a</td>
</tr>
<tr>
<td>Traffic policemen (n.170)</td>
<td>Blood Nickel</td>
<td>r: -0.372</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p: 0.000 a</td>
</tr>
<tr>
<td>Police drivers (n. 59)</td>
<td>Blood Nickel</td>
<td>r: -0.526</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p: 0.000 a</td>
</tr>
<tr>
<td>Police motorcyclists (n.15)</td>
<td>Blood Nickel</td>
<td>r: -0.740</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p: 0.002 a</td>
</tr>
<tr>
<td>Policemen with other outdoor activities (n. 30)</td>
<td>Blood Nickel</td>
<td>r: -0.814</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p: 0.000 a</td>
</tr>
</tbody>
</table>

*a* = The correlation is statistically significant at p 0.01 level (two-tailed).
Table 5 - Multiple linear regression analysis, in the total group of subjects studied, between the Log Testosterone plasma values (dependent variable) and Log urinary Ni with the main confounding factors (independent variables).

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Total sample</th>
<th>Traffic policemen</th>
<th>Police drivers</th>
<th>Police motorcyclists</th>
<th>Policemen with other outdoor activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t (beta)</td>
<td>p</td>
<td>t (beta)</td>
<td>t (beta)</td>
<td>t (beta)</td>
</tr>
<tr>
<td>Log Urinary nickel</td>
<td>-8,631</td>
<td>0,000</td>
<td>-5,224</td>
<td>0,000</td>
<td>-4,579</td>
</tr>
<tr>
<td></td>
<td>(-0,466)</td>
<td></td>
<td>(-0,375)</td>
<td></td>
<td>(-0,526)</td>
</tr>
<tr>
<td>Age (ys)</td>
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<td>0,358</td>
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<td>0,101</td>
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<tr>
<td></td>
<td>(0,074)</td>
<td></td>
<td>(0,169)</td>
<td></td>
<td>(-0,182)</td>
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<td>Working life (ys)</td>
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<td>0,361</td>
<td>-0,724</td>
<td>0,470</td>
<td>0,705</td>
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<tr>
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<td></td>
<td>(-0,075)</td>
<td></td>
<td>(0,119)</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>0,245</td>
<td>0,807</td>
<td>-1,043</td>
<td>0,298</td>
<td>-0,431</td>
</tr>
<tr>
<td></td>
<td>(-0,14)</td>
<td></td>
<td>(-0,075)</td>
<td></td>
<td>(-0,053)</td>
</tr>
<tr>
<td>Model</td>
<td>F (R² Ad.)</td>
<td>p</td>
<td>F (R² Ad.)</td>
<td>p</td>
<td>F (R² Ad.)</td>
</tr>
<tr>
<td></td>
<td>19,194</td>
<td>0,000*</td>
<td>7,892</td>
<td>0,000*</td>
<td>5,534</td>
</tr>
<tr>
<td></td>
<td>(0,210)</td>
<td></td>
<td>(0,140)</td>
<td></td>
<td>(0,238)</td>
</tr>
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</table>

ys = years
R² Ad. = R² Adjusted
* = Statistically signific
can significantly affect the reproductive functions, the hormones of the hypothalamic-pituitary-gonadal axis and the fertility (42, 52-55).

The PM components responsible for these effects, however, continue to remain not fully elucidated, and literature offers few and controversial results about it. It has been however demonstrated that the toxicity of PM2.5 depends, at least in part, on specific chemicals that are adherent to it and that the metals are often implicated as causative agents (31, 71).

The majority of reports on metal toxicity on the reproductive system are coming from experimental studies on animals, studies that are usually performed with high doses of exposure and / or short-term exposures, thus providing models that can be applied to the most common situations of human exposure. Moreover the potential for fertility and the endocrine system in man may differ from those of other mammals, as well as his susceptibility to different metals (35, 40, 72).

Epidemiological studies are therefore needed to validate the effect identified in experimental models. The data on the toxicity of metals on the human reproductive system for the moment are few and usually limited to groups of subjects non-occupationally exposed and residents near areas with high levels of air pollution (73) and to groups of subjects exposed to metals through consumption of contaminated food or water (74). These data, reporting the toxicity of metals on the human reproductive system, are also limited to only a few metal ions, lead above all. The effects on the reproduction of other metals such as Ni, are very few and usually limited to subjects with occupational exposures to high concentrations as workers employed in the mining facilities, refineries, electro-plating and foundries (75).

Occupational exposure to low doses of Ni and its possible effects on health and in particular on the reproductive system have not been well studied yet (35, 40, 72, 76).

This is the first research focused on occupational exposure to Ni in outdoor workers exposed to urban pollutants and on the effects of such exposure on Testosterone.

Our study was conducted in the largest city of central Italy in which there are about 2,700,000 inhabitants (77) with a density of approximately 1471 vehicles per km² (78). In the city covered by the study, the Ni pollution in the air is mainly adhering to respirable dusts (71).

In the city under study there are fixed stations for the monitoring of pollutants that show average annual values of Ni in urban air slightly decreasing from 4.9 in 2008 to 4.4 ng/m³ in 2010 (1) and similar to those observed in other cities where the average values range from 2.01 to 4.5 ng/m³ (79-81). These values indicate that urban air pollution by airborne Ni on particulate suspended matter (PTS) in the city under study can be considered at low doses.

The results of the individual dosimetry are in agreement with the data obtained by the control units. Although they were, on the average, higher than the values monitored by fixed stations, no samples exceeded the limit value of 1.5 mg/m³ proposed by ACGIH for subjects occupationally exposed to Ni. The occupational exposure of outdoor workers evaluated in the present study is also lower than the indoor exposure of industrial workers (82, 83).

In our study we did not perform statistical comparisons between the values of Ni obtained by fixed monitoring stations and testosterone plasma values because the fixed stations are less representative than personal dosimeters about the real Ni exposure of workers during their work shift. It should be considered that fixed stations are often placed higher (about 3.5 meters) (1) than personal dosimeters which are at the worker’s collar in the breathing area. Moreover, fixed stations do not provide data about the exposure of
workers during their movements connected to the execution of their individual tasks.

Finally, fixed stations take measurements over 24 hours that is during the night hours too, when pollutants values are much lower and not all workers are on duty.

All this may explain why the average pollutants values obtained from individual dosimeters were higher than those obtained from the fixed monitoring stations, and let us understand that outdoor workers have, during their work shift, a different pollutants exposure, often much higher than that of the general population.

The average values obtained for Ni individual dosimetry (Table 1) are, indeed, higher than the target value for the general population of 20 ng/m³, proposed by ARPA Lazio and in accordance with the Legislative Decree 03/08/2007 n 152, which transposes the European Directive 2004/107/EC into the Italian legislation (1).

Considering the results obtained, we believe that, even at low doses, the Ni can have the effects of endocrine disruptor for outdoor workers occupationally exposed. This figure is confirmed by the statistically significant negative correlation between the values of urinary Ni and plasma testosterone values in the studied subjects. This negative correlation was confirmed by the multiple linear regression (Table 5) which showed that the main confounding factors studied (age, length of service and smoking habit) did not contribute significantly to influence the results of the correlation and that the urinary Ni persisted as the only significant variable capable of influencing the values of plasma testosterone.

The multiple linear regression analysis also showed a statistically significant and positive correlation between low doses of the values of atmospheric Ni measured in individual dosimetry and the values of urinary Ni in both the total sample and after subdivision on the basis of the job (traffic policemen and drivers, Table 3).

These results comply with to those we had in other research, which showed the effects of pollution on human health, above all on the endocrine parameters and sex hormones and on other organ’s diseases (42, 43, 46, 48-52, 84-99).

The Ni can affect the male reproductive system directly, when the reproductive organs are the specific target organs, or indirectly when its action is exerted on the neuroendocrine system.

Since it accumulates in the epididymis, in the prostate, the seminal vesicles or in the seminal fluid, the Ni can alter the motility and the vitality of sperm (100, 101) and cause hormonal imbalances affecting the gonadal and neuroendocrine system. According to Jensen and colleagues (2006), occupational exposure to Ni and other metals can disrupt the androgen secretion by the Leydig cells or the inhibin B by the Sertoli cells (102).

One of the main pathogenetic mechanisms that can explain this type of alterations is the oxidative stress, frequently involved in the pathogenesis of the male infertility (103-105). According to many authors many metals, including Fe, Cu, Ni, Pb and Cd, may increase the production of reactive oxygen species (ROS), decrease the levels of glutathione and other antioxidants, improve lipid peroxidation of the cell membrane, and cause apoptosis and oxidative damage to the DNA (106, 107).

Clinical studies on this topic have associated the exposure to Ni with an increased risk of toxicity of the prostate (34), infertility and testicular toxicity (35, 36). Studies on laboratory animals and in vitro studies have observed that the Ni may interfere with the reproductive hypothalamic hormones LH and FSH and the testosterone (37, 38). These results were partly confirmed also in studies on human subjects exposed to Ni (35, 39, 40).

This correlation, however, has never been investigated by other Authors for occupational exposure to low doses of Ni.
pollution such as those observed in the subjects of the present study.

In our study, the T tests at independent-samples, the Mann-Whitney U test, ANOVA test and Kruskal Wallis test were not significant, showing that the testosterone and the urinary Ni did not vary with age, length of service and the smoking habit.

The urinary concentration of Ni is a good indicator of recent exposure to metallic Ni and its compounds (108, 109). The Ni in fact is not a cumulative toxic and practically the entire absorbed amount is excreted primarily in the urine, so the absorption of the metal by exposed workers is easily detectable by the biological monitoring of urinary Ni, which is the best indicator of internal dose for continuous occupational exposures. At the end of the exposure, the levels of urinary Ni can gradually return to normal limits (108, 109). The fact that Ni is not a metal with cumulative properties may explain why, in our research, it did not vary at different age and length of service (Table 2).

Ni is also contained in the tobacco, and therefore present in cigarettes, along with a multitude of different metals and other substances likely absorbed by the ground or contained in fertilizers or pesticides. It has been said that the Ni present in cigarettes could form volatile gaseous compounds, such as the Ni tetracarbonyl, which are introduced into the respiratory tract of smokers (110). The results reported in the literature about the health effects of the Ni content in cigarettes, however, are still controversial (110, 111). According to Torjussen (110) the Ni pollution is the main source of exposure to Ni in outdoor smoking workers occupationally exposed to urban pollution, compared with the Ni content in cigarettes.

This result is in agreement with those found in our study, where the mean values of urinary Ni were higher in smokers than in non-smokers, but not in a statistically significant way (univariate ANOVA test and Kruskal Wallis test: p > 0.05). The index of correlation between the urinary Ni and the plasma testosterone, however, was statistically significant and the test of multiple linear regression showed that the habit of cigarette smoking did not contribute significantly to influence the results of the correlation. We believe that these results show, in exposed workers, a higher effect of Ni pollution on the testosterone, compared with the Ni content in cigarette smoking.

Considering the results of multiple linear regression, we can assume that the ratio of urinary Ni and the decrease of plasma testosterone depends on the action of the metal on the hypothalamic-pituitary-gonadal axis.

In our study, no significant differences were observed regarding the presence of pathologies in fertility. This fact could be explained taking into account that chronic occupational exposure to urban pollutants is the basis for the development of a chronic and slowly progressive disease process of which our results represent only the initial phase.

The Ni content in cigarettes is 1-3 μg and you can inhale 2-12 μg of Ni for each pack of cigarettes smoked (20). Our results show that the habit of cigarette smoking does not seem to affect the relationship between Ni and the values of plasma testosterone, given the lack of significant correlations between these factors. This result can probably be explained by the fact that the study subjects abstained from smoking during examinations. However, this figure needs to be confirmed by further studies.

The relationship between the exposure to Ni and the values of plasma testosterone was already documented for medium-high doses but not for low doses, particularly in outdoor workers. In this study, for the first time in the literature, we evaluated the possible correlation between the exposure to Ni present in the urban environment and
the testosterone plasma values in outdoor workers, using the values of individual dosimetry and biological monitoring of urinary Ni.

The results obtained confirmed the association between the occupational exposure to low doses of atmospheric Ni and the indices of the hypothalamic-pituitary-gonadal axis. These results should lead to further studies on the effects of Ni on the working population exposed to urban pollutants, and on the effects of other hormones of the hypothalamic-pituitary-gonadal axis line as the GnRH, LH and FSH. Preventive measures should be taken to protect the health of all categories of outdoor exposed workers.

Testosterone may also be used as an early biological marker, valid for the group, to be used in subjects occupationally exposed to low doses of Ni before the onset of values out of the range and of the fertility disorders.

**Ethics fulfilment**

The present study was performed according to all the requirements concerning approval by ethical regulations and all the human subjects were informed about the meaning and the goals of the research.

**Riassunto**

*Correlazione tra nickel urinario e valori di plasmatici di testosterone in lavoratori professionalmente esposti a stressor urbani*

**Background:** Lo scopo del presente studio è valutare se l’esposizione occupazionale alle basse dosi di Nichel (Ni) presenti nell’aria urbana possa provocare alterazioni della concentrazione di testosterone plasmatico in lavoratori della Polizia Municipale di una grande città italiana assegnati a differenti tipologie di mansioni outdoor.

**Metodi:** 359 soggetti di sesso maschile sono stati inclusi nello studio e suddivisi sulla base della mansione, età, anzianità lavorativa e abitudine al fumo di sigaretta. Ad un campione dei lavoratori inclusi nello studio è stato effettuato il dosaggio del Ni atmosferico tramite dosimetrie personali. Per ciascun lavoratore incluso nello studio è stato effettuato il dosaggio del Ni totale ematico e del testosterone plasmatico. Sul campione totale è stato effettuato il test T a campioni indipendenti e il test Mann-Whitney per le variabili a 2 modalità (abitudine al fumo di sigaretta) e il test ANOVA univariata e il test di Kruskal Wallis per le variabili con più di 2 modalità (età, anzianità lavorativa e mansione lavorativa). È stato valutato l’indice di correlazione di Pearson con p a 2 code tra le variabili nel campione totale e dopo suddivisione sulla base dell’abitudine al fumo e sulla base della mansione. Dopo aver tenuto conto dei principali fattori di confondimento è stata effettuata la regressione lineare multipla sia sul campione totale dopo suddivisione per mansioni.

**Risultati:** È stata rilevata una correlazione negativa costante tra i valori di Ni urinario e i valori dei testostereonne plasmatici sia sul campione totale che per tutte le classi di suddivisione. Tali risultati sono stati confermati dalla regressione lineare multipla, che ha indicato il Ni come unica variabile significativa in grado di contribuire alle alterazioni del testosterone.

**Conclusioni:** Sulla base dei risultati ottenuti gli autori ipotizzano che l’esposizione occupazionale alle basse dosi di Ni presente nell’inquinamento urbano sia in grado di influenzare alcune linee del sistema ipotalamo-ipofisi-gonadi nei lavoratori esposti.

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