ISSN:2240-2594

CORRELATION BETWEEN URINARY NICKEL AND FSH PLASMA VALUES IN WORKERS OCCUPATIONALLY EXPOSED TO URBAN STRESSORS.

CORRELAZIONE TRA NICHEL URINARIO E VALORI DI FSH PLASMATICO IN LAVORATORI OUTDOOR ESPOSTI OCCUPAZIONALMENTE A STRESSOR URBANI.

De Sio S[1], Casale T[1], Rosati MV[1], Caciari T[1], Giubilati R[1], Gioffrè PA[1], Scala B[1], Nardone N[1], Suppi A[1], Di Pastena C[1], Loreti B[1], Sacco C[1], Sancini A[1], Tomei G[2], Tomei F[1].

[1] Department of Anatomy, Histology, Medical-Legal and Orthopaedics, Unit of Occupational Medicine, "Sapienza" University of Rome, Italy
[2] Department of Neurology and Psychiatry, "Sapienza" University of Rome, Italy

[1] Dipartimento di Anatomia, Istologia, Medicina Legale e Ortopedia, Unità di Medicina del Lavoro, "Sapienza" Università di Roma
[2] Dipartimento di Neurologia e Psichiatria, "Sapienza" Università di Roma

Citation: De Sio S, Casale T, Rosati MV, et al. Correlation between urinary Nickel and FSH plasma values in workers occupationally exposed to urban stressors. Prevent Res 2013; 3 (4): 263-279.
Available from: http://www.preventionandresearch.com/

Key words: environmental health, outdoor workers, FSH, urinary nickel, biological monitoring.

Parole chiave: salute ambientale, lavoratori outdoot, FSH, nichel urinario, monitoraggio biologico.

Abstract

Background: There is a growing concern about the adverse health effects of air pollution on the exposed populations. An important aspect of these effects concerns the endocrine disruption. Diesel exhaust particles, in particular, possess estrogenic, anti-estrogenic, and anti-androgenic disruptor properties that may have a potential negative impact on both male and female reproductive function. Clinical studies on this topic associated Ni exposure with an increased risk of toxicity of the prostrate, infertility, and testicular toxicity.

Objectives: The aim the study is to assess the relationship between occupational exposure to airborne nickel (Ni) and alterations of plasma FSH in workers of the Municipal Police assigned to different types of outdoor tasks.

Methods: 359 male subjects were enrolled and divided on the basis of job, age, length of service, and smoking habit. Exposure to airborne Ni, dosage of urinary Ni and plasma FSH were carried out.

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

Discussion: The endocrine disruptors are exogenous agents that have the ability to interfere in the functioning of the endocrine system by altering the production, release, transport, metabolism, and mechanisms of hormone actions. The alterations caused by these agents may be temporary or permanent. Exposure to these endocrine disruptors can alter hormone metabolism of the exposed subjects, altering the synthesis and/or release of testosterone, FSH, and LH.

Conclusions: Based on the results, it is suggested that occupational exposure to low doses of airborne Ni is able to influence some lines of the hypothalamic-pituitary-gonadal axis in exposed workers.

Abstract

Introduzione: Vi è una crescente preoccupazione per gli effetti negativi dell'inquinamento atmosferico sulla salute delle popolazioni esposte, in particolare per gli effetti che inducono disturbi endocrini. Le particelle di scarico, in particolare, hanno proprietà di interferente endocrino sia per gli estrogeni che per gli androgeni, e possono avere un potenziale impatto negativo sulla funzione riproduttiva maschile e femminile.
Studi clinici su questo argomento associano l'esposizione al Ni ad un aumentato rischio di tossicità per la prostata, infertilità e tossicità testicolare.

Obiettivi: Scopo dello studio è quello di valutare la relazione tra l'esposizione professionale a nichel (Ni) presente nell'aria e alterazioni dell'FSH plasmatico, in lavoratori della Polizia Municipale con diverse mansioni outdoor.

Metodi: 359 soggetti di sesso maschile sono stati coinvolti nello studio e suddivisi in base al lavoro, l'età, l'anzianità lavorativa e l'abitudine al fumo di sigaretta. Sono stati effettuati dosaggi di Ni presente nell'aria, di Ni ed FSH urinari e plasmatici.

Risultati: E' stata mostrata una correlazione positiva tra i valori di Ni ed FSH urinari e plasmatici sia sul campione totale, che per tutte le sottoclassi. Questi risultati sono stati confermati dall'analisi di regressione lineare multipla, che indica il Ni come unica variabile significativa che può contribuire alle alterazioni FSH.

Discussione: Gli interferenti endocrini sono agenti esogeni che hanno la capacità di interferire nel funzionamento del sistema endocrino alterando la produzione, il rilascio, il trasporto, il metabolismo e i meccanismi d'azione ormonali. Le alterazioni causate da questi agenti possono essere temporanei o permanenti. L'esposizione a questi interferenti endocrini può alterare, nei soggetti esposti, il metabolismo, la sintesi e/o il rilascio di testosterone, FSH e LH.

Conclusioni: I risultati ottenuti suggeriscono che l'esposizione professionale a basse dosi di Ni presente nell'aria è in grado di influenzare l'asse ipotalamo-ipofisi-gonadi nei lavoratori esposti.

Background

There is a growing international concern about the adverse health effects of air pollution on the exposed populations. In many industrialized and developing countries, this issue has become a major public health problem (1).

Diesel exhaust particles contain a heterogeneous mixture of chemical substances, heavy metals, and large amounts of total suspended particulates (PTS), which are able to adsorb on the external surface of a large part of other pollutants, in particular polycyclic aromatic hydrocarbons and heavy metals (2). This property was also investigated by the World Health Organization, according to which, the PTS is an important vehicle of exposure of all other air pollutants (3).

It is known that the organic compounds adsorbed on these suspended particles have lipophilic properties and are therefore able to pass through various organic barriers and cell membranes and potentially cause alterations in various organs and systems (4).

Several studies have shown a direct association between chronic exposure to diesel exhaust particles and development of respiratory, kidney, brain, and endocrine diseases, as well as cardiological, vascular, neoplastic, and hematopoietic abnormalities, with increased morbidity and mortality in the exposed populations (5-13).

An important aspect of these effects concerns the endocrine disruption. It has been shown in the literature that diesel exhaust particles, in particular, possess estrogenic, anti-estrogenic, and anti-androgenic disruptor properties (14-21) that may have a potential negative impact on both male and female reproductive function (22).

According to Yoshida et al., diesel exhaust particles are capable of suppressing spermatogenesis and lower levels of testosterone in laboratory mice (23). These results were also confirmed by other authors, who showed that urban pollution contains compounds that can modulate the estrogenic and anti-androgenic activity of the hypothalamic-pituitary-gonadal axis (24).

However, the specific pollutant responsible for these phenomena has not yet been identified, although various studies have suggested heavy metals present in urban pollution as potential elements able to influence the male reproductive system (25).

Nickel (Ni), in particular, has been recognized as a potential endocrine disruptor because of its adverse effects on reproduction (26) as well as interruption of steroidogenesis and spermatogenesis both in vivo and in laboratory animals (27, 28).

Clinical studies on this topic associated Ni exposure with an increased risk of toxicity of the prostrate (29), infertility, and testicular toxicity (30). Studies carried out on lab animals and in vitro studies observed that Ni may interfere with the reproductive hypothalamic hormones LH and FSH as well as testosterone (31, 32). These results were also partially confirmed in studies on exposed human subjects (30, 33, 34).

In correlation with LH and FSH gonadotropins, testosterone in men regulates the sexual functioning and controls sexual desire, sexual development, and semen production and maturation in the testes. Chronic alterations of these hormones can harm both physical and sexual aspects of the male anatomy. The most common side effects are erectile dysfunction, low sperm production, and poor male fertility.

Ni is an immunotoxic, neurotoxic, genotoxic, hepatotoxic, and nephrotoxic metal widely distributed in the environment, and it is one of the most heavy metals present as pollutants in urban air in the form adsorbed to the PM (3).

The International Agency for Research on Cancer attributes a certain carcinogenicity to humans (Group 1) for Ni compounds (35), and the European Union also includes it in the list of carcinogenic and/or mutagenic substances, assigning to it the risk phrase R49 (i.e. "may cause cancer by inhalation").

Ni present in urban air originates from natural sources (such as volcanic eruptions, forest fires, and windblown dust from rocks and soil) and artificial sources, especially, fossil combustion processes, which account for 62% of the total anthropogenic emissions (35). Other anthropogenic sources are related to Ni industrial refining processes (17% of total emissions), incineration of waste (12%), production of steel (3%), production of other metal alloys containing Ni (2%), and coal combustion (2%) (36). The compounds of Ni are also present as additives in unleaded gasoline (37), as catalysts in catalytic converters (38), and in paints, solvents (39), and some pesticides (40).

Outdoor workers, such as traffic policemen, are daily exposed to a large number of pollutants arising from traffic and to various other psychosocial stressors, which have been associated in the literature with alterations in the mean values of plasmatic androstenedione, testosterone, FSH, and LH (14, 15, 18-20, 41-44).

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

Materials and methods

Study Population

The study was conducted on a sample of 385 male outdoor workers who were employees in the Municipal Police of a large Italian city and assigned to different types of outdoor tasks such as traffic policemen, drivers, and other outdoor tasks. All subjects included in the study joined our program of health promotion in the workplace. This program was conducted in accordance with the directions of the current legislation and aimed to investigate the health status of individuals occupationally exposed to urban pollutants.

Traffic policemen were assigned to the control of vehicular traffic in streets and areas of high and medium traffic density, monitoring and controlling traffic at intersections, parking lots, and limited traffic areas. Drivers 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 core support marginalized workers, outdoor activities in the field of construction or the Judicial Police, Environmental Police, etc. Most of these activities were carried out in outdoor environments (only for the drivers, they were carried out in cars) for at least 80% of the working time (8 h a day for 5 days a week). All workers were not equipped with protective equipment against dust and fumes from traffic.

A sample comprising 385 subjects was chosen from different areas of the city examined. We divided the city into eight areas and selected 45 workers for each area (22 traffic policemen, 13 drivers, and 10 employees in other outdoor tasks); in the busiest central area, we selected 70 employees (40 traffic policemen, 15 drivers, and 15 employees in other outdoor tasks).

All workers were monitored once during the morning shift (7:00–14:00) on a working day between September 2010 and April 2011. Each worker completed a clinical case history on the same day of sampling. The questionnaire included information on age, area of residence in the last 5 years, physiological anamnesis (especially focused on diet, consumption of water from the water supply and/or mineral water, and exposure to cigarette smoking), and near and remote medical history. Following the recommendations of the WHO, we classified subjects who reported having smoked at least 100 cigarettes in their life, having stopped smoking less than 6 months ago, or being a smoker as smokers (45).

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 to verify the assumption of drugs 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 (40) as well as subjects using 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) (46). We also excluded subjects older than 50 years of age, those working on shift work and/or night shifts (47), those practicing competitive sports activities (48), and those performing outdoor tasks for less than 1 year. Those with levels of urinary Ni below the lower limit of detection (LOD) were also excluded

because they were nonrepresentative. The final number of workers included was 264.
For the purposes of statistical evaluation, we considered the following factors: job positions (traffic policemen, drivers, and outdoor workers with other tasks), age, seniority, and smoking habit. We stratified the sample based on age and 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) to better evaluate the influence of these variables on the parameters studied.
All subjects included in the study obtained written information referred to Art. 13 of Legislative Decree no. 196/03 ("Code regarding the protection of personal data") and were informed that the processing of their personal data are necessary to fulfill specific hygiene and work prevention requirements and are used for purposes related to the protection of workers' health in the workplace and for the formulation of judgment of suitability for the specific task by the physician's. All subjects also agreed to make their personal information available and were aware that such data would be classified as "sensitive information"; they also agreed that the 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.
All subjects signed confirmation of the information provided and declares to have been informed about the importance and the results of the investigations carried out.

Environmental monitoring of Ni: personal dosimetry

The characterization of the exposure to atmospheric Ni was evaluated through the execution of personal dosimetry. In total, personal dosimetry was provided to eight traffic policemen selected from eight different work areas considered to be most representative of the city's air quality in this study, as well as to four police drivers of cars with at least two policemen for each shift (so that 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 measured on the same day to avoid the influence of weather conditions on Ni in the air. All subjects were asked not to smoke during the sampling period. The personal air samples were collected using Dorr-Oliver cyclones (Sensidyne, Industrial Health & Safety, USA) of the type with a cut-point for the 5-microns 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 of air per minute, following the directions of the NIOSH (National Institute for Occupational Safety and Health). Each cyclone was fitted with a cassette holding a polyvinyl chloride (PVC) membrane filter of 37 mm. 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 Ni according to the method indicated by the NIOSH 7521 (49). 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 (7h). For each sample of air, the level of personal TWA exposure to Ni for 7 h was calculated. The American Conference of Governmental Industrial Hygienists (50) proposed a limit value (TLV-TWA) of 1,5 mg/m^3 for subjects occupationally exposed to Ni.

Urinary Ni and plasma FSH

The assay of urinary Ni and examination of plasma FSH 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, and fresh and dried legumes during the 4 days prior to the examination (51). For the urinary Ni, urine samples were transported to the lab within an appropriate thermal bag at the temperature of +4°C, and then were stored in a refrigerator at −20°C until the analytical determination of Ni and urinary creatinine were performed. Urinary Ni was determined 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. Determination of urinary creatinine to adjust the values of the biological indicators was carried out by using the Jaffe method (52). To take into account the dilution of the concentration of Ni in different urine samples, we divided the concentration of Ni (g/l) for urinary creatinine (g/l) and expressed the urinary concentration of Ni in terms of mg/g creatinine.
For the plasma FSH, 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°C until the moment when they were transferred (in a suitable container

and at the same temperature) to the laboratory where they were centrifuged and then stored at −20°C until analysis (within 3 days). The immunoassay method (EIA) was used to analyze the plasma FSH. Normal levels of plasma FSH were those routinely used by the laboratory for the clinical analysis of male subjects (1,0–14,0 µIU/ml).

Statistical analysis

The normal distribution of variables was assessed using the Kolmogorov–Smirnov test, which was statistically significant for the urinary Ni and FSH; hence, these parameters were converted into logarithmic form for the analysis of the index of correlation and multiple linear regression. The results of atmospheric Ni measured using the individual dosimetry, urine Ni, plasma FSH levels, and 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 was used for variables with two modes (smoking cigarette) and ANOVA and Kruskal-Wallis test were used for variables with more than two modes (age, length of service, and job function). Pearson correlation coefficient was applied after the logarithmic transformation of the data to evaluate the correlation between urinary Ni and FSH. Multiple linear regression analysis was performed after the logarithmic transformation of the data, considering the plasma FSH as a dependent variable and urinary Ni, age, length of service, and smoking cigarette as independent variables. Furthermore, multiple linear regression analysis was repeated using the urinary Ni as dependent variable and atmospheric Ni, age, length of service, and smoking cigarette as independent variables. The results were considered significant if p values were less than 0,05. All statistical analyses were performed using the software SPSS ® 10.0 Advanced StatisticalTM.

Results

Characteristics of the study population

The total sample of 264 male subjects was composed as follows: 184 were nonsmokers and 80 were smokers; 157 subjects were traffic policemen, 62 were drivers and 2 were patrol; and 45 were subjects with other outdoor tasks. These characteristics are shown in Table 1. The average values of urinary Ni and plasma FSH levels were 4,36 (SD = 3,12) µg/g creatinine and 4,56 (DS=4,5) µIU/ml in smokers and 4,15 (DS=3,64) µg/g creatinine and 4,5 (DS=7,9) µIU/ml in nonsmokers.

No statistically significant differences between the values of urinary Ni and FSH (test variables) and the habit of cigarette smoking (grouping variable) were found in t-test for independent samples and Mann-Whitney U test. Furthermore, no statistically significant differences between the values of urinary Ni and FSH (dependent variable) and age and length of service (independent variables – Age: Group A: 20–35 years; Group B: 36–45 years; Group C:> 45 years; Length of service: Group A: <10 years; Group B: 10–20 years; Group C: 21–40 years) were found in the univariate ANOVA and Kruskal-Wallis test. The results of the above-mentioned statistical tests are presented in Table 2. There were no statistically significant differences among the different outdoor tasks (traffic policemen, drivers, and subjects with other tasks) in relation to the average values and the distribution by age, length of service, and smoking habit (smokers and nonsmokers). In the sample studied, no subjects reported being diagnosed or treated for fertility disorders, either in the past or present.

Environmental monitoring of Ni: individual dosimetry

The values of individual exposure to atmospheric Ni are shown in Table 1. All the subjects reported that they had not smoked during the sampling period. No sample exceeded the limit value of 1,5 mg/m^3 ACGIH proposed for occupationally exposed subjects. Multiple linear regression analysis revealed a significant correlation ($p < 0,01$) between the atmospheric Ni and urinary Ni in both the total sample and in sample classified on the basis of job (traffic policemen and drivers; Table 3).

Ni urinary and plasma FSH

All 264 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, and fresh and dry vegetables during the 4 days prior to collection of blood for the determination of Ni. Dietary habits and consumption of water from the

water supply and/or mineral water were similar in all the subjects studied. All values of the urinary creatinine analyzed were within the normal range (0,3–3,0 g/l) recommended by WHO (53).
The values of the concentrations of urinary Ni and plasma FSH were expressed in terms of mean, standard deviation (SD), geometric mean, median, and range (min-max), and are shown in Table 1. ANOVA and Kruskal-Wallis test showed no statistically significant differences in the comparison between different tasks (independent variable) and urinary Ni (dependent variable), and between the different tasks and plasma FSH, as shown in Table 2.
In the total sample and in all subgroups stratified on the basis of smoking habit and job position, Pearson's correlation analysis showed values of urinary Ni positively and significantly correlated (two-tail p) with the values of plasma FSH, as a result of which the concentration of plasma FSH increased in a statistically significant manner when the urinary Ni increased (Table 4). Multiple linear regression analysis confirmed the significance of the positive correlation between plasma FSH and urinary Ni ($R = -0,240$, $p = 0,027$), when compared with 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 FSH outside the normal laboratory range for males (1,0–14,0 μIU/ml).

Discussion

Until relatively recently, the role of exposure to external environmental factors in the alterations of male reproduction had only been studied in experimental animals. The interest on this topic for occupationally exposed populations is relatively recent similar to the promotion of research in this area (54).
The endocrine disruptors are exogenous agents that have the ability to interfere in the functioning of the endocrine system by altering the production, release, transport, metabolism, and mechanisms of hormone actions. The alterations caused by these agents may be temporary or permanent (55) and involve, as part of the male gonadal system, anatomical, hormonal, and genetic alterations (56, 57).
It has been shown in studies that various environmental toxicants contained in urban PTS are endocrine disruptors able to significantly affect the reproductive functions, hormones of the hypothalamic-pituitary-gonadal axis, and the main characteristics of the seminal fluid (changes in viscosity, mobility, and sperm vitality) (19, 58, 59).
According to Yang et al. (2013), exposure to these endocrine disruptors can alter hormone metabolism of the exposed subjects, altering the synthesis and/or release of testosterone, FSH, and LH (60). These results were also confirmed by other authors in the literature and from our previous research (14, 18, 20, 41-43, 61, 62).
However, it should be noted that a number of questions on this topic have not yet been fully resolved.
First, the mechanism of action of urban pollutants in the pathogenesis of hormonal changes has not yet been clarified, and the results of literature studies are not only unique, but even sometimes contradictory. The sites of attachment and mechanisms of action of endocrine disruptors in the male reproductive system are in fact extremely heterogeneous, and endocrine disruption could be linked to the suppression of the neuroendocrine control in the testicles (with effects on the synthesis and release of testosterone), central nervous system (with effects on GnRH, FSH, and LH), or both the locations simultaneously (63). According to some research, in addition, some chemical compounds can exert their toxicity through structural similarity with the steroid and reproductive hormones (64).
Some studies have also emphasized on the importance of contamination of semen by the environmental toxicants and have demonstrated that these chemicals are able to pass into the semen via testicular plasma, epididymal plasma, vas deferens and ampullary secretions, and secretory fluids of seminal vesicles and other accessory glands (65).
According to other authors, the causes of interference may be associated with the liver, which is one of the sites of metabolism of steroid hormones as well as the primary target organ of exogenous toxicants (66).
Second, another important question is about the safety of the current urban pollutant exposures limit values, which appear to be inadequate for the health of exposed population.
In several studies, the development of alterations in hormone concentrations even after exposure to urban pollutants below the provided limit values has been demonstrated (54, 67).
The PM components responsible for these endocrine effects, however, are still not fully elucidated, and literature offers few and controversial results. It has been demonstrated that the toxicity of PM depends, at least in part, on specific chemicals that adhere to it and that the metals are often implicated as causative agents (3, 28).
The majority of results on metal toxicity on the reproductive system are obtained from experimental studies on animals, studies that are usually performed with high doses of exposure, and/or short-term exposures, thus providing

models that cannot be applied to the most common situations of human exposure. Moreover, the potential for fertility and endocrine system in man may differ from those of other mammals as well as the susceptibility to different metals (30, 34).

Therefore, epidemiological studies are needed to validate the effect identified in experimental models.

Currently, the data concerning occupationally exposed subjects are few and usually limited to groups of subjects with occupational exposures to high concentrations of metals such as workers employed in the mining facilities, refineries, electroplating, foundries, and welding. In addition, the existing data are limited to only a few metals such as lead, mercury, and cadmium. For other heavy metals such as Ni, literature studies are inadequate or missing (30, 54, 12, 34).

This study represents the first research focused on occupational exposure to chronic low doses of Ni in outdoor workers exposed to urban pollutants and the effects of such exposure on FSH.

Our study was conducted in one of the largest cities of central Italy in which there are about 2.700.000 inhabitants (68) with a density of approximately 1.471 vehicles per km^2 (69).

In the city studied, there are fixed stations for monitoring pollutants, which showed that the average annual values of Ni in urban air slightly decreased from 4,9 in 2008 to 4,4 ng/m^3 in 2010 (70). These values indicate that urban air pollution by airborne Ni on PTS in the city studied can be considered at low doses. Furthermore, the results are in agreement with the data obtained by the control units. Although the Ni mean values in individual dosimetry (Table 1) were higher than the target value proposed by ARPA Lazio for the general population (20 ng/m^3) (70), no samples exceeded the limit value of 1,5 mg/m^3 proposed by ACGIH for subjects occupationally exposed to this metal.

The occupational exposure of outdoor workers evaluated in the present study also appeared to be several orders of magnitude lower than that of industrial workers operating in indoor environments (71).

Considering the results obtained, we believe that even at low doses, Ni can produce the effects of endocrine disruptor on the hypothalamic-pituitary-gonadal axis of occupationally exposed outdoor workers. This figure was confirmed by the statistically significant positive correlation between the values of urinary Ni and plasma FSH in the studied subjects.

This correlation was also confirmed in the multiple linear regression analysis (Table 5), which showed that the main confounding factors studied (age, length of employment, and smoking habit) did not significantly contribute to influence the results of correlation and that the urinary Ni persisted as the only significant variable capable of influencing the values of plasma FSH.

Multiple linear regression analysis also showed a statistically significant positive correlation between low concentrations of atmospheric Ni measured in individual dosimetries 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).

Finally, in our study, independent-samples t-test, Mann-Whitney U test, ANOVA, and Kruskal-Wallis test were not significant, showing that urinary FSH and Ni did not vary with age, length of service, and smoking habit.

These results could be explained by considering the fact that the urinary Ni represents a good indicator of recent exposures, but not late exposures, to Ni metal and its compounds (72, 73).

Ni, in fact, is not a cumulative toxicant, and, practically, the entire absorbed amount is excreted primarily in the urine. This makes the urinary Ni the best biological indicator of internal dose for continuous occupational exposures; however, at the end of the exposure, the levels of urinary Ni can gradually return to normal limits (72, 73). 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 (one-way ANOVA to Kruskal-Wallis test, Age: Group A: 20–35 years, Group B: 36–45 years, Group C:> 45 years; Length of service: Group A: <10 years, Group B: 10–20 years, Group C: 21–40 years; Table 2).

Ni is also a metal content in tobacco and is present in cigarettes along with a multitude of different metals and other substances. It has also been reported that Ni present in cigarette could form volatile gaseous compounds such as Ni tetracarbonyl, which are introduced into the respiratory tract of smokers, although the results regarding the effects of these inhalations on health remain, to date, controversial (74).

According to Torjussen, Ni pollution is the main source of exposure to Ni in outdoor workers and smokers occupationally exposed to urban pollution, when compared with the Ni content in the cigarettes they smoked (74).

This observation is in agreement with those found in our study, where the mean values of urinary Ni were higher in smokers than in nonsmokers, but not in a statistically significant way (univariate ANOVA test and Kruskal-Wallis test: p> 0,05).
However, the index of correlation between the urinary Ni and plasma FSH in workers who smoke was statistically significant, and multiple linear regression analysis showed that the habit of cigarette smoking did not significantly influence the results of this correlation. These results seemed to indicate a higher effect of Ni pollution on the FSH in exposed workers who smoke, when compared with the Ni content in cigarette smoke.
Finally, in our study, no significant differences were observed with regard to the presence of pathologies in fertility. This fact could be explained by taking into account that chronic occupational exposure to urban pollutants is the basis for the development of chronic and slowly progressive disease process, and our results represent only its initial phase.

Conclusions

The relationship between exposure to Ni and values of plasma FSH has never been documented in outdoor workers chronically exposed to low doses of this metal.
This study is the first to evaluate the possible correlation between exposure to low doses of Ni present in the urban environment and values of FSH in outdoor workers, using the values of personal dosimetry and biological monitoring of urinary Ni.
In agreement with the results obtained, we can assume that the relationship between urinary Ni and increase in plasma FSH depends on the action of this metal, even at low doses, on 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 such as GnRH, LH, and testosterone.
Preventive measures should be taken to protect not only the health of the category of outdoor workers studied in the present study, but also the health of all categories of exposed workers.
FSH may also be used as an early biological marker, valid for the group, in occupational subjects exposed to low doses of Ni before the onset of values out of range and fertility disorders.

References

1. Markert B, Wuenschmann S, Fraenzle S, et al. Bioindication of atmospheric trace metals--with special references to megacities. Environ Pollut 2011; 159 (8-9): 1991-1995.
2. Strandell M, Zakrisson S, Alsberg T, et al. Chemical analysis and biological testing of a polar fraction of ambient air, diesel engine, and gasoline engine particulate extracts. Environ Health Perspect 1994; 102: 85-92.
3. WHO, World Health Organization. (2000). Air quality guidelines for Europe. 2nd ed.
Available from: http://www.euro.who.int/__data/assets/pdf_file/0005/74732/E71922.pdf
4. Oehme M, Larssen S, Brevik EM. Emission factors of PCDD and PCDF for road vehicles obtained by tunnel experiment. Chemosphere 1991; 23:1699-1708.
5. Ciarrocca M, Tomei G, Fiaschetti M, et al. Assessment of occupational exposure to benzene, toluene and xylenes in urban and rural female workers. Chemosphere2012; 87:813-819.
6. Ciarrocca M, Tomei F, Caciari T, et al. Environmental and biological monitoring of benzene in traffic policemen, police drivers and rural outdoor male workers. J Environ Monit 2012; 14: 1542-1550.
7. Dominici F, Peng RD, Bell ML, et al. Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 2006; 295: 1127-1134.
8. Sancini A, Tomei F, Schifano MP, et al. Stress characteristics in different work conditions: is it possible to identify specificity of risk factors by the questionnaire method? Eurpean Journal of Inflammation 2010; 8: 117-123.
9. Sancini A, Caciari T, Fioravanti M, et al. Meta-analisi: efficacia degli interventi preventivi negli infortuni in agricoltura. G Ital Med Lav Erg 2010; 32: 25-30
10. Sancini A, Ciarrocca M, Capozzella A, et al. Shift work and night work and mental health. G Ital Med Lav Erg 2012; 34: 76-84.
11. Theophanides M, Anastassopoulou J, Vasilakos C, et al. Mortality and pollution in several Greek cities. J Environ Sci Health A Tox Hazard Subst Environ Eng 2007; 42: 741-746.

12. Tomei F, Rosati MV, Ciarrocca M, et al. Urban pollution and nickel concentration in serum. Int J Environ Health Res 2004; 14 (1): 65-74.
13. Tomei G, Ciarrocca M, Scimitto L, et al. Mental health and women's work: Is balance possible? Minerva Psichiatr. 2012; 53: 79-89.
14. Ciarrocca M, Caciari T, Ponticiello BG, et al. Follicle-stimulating hormone levels in female workers exposed to urban pollutants. Int J Environ Health Res 2011; 21 (6): 391-401.
15. Ciarrocca M, Capozzella A, Tomei F, et al. Exposure to cadmium in male urban and rural workers and effects on FSH, LH and testosterone. Chemosphere 2013; 90 (7): 2077-2084.
16. Tomei F, Rosati MV, Baccolo TP, et al. Plasma concentration of adrenocorticotropic hormone in traffic policemen. Journal of Occupational Health 2003; 45 (4): 242-247.
17. Tomei F, Rosati MV, Baccolo TP, et al. Occupational exposure to urban pollutants and plasma growth hormone (GH). J Environ Sci Health A Tox Hazard Subst Environ Eng 2003; 38 (6): 1017-1024.
18. Tomei G, Ciarrocca M, Fiore P, et al. Exposure to urban stressor and effects on free testosterone in female workers. Sci Total Environ 2008; 392: 198-202.
19. Tomei G, Sancini A, Cerratti D, et al. Effects on plasmatic androstenedione in female workers exposed to urban stressor. Eur J Inflamm 2009; 7: 175-182.
20. Tomei G, Tomao E, Ciarrocca M, et al. Follicle-stimulating hormone levels in male workers exposed to urban chemical, physical, and psychosocial stressors. Toxicol Ind Health 2009; 25 (6): 395–402.
21. Okamura K, Kizu R, Toriba A, et al. Antiandrogenic activity of extracts of diesel exhaust particles emitted from diesel-engine truck under different engine loads and speeds. Toxicology 2004; 195: 243-254.
22. Quigley CA, De Bellis A, Marschke KB, et al. Androgen receptor defects: historical, clinical, and molecular perspectives. Endocr Rev 1995; 16: 271–321.
23. Yoshida S, Sagai M, Oshio S, et al. Exposure to diesel exhaust affects the male reproductive system of mice. Int J Androl 1999; 22: 307–315.
24. Tsukue N, Toda N, Tsubone H, et al. Diesel exhaust (DE) affects the regulation of testicular function in male Fischer 344 rats. J Toxicol Environ Health A 2001; 63: 115–126.
25. Li C, Taneda S, Suzuki AK, et al. Estrogenic and anti-androgenic activities of 4-nitrophenol in diesel exhaustparticles. Toxicol Appl Pharmacol 2006; 217 (1): 1-6.
26. Hjollund NH, Bonde JP, Jensen TK, et al. Semen quality and sex hormones with reference to metal welding. Reprod Toxicol 1998 ; 12 (2): 91-95.
27. Sun Y, Ou Y, Cheng M, et al. Binding of nickel to testicular glutamate-ammonia ligase inhibits its enzymatic activity. Mol Reprod Dev 2011; 78 (2): 104-115.
28. Xu X, Rao X, Wang TY, et al. (2012). Effect of co-exposure to nickel and particulate matter on insulin resistance and mitochondrial dysfunction in a mouse model.
Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3545913/
29. Kizu R, Okamura K, Toriba A, et al. Antiandrogenic activities of diesel exhaust particle extracts in PC3/AR human prostate carcinoma cells. Toxicol Sci 2003; 76: 299–309.
30. Apostoli P, Catalani S. Metal ions affecting reproduction and development. Met Ions Life Sci 2011; 8: 263-303.
31. Lukac N, Bardos L, Stawarz R, et al. In vitro effect of nickel on bovine spermatozoa motility and annexin V-labeled membrane changes. J Appl Toxicol 2011; 31 (2): 144-9.
32. Murawska-Ciałowicz E, Bal W, Januszewska L, et al. (2012). Oxidative stress level in the testes of mice and rats during nickel intoxication. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3290116/
33. Pizent A, Tariba B, Živković T. Reproductive toxicity of metals in men. Arh Hig Rada Toksikol 2012; 1: 35-46.
34. Wirth JJ, Mijal RS. Adverse effects of low level heavy metal exposure on male reproductive function. Syst Biol Reprod Med 2010; 56 (2):147-167.
35. IARC, International Agency for Research on Cancer. (2012). Diesel Engine Exhaust Carcinogenic, Press release. Available from: http://www.iarc.fr/en/media-centre/pr/2012/pdfs/pr213_E.pdf.
36. ATSDR, Agency for Toxic Substances and Disease Registry. (2005). Toxicological Profile for Nickel. US Department of Health and Human Services. Available from: http://www.atsdr.cdc.gov/toxprofiles/tp15.pdf.

37. Espinosa AJF, Rodrıguez MT, Barragan de la Rosa FJ, Jimenez Sanchez JC. A chemical speciation of trace metals for fine urban particles. Atmospheric Environment 2002; 36: 773-780.
38. Han FS. Transition-metal-catalyzed Suzuki-Miyaura cross-coupling reactions: a remarkable advance from palladium to nickel catalysts. Chem Soc Rev 2013; 42 (12): 5270-5298.
39. Stewart WF, Stewart PA. Occupational case-control studies: I. Collecting information on work histories and work-related exposures. Am J Ind Med 1994; 26 (3): 297-312.
40. Noone P. Pesticides, nickel exposure and retirement planning. Occup Med (Lond) 2010; 60 (4): 323.
41. Monti C, Ciarrocca M, Cardella C, et al. Exposure to urban stressor and effects on luteinizing hormone (LH) in female outdoor workers. J Environ Sci Health A Tox Hazard Subst Environ Eng 2006; 41 (8): 1437-1448.
42. Sancini A, Tomei F, Tomei G, et al. Exposure to urban stressors and free testosterone plasma values. Int Arch Occup Environ Health 2011; 84 (6): 609-616.
43. Tomao E, Tomei G, Rosati MV, et al. Luteinizing hormone (LH) levels in male workers exposed to urban stressors. Sci Total Environ 2009; 407 (16): 4591-4595.
44. Tomei G, Ciarrocca M, Bernardini A, et al. Plasma 17-alpha-OH-progesterone in male workers exposed to traffic pollutants. Ind Health 2007; 45 (1): 170-176.
45. BRFFS, Behavioral Risk Factor Surveillance System. (2013). Chronic Disease Indicators: Indicator Definition. Available from: http://apps.nccd.cdc.gov/cdi/IndDefinition.aspx?IndicatorDefinitionID=17
46. Saunders JB, Aasland OG, Babor TF, et al. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II. Addiction 1993; 88 (6): 791-804.
47. Davis S, Mirick DK, Chen C, Stanczyk FZ. Night shift work and hormone levels in women. Cancer Epidemiol Biomarkers Prev 2012; 21 (4): 609-618.
48. Shiels MS, Rohrmann S, Menke A, et al. Association of cigarette smoking, alcohol consumption, and physical activity with sex steroid hormone levels in US men. Cancer Causes Control 2009; 20 (6): 877-886.
49. EPA, Environmental Protection Agency. (1983). Methods for the Chemical Analysis of Water and Wastes. Available from: http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=30000Q10.txt
50. AIDII, Associazione Italiana degli Igienisti Industriali per l'igiene industriale e per l'ambiente. TLV e IBE ACGIH 2012 basati sulla documentazione per i valori limite di soglia per sostanze chimiche ed agenti fisici e gli indici biologici di esposizione. IJOEHY 2012; suppl. vol.3 n.1
51. Sharma AD. Relationship between nickel allergy and diet. Indian Journal of Dermatology, Venereology and Leprology 2007; 73: 307-312.
52. Liu WS, Chung YT, Yang CY, et al. Serum creatinine determined by Jaffe, enzymatic method, and isotope dilution-liquid chromatography-mass spectrometry in patients under hemodialysis. J Clin Lab Anal 2012; 26 (3): 206-214.
53. WHO, World Health Organization. (2007). Nickel in Drinking Water. Guidelines for Drinking-water Quality. Available from: http://www.who.int/water_sanitation_health/gdwqrevision/nickel2005.pdf
54. Figà-Talamanca I, Traina ME, Urbani E. Occupational exposures to metals, solvents and pesticides: recent evidence on male reproductive effects and biological markers. Occup Med (Lond) 2001; 51 (3): 174-188.
55. Waissmann W. Health surveillance and endocrine disruptors. Cad Saude Publica 2002; 18 (2): 511-517.
56. Nelson P. Epidemiology, biology, and endocrine disrupters. Occup Environ Med 2003; 60: 541-542.
57. Pflieger-Bruss S, Schuppe HC, Schill WB. The male reproductive system and its susceptibility to endocrine disrupting chemicals. Andrologia 2004; 36: 337-345.
58. Dawson EB, Ritter S, Harris WA, et al. Comparison of sperm viability with seminal plasma metal levels. Biol Trace Elem Res 1998; 64: 215-219.
59. De Rosa M, Zarrilli S, Paesano L, et al. Traffic pollutants affect fertility in men. Hum Reprod 2003; 18: 1055-1061.
60. Yang Y, Lu XS, Li DL, Yu YJ. Effects of Environmental Lead Pollution on Blood Lead and Sex Hormone Levels among Occupationally Exposed Group in An E-waste Dismantling Area. Biomed Environ Sci 2013; 26 (6): 474-484.
61. Queiroz EK, Waissmann W. Occupational exposure and effects on the male reproductive system. Cad Saude Publica 2006; 22 (3): 485-493.
62. Yu T, Li Z, Wang X, et al. Effect of lead exposure on male sexual hormone. Wei Sheng Yan Jiu 2010; 39 (4): 413-415.

63. Rodamilans M, Martinez-Osaba MJ, To-Figueras J, et al. Inhibition of intratesticular testosterone synthesis by inorganic lead. Toxicol Lett 1988; 42 (3): 285-390.
64. Roy JR, Chakraborty S, Chakraborty TR. Estrogen-like endocrine disrupting chemicals affecting puberty in humans--a review. Med Sci Monit 2009; 15 (6): RA137-145.
65. Mann T, Lutwak-Mann C. Passage of chemicals into human and animal semen: mechanisms and significance. Crit Rev Toxicol 1982; 11 (1): 1-14.
66. Saiyed H, Dewan A, Bhatnagar V, et al. Effect of endosulfan on male reproductive development. Environ Health Perspect 2003; 111 (16): 1958-1962.
67. Caciari T, Casale T, Ciarrocca M, et al. Correlation between total blood lead values and peripheral blood counts in workers occupationally exposed to urban stressors. J Environ Sci Health A Tox Hazard Subst Environ Eng 2013; 48 (12): 1457-1469.
68. ISTAT, Italian National Institute of Statistic. (2008). Bilancio demografico anno 2008 e popolazione residente al 31 Dicembre 2006. Available from: http://demo.istat.it/bil2008/index.html
69. ISPRA, Institute for Protection and Environmental Research. (2007). Rete del Sistema Informativo Nazionale Ambientale. Urban Areas Indicators. Report.
Available from: http://www.areemetropolitane.sinanet.apat.it/OSTlist.php
70. ARPA Lazio, Regional Agency for the Environmental Prevention. (2007). Quarto rapporto sulla qualità delle acque superficiali e sotterranee della Provincia di Roma. Available from: http://www.arpalazio.net/main/
71. Yokota K, Johyama Y, Kunitani Y, et al. Urinary elimination of nickel and cobalt in relation to airborne nickel and cobalt exposures in a battery plant. Int Arch Occup Environ Health 2007; 80 (6): 527-531.
72. Gil F, Hernández AF, Márquez C, et al. Biomonitorization of cadmium, chromium, manganese, nickel and lead in whole blood, urine, axillary hair and saliva in an occupationally exposed population. Sci Total Environ 2011; 409 (6): 1172-1180.
73. Weiss T, Pesch B, Lotz A, et al. Levels and predictors of airborne and internal exposure to chromium and nickel among welders--results of the WELDOX study. Int J Hyg Environ Health 2013; 216 (2): 175-183.
74. Torjussen W, Zachariasen H, Andersen I. Cigarette smoking and nickel exposure. J Environ Monit 2003; 5 (2): 198-201.

Table 1. Characteristics of the study population divided for task

Variables	Traffic policemen	Police drivers	Policemen with other outdoor activities
Number of subjects	**(n.157)**	**(n.62)**	**(n.45)**
Smoking habit			
n°(%)	38 (24,2)	19 (30,6)	13 (28,8)
Age (ys)			
Mean (SD)	45,65 (7,68)	45,74 (8,02)	46,84 (8,36)
Geometric Mean	45,02	45,03	46,04
Min-Max	29-64	28-63	28-60
Median	44	45	48
Working life (ys)			
Mean (SD)	14,23 (8,4)	15,66 (7,33)	17,53 (8,21)
Geometric Mean	11,85	13,97	15,25
Min-Max	1-36	5-35	4-34
Median	13	16	17
Urinary Nickel (µg/g creatinine)			
Mean (SD)	4,79 (4,5)	4,21 (2,6)	4,34 (4,32)
Geometric Mean	3,05	3,31	3,25
Min-Max	1-10,1	1-24,9	1-22,1
Median	3,1	3,15	3,1
FSH (µIU/ml)			
Mean (SD)	4,92 (8,47)	4,14 (2,42)	4,48 (5,76)
Geometric Mean	3,21	3,27	3,13
Min-Max	1,1-14	1,1-10,3	1,1-13,2
Median	2,95	3,6	3,1
Air Nickel (ng/m^3)			
Number of subjects	8	4	
Mean (SD)	113,22 (123,46)	103,39 (62,32)	
Geometric Mean	85,18	83,62	
Min-Max	30,2-538,7	11,6-253	
Median	78	84,95	

SD: Standard Deviation
ys: years

Table 2. Indipendent sample T test and ANOVA univariate test between dependent variables (FSH and Urinary Ni) and independent variables (smoking habit, age, working life and kind of task).

	Statistical analysis with dependent variable: Plasmatic FSH				Statistical analysis with dependent variable: Urinary Nickel			
	Indipendent sample T test (p)	Univariate Anova test (p)	Mann-Whitney U test (p)	Kruskal Wallis Test (p)	Indipendent sample T test (p)	Univariate Anova test (p)	Mann-Whitney U test (p)	Kruskal Wallis Test (p)
Smoking habit	0,84		0,32		0,75		0,31	
Age		0,81		0,13		0,62		0,61
Working life		0,54		0,30		0,17		0,12
Kind of task		0,53		0,68		0,96		0,51

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).

Indipendent variables	Dependent variable: Log Urinary Nickel					
	Total sample		Traffic policemen		Police drivers	
	t (beta)	p	t (beta)	p	t (beta)	p
Log Air Nickel	19,344 (0,876)	0,000	23,431 (0,924)	0,000	5,481 (0,863)	0,000
Age (ys)	0,132 (0,008)	0,895	-0,261 (-0,014)	0,795	-0,537 (-0,112)	0,603
Working life (ys)	0,285 (0,018)	0,776	0,430 (0,024)	0,668	0,275 (0,059)	0,789
Smoking habit	-1,064 (-0,048)	0,290	0,505 (0,020)	0,615	-1,382 (-0,221)	0,197
Model	F (R^2 Ad.)	p	F (R^2 Ad.)	p	F (R^2 Ad.)	p
	98,361 (0,772)	0,000 [a]	140,339 (0,845)	0,000 [a]	9,575 (0,710)	0,002[a]

Ys: years

R^2 Ad.: R^2 Adjusted

[a]: Statistically significant

Table 4. Pearson correlation coefficient (R) between log FSH plasma values and log total blood Ni in the total sample and after subdivision on the basis of cigarette smoking habit and kind of task.

Variables	Biological indicator	Log FSH
Total sample (n.264)	Log Urinary Nichel	r: 0,409 p: 0,000 [a]
Non smoker subjects (n.184)	Log Urinary Nichel	r: 0,363 p: 0,000 [a]
Smoker subjects (n.80)	Log Urinary Nichel	r: 0,516 p: 0,000 [a]
Traffic policemen (n.157)	Log Urinary Nichel	r: 0,314 p: 0,000 [a]
Police drivers (n.62)	Log Urinary Nichel	r: 0,389 p: 0,002 [a]
Policemen with other outdoor activities (n.45)	Log Urinary Nichel	r: 0,838 p: 0,000 [a]

[a] : The correlation is statistically significant at the 0.01 level (two-tailed).

Table 5. Multiple linear regression analysis, in the total group of subjects studied, between the Log FSH plasma values (dependent variable) and Log urinary Ni with the main confounding factors (independent variables).

Indipendent variables	Dependent variable: Log FSH							
	Total sample		Traffic policemen		Police drivers		Policemen with other outdoor activities	
	t (beta)	p	t (beta)	p	t (beta)	p	t (beta)	p
Log Urinary Nickel	5,101 (0,293)	0,000	4,100 (0,301)	0,000	3,283 (0,391)	0,002	3,051 (0,388)	0,02
Age (ys)	0,323 (0,028)	0,747	-0,021 (-0,002)	0,983	0,417 (0,075)	0,679	-0,361 (-0,100)	0,720
Working life (ys)	1,416 (0.121)	0,158	1,458 (0,154)	0,147	0,448 (0,078)	0,656	0,793 (0,217)	0,432
Smoking habit	-0,913 (-0,054)	0,362	0,260 (0,019)	0,795	-1,675 (-0,213)	0,100	-1,259 (-0,209)	0,215
Model	F (R^2 Ad.)	p	F (R^2 Ad.)	p	F (R^2 Ad.)	p	F (R^2 Ad.)	p
	9,395 (0,110)	0,000 [a]	5,714 (0,100)	0,000 [a]	4,191 (0,237)	0,005 [a]	3,945 (0,131)	0,03 [a]

ys: years

R^2 Ad.: R^2 Adjusted

[a] : Statistically significant

Corresponding Author: Gianfranco Tomei
Department of Neurology and Psychiatry, "Sapienza" University of Rome, Italy
e-mail: info@preventionandresearch.com

Autore di riferimento: Gianfranco Tomei
Dipartimento di Neurologia e Psichiatria, "Sapienza" Università di Roma
e-mail: info@preventionandresearch.com